A MODEL SYSTEM FOR COMPARING HOST RESPONSES TO MULTIPLE INFECTIONS ON INTACT ONIONS I. EFFECTS OF LIGHT

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ABSTRACT

Fleshy leaf-bases of white, yellow, and red onions were exposed to ambient air and inoculated at 3 locations with Colletotrichum dematium *var* circinans *and* Botrytis allii *before incubation in light or dark. Other areas, on the same bulbs, were puncture wounded without inoculation, or not treated after exposure. Largest lesions were produced by* B. allii. *On tissue incubated in dark, lesions produced by* B. allii *were larger than those produced on tissue exposed to light. The converse was true for lesions produced by* C. dematium *var* circinans. *Basal portions of bulbs were less susceptible than equatorial and apical regions of all bulbs. The pH of lesions induced by these pathogens was significantly lower (range, 3.9 to 5.3) than that of uninfected tissue (pH 5.9). In areas adjacent to lesions, pH was generally unchanged from the norm (range 5.2 to 5.9). Wounding did not affect pH. Tissue pH (lesion or adjacent) was not affected by presence of light. Most nuclei in cells adjacent to lesions moved toward the infection. More nuclei moved toward* B. allii *lesions than* C. dematium *var* circinans *lesions. Nuclear movement was not related to lesion size, lesion pH, section of bulb inoculated, or presence or absence of light.*

The outcome of a host-parasite interaction is dependent on many external variables, as well as the physiologies of the host and the pathogen and their responses. Presence or absence of phenolic compounds, in outer dry leaf-bases of onion, are important in determining host response to infection (Link and Walker 1933, Clark and Lorbeer 1975). Once the protective layers (dry leaf-bases) are penetrated inner tissue susceptibility to infection increases (Hatfield *et al.* 1948). Exposure of underlying leaf-base epidermal tissue to ambient air increases nuclear dry mass, nuclear RNA, and nuclear protein over a 48 hr period, with attendant increase in nucleolar size (Courtis and Pappelis 1977 a, b). These reactions are interpreted as activation of quiescent cells due to exposure, and are accompanied by increased levels of caffeic and chlorogenic acids, and increased resistance to spread of *Botrytis allii* (Somasekhara 1974). These studies were limited to 48 hrs following exposure and were carried out in the dark, as were all studies of host-pathogen interactions conducted by Kulfinski and Pappelis (Pappelis *et al.* 1974, Kulfinski and Pappelis 1976).

Pappellis *et al.* (1976) reported that caffeic and chlorogenic acids inhibited germination of *B. allii* spores at pH levels found in onion bulb tissue. Tichelaar (1967) reported a pH gradient exists between colonized and uncolonized tissue, extending through several rows of cells in advance of hyphae. This gradient may affect activation of host phenols and fungal metabolism. Clark and Lorbeer (1975) reported that mycelium was not inhibited by levels of phenols that was found by Somasekhara (1974) to inhibit germination of *B. allii* spores.

Reports on onion physiology and the physiology of onion pathogens are common (Walker and Lindegren 1924, Walker and Stahman 1955, Kulfinski and Pappelis (1971). Light influences fungal pathogenicity (Link and Walker 1933, Venateswarhu and Sirohi 1974, Antenhill and Nicholson 1978), yet studies on the physiology of host-pathogen interactions on onion, especially

in light, are infrequent. The onion-pathogen interactions can serve as model systems under various conditions of storage, temperature, presence of gases, relative humidity, and light so that lesion development, nuclear movement in host cells, pH shifts, synthesis of fungitoxic compounds by the host, and synthesis of toxins by the pathogen in response to infection can be better understood.

The purpose of this study was to determine the effect of light on three variables (lesion size, tissue pH, and nuclear movement in tissue) following exposure of turgid leaf-bases to the ambient atmosphere, and inoculation with *Colletotrichum dematium* var *circinans* (Berk.) v. Arx and *B. allii* Munn.

This study was undertaken by students in Introductory Plant Pathology as part of an effort to incorporate the process skills used by scientists into the classroom laboratory. The introduction of process skills training into several university classes, greatly aided the improvement of problem solving by undergraduates and graduates majoring in science or non-science specialties (Pohlman and Pappelis 1977, Pappelis *et al.* 1980). "Hands-on" approaches to pest management and research techniques in plant pathology classes are gaining acceptance (Garrett *et al.* 1980).

This manuscript represents the success of these students in a guided group research effort.

MATERIALS AND METHODS

White, yellow, and red onions were obtained from local, commercial sources in 50 lb lots for use throughout these experiments. Varieties were not known. However, it has been shown that repeatable results could be obtained on otherwise like onions, *i.e.* bulbs having the same color and size and being free of damage or disease (Pappelis *et al.* 1974, Mayama and Pappelis 1977, Kulfinski and Pappelis 1978). Bulbs were stored in the laboratory in mesh sacks, simulating commercial storage. Only bulbs 8 cm in diameter, free from injury and disease, were selected for study. All dry leaf-bases were removed from selected bulbs to expose turgid leaf-bases. Bulbs were then rinsed with deionized water before treatment.

Four treatments were applied to each bulb. These were: exposure to ambient atmosphere without wounding or inoculation; exposure with wounding; and exposure with inoculation with both pathogens, *B. allii* and *C. dematium* var *circinans.* The same treatment was applied at three locations on each of four axes of each bulb. These locations were: 3 cm from the apex (apical); mid-point of the bulb (equatorial); and, 3 cm from the base (basal). Treatments and resulting lesions were always more than 2 cm apart so that little or no treatment interactions were expected (Kulfinski and Pappelis 1976).

To inoculate bulbs a sterile transfer needle was scraped across 10- to 14-day-old cultures, grown on white onion infusion agar, to accumulate spores. The infested needle was inserted into turgid leaf-bases to a depth of 0.5 cm. Pathogens were inoculated into bulbs at points 180° opposed to each other at each of the three points of inoculation (apical, equatorial, basal).

Wounding was performed using a sterile transfer needle inserted into turgid leaf-bases to a depth of 0.5 cm at points equidistant between inoculations. Tissue that was exposed, without wounding or inoculation, was located 180° opposite exposed-wounded tissue.

Treated white, yellow, and red onions were incubated at 24 ± 1 °C under continuous cool-white fluorescent light (Sylvania® , 1,133 lux, 320-750 nm) or in the dark for up to 7 days.

At 3 and 7 days, three of each bulb type were used to determine areas of ellipsoid shaped lesions. For each treatment short-range pH paper (pHydrion, Micro Essential Laboratory, N.Y., N.Y.) was inserted into an incision to determine pH. For lesions, a horizontal incision was made in the colonized tissue and 1 cm beyond the edge of the lesion. For wounds (punctures in exposed tissue), pH was determined in incisions made horizontally through the wound. For exposed tissue, a horizontral incision was made midway between the two adjacent inoculation treatments and on the side opposite wounds.

For histological observations, pieces of leaf-bases, approximately 2 cm^2, including the wound site or edge of lesions, were removed and the lower (abaxial) epidermis stripped using forceps. The epidermal piece was immediately placed in FAA. Using flat-field light microscopy, nuclear position was determined in 20 cells in two vertical rows of cells adjacent to the wound or inoculation site. Tissue was stained with a dilute solution of methylene blue to facilitate viewing. Nuclear position was classified as being in the central longitudinal third of the cell, or in the third toward or away from wounds or inoculations. For control tissue, nuclei in the right hand third of the cell were arbitrarily assigned as "side toward" and in the left hand third of the cell as "side away" for comparison to nuclei in tissue adjacent to wounded or in fected tissue. Distribution of nuceli in control tissue was used as the base from which nuclei position (distribution) percentages for other treatments could be computed. Means for all treatments were tested statistically for differences using Analysis of Variance (a = .05) and by regression analysis (p = .05) (Barr *et al.* 1976).

RESULTS AND DISCUSSION

Lesion Formation and Size

Lesions were gray, water-soaked, sunken areas of tissue surrounding a puncture inoculation site. After 3 days of incubation, few lesions had formed in *C. dematium* var *circinans*-inoculated tissue. In the case of *B. allii,* lesions had formed at all inoculation sites except those in apical regions of red onions incubated in light. At the end of 7 days incubation, *B. allii* lesions were present in all inoculation sites except those of apical areas of red onions incubated in light.

After 7 days of incubation, lesions produced by *B. allii* were larger than *C. dematium* var *circinans* lesions on the same bulbs incubated under identical conditions. For *C. dematium* var *circinans,* lesions produced in light were larger than those in the dark. The reverse was observed for lesions produced by *B. allii* (Table 1).

In general lesions caused by both pathogens in white, yellow, and red bulbs, were smaller in basal inoculation sites than those produced at the apical and equatorial sites regardless of incubation regime (Table 2).

Clark and Lorbeer (1975) inferred that the reason symptons of *Botrytis* brown stain are more severe in the neck of the onion bulb is due to the lower concentrations of phenols in the neck (Walker and Stahman 1955, Clark and Lorbeer 1973) and in the shoulder of the bulb (Clark and Lorbeer 1975). Data presented here support these inferences since larger lesions were produced in neck (apical) and shoulder (equatorial) regions than in basal regions of bulbs of each color type for both pathogens studied.

Hammerschmidt and Nicholson (1977) indicated that lesion development of *C. graminicola* on corn is light-dependent. Responses recorded here for the two fungal pathogens of onion indicate that light and bulb location affect lesion size. MacLean and Tommerup (1979) determined that quality of light and length of exposure affected lesion development in *Poa pratensis* inoculated with *Drechslera sorokiniana.* Antenhill and Nicholson (1978) determined that dark treatment of corn, inoculated with *C. graminicola,* allowed for lesion enlargement due to lack of production of phenols that would normally be produced in light. This similar condition was reported for wheat rust caused by *Puccinia recondita* (Venateswarhu and Sirohi 1974).

Possible explanations for differences in lesion sizes due to exposure to light may be that 1.) inhibitors or stimulators are synthesized by activated host tissue (Somasekhara 1974, Courtis and Pappelis 1977a, b), 2.) effects on pathogens (no effect, stimulation, inhibition) are mediated by different concentrations of the compound(s), 3.) cells in the three locations (apical, equatorial, basal) are activated to different levels producing, or accumulating, different concentrations of the same substance, or 4.) different substances are synthesized in the different locations. Bhullar and Daly

Table 1. Mean lesion area (cm^2) for all lesions on white, yellow, and red onions inoculated with *Botrytis allii* and *Colletotrichum dematium* var *circinans* incubated in light and dark at 24 °C. Values represent means for three bulbs per onion scale color, each with six lesions *1/*, measured seven days after inoculation.

Pathogen	*Incubation Regime*	*Scale Color*	*Lesion Area*
Botrytis allii	Dark	Red	10.09a *2/*
		Yellow	4.00 b
		White	2.41 c
	Light	Red	3.41 b
		Yellow	3.08 bc
		White	1.90 d
Colletotrichum dematium var *circinans*	Dark	Red	0.04 f
		Yellow	0.61 e
		White	0.39 e
	Light	Red	1.01 d
		Yellow	1.20 d
		White	1.19 d

1/ 3 lesions per pathogen, 108 total lesions

2/ Values followed by the same postscript are not significantly different at the 5% level (SNK).

(1975) determined that light stimulated toxin production in *Helminthosporium maydis*. Light may activate synthesis of fungal toxins in *C. dematium* var *circinans* and inhibit synthesis of *B. allii*.

Tissue pH

Mean pH of control tissue throughout remained at 5.9 regardless of onion color. Wounding did not alter pH. After 7 days of incubation, the pH of tissue adjacent to lesions of *C. dematium* var *circinans* was the same as that for controls. Tissue adjacent to *B. allii* lesions at equatorial and basal sites of yellow onions incubated for 7 days in dark, and at equatorial sites of red onions incubated for 7 days in light had a mean pH of 5.4 (range, 5.2 to 5.5). This difference was statistically significant from that of controls.

Table 2. Mean lesion area (cm^2) for all lesions on apical, equatorial, and basal sections of onion for all leaf-base colors inoculated with *Botrytis allii* and *Colletotrichum dematium* var *circinans* incubated in light and dark at 24 °C. Values represent means for three bulbs per leaf-base color, each with six lesions *1/*, measured seven days after inoculation.

Pathogen	*Incubation Regime*	*Bulb Section*	*Lesion Area*
Botrytis allii	Dark	Apical	6.28a *2/*
		Equatorial	6.66a
		Basal	3.56 c
	Light	Apical	4.52 b
		Equatorial	3.14 c
		Basal	0.72 e
Colletotrichum dematium var *circinans*	Dark	Apical	0.64 e
		Equatorial	0.27 f
		Basal	0.14 f
	Light	Apical	1.37 d
		Equatorial	1.77 d
		Basal	0.27 f

1/ 3 lesions per pathogen, 108 total lesions

2/ Values followed by the same postscript are not significantly different at the 5% level (SNK).

Few lesions, which had developed after 3 days, showed shifts in pH. Where changes did occur pH values, although lower than those of control tissue (range, 5.7 to 5.8) were not statistically different. After 7 days, lesion pH was significantly below control tissue pH (range 3.9 to 5.3). Exceptions occurred in *C. dematium* var *circinans* inoculated equatorial and basal sites of yellow onions incubated in dark, and basal sites of *C. dematium* var *circinans* inoculated yellow onions incubated in light. Lesion pH in those areas was generally 5.8 (range, 5.6 to 5.8). There were no statistically significant changes in shifts of pH due to presence or absence of light, and shifts in pH were not related to lesion size ($r^2 = 0.23$).

Somasekhara (1974) reported a sharp transition of pH at boundaries of dark-induced *B. allii* lesions. This was the case here for both fungi incubated in light and dark.

Nuclear Movement

Nuclear distribution patterns for exposed wounded and unwounded tissue, and tissue adjacent to wounds and lesions, were consistent for tissue treatments over bulb locations, onion color, incubation regime, length of incubation, lesion size, and lesion pH. All means were combined for statistical analysis. The means for nuclear distribution: 1.) in uninoculated tissue and tissue adjacent to lesions were significantly different, 2.) in exposed and exposed-wounded tissue were not statistically different, and 3.) in tissue adjacent to *B. allii* and *C. dematium* var *circinans* lesions were statistically different. For exposed and exposed-wounded tissue, approximately 28% of the nuclei were located at the "side toward" and "side away"; 44% were located in the center of cells. For tissue adjacent to *C. dematium* var *circinans* lesions, about 50% of the nuclei were located on the "side toward" infection, about 19% on the "side away", and about 31% in the center of cells. For tissue adjacent to *B. allii* lesions, approximately 64% of the nuclei were on the "side toward" infection, 13% on the "side away", and about 23% in the center of cells.

It is accepted that onion bulb color is a stable genetic character. This study established that a model system for studying host responses to multiple infection, with comparisons made to genetically identical control tissue on the same intact bulbs is feasible, and that responses between color groups (white, yellow, red) vary. It is expected that further experimentation may indicated differences for measured responses within varieties of a particular color group.

Specifically, this preliminary study has produced data which indicate that 1.) light plays a role in development of lesions in onion tissue infected with *C. dematium* var *circinans* and *B. allii,* 2.) that different locations on bulbs (basal vs apical or equatorial) can be used to study response capacities of the host, and 3.) two or more pathogens can be studied and compared to uninfected tissue on the same bulb. However, many more questions on the effect of some variable of light (wavelength, time of exposure, intensity) on host and pathogen metabolism need further study. Using the model, studies of host-pathogen interactions with other variables (*i.e.* temperature, presence of gases, and relative humidity), can be undertaken to clarify the mechanisms of lesion development, pH shift, and nuclear movement in a host that is well known for the involvement of phenolic compounds in the resistance mechanism.

LITERATURE CITED

ANTENHILL, F.J., and R.L. NICHOLSON. 1978. Involvement of pheonols in resistance of corn to a fungal pathogen: Light a mediating factor. Phytopathology News, 12: 163 (Abstr.).

BARR, A.J., J.H. GOODNIGHT, J.P. SALL, and J.T. HELWIG. 1976. A user's guide to SAS 76. SAS Institute, Raleigh, N.C.

BHULLAR, B.S., and J.M. DALY. 1975. Light stimulated toxin production in *Helminthosporium maydis* race T cultures. Can. J. Bot. 53: 1796-1800.

CLARK, C.A., and J.W. LORBEER. 1973. Symptomology, etiology, and histopathology of *Botrytis* brown stain on onion. Phytopathology 63: 1231-1235.

CLARK, C.A., and J.W. LORBEER. 1975. The role of phenols in *Botrytis cinerea* brown stain on onions. Phytopathology, 65: 338-341.

COURTIS, W.S., and A.J. PAPPELIS. 1977a. Increase in *Allium cepa* nuclear dry mass following exposure to ambient atmosphere. J. Exp. Bot. 28: 719-723.

COURTIS, W.S., and A.J. PAPPELIS. 1977b. Effect of exposure to ambient atmosphere on nucleolar size and DNA, RNA, and protein content of *Allium cepa* epidermal nuclei. J. Exp. Bot. 28: 724-728.

GARRETT, W.N., F.F. HENDRIX, and W.M. POWELL. 1980. Plant protection and pest management training at the University of Georgia. Plant Dis. 64: 156-160.

HAMMERSCHMIDT, R., and R.L. NICHOLSON. 1977. Resistance of maize to anthracnose: effect of environmental factors. Phytopathology, 67: 247-250.

HATFIELD, W.C., J.C. WALKER, and J.H. OWEN. 1948. Antibiotic substances in onion in relation to disease resistance. J. Agric. Res. 77: 115-135.

KULFINSKI, F.B., and A.J. PAPPELIS. 1971. Interference microscopy of onion epidermal nuclei in response to three fungal pathogens. Physiol. Plant Pathol. 1: 489-494.

KULFINSKI, F.B., and A.J. PAPPELIS. 1976. The use of quantitative interference microscopy in the study of onion epidermal tissue. In: Microbiology of Aerial Surfaces (C.H. Dickinson and T.F. Preece, eds.). pp. 637-657, Academic Press, London.

KULFINSKI, F.B., and A.J. PAPPELIS. 1978. Appropriate control tissues for host-pathogen studies in Onion. Cytologia, 43: 411-414.

LINK, K.P., and J.C. WALKER. 1933. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. J. Biol. Chem. 100: 379-383.

MACLEAN, D.J., and I.C. TOMMERUP. 1979. Histology and physiology of compatibility and incompatibility between lettuce and the downy mildew fungus, *Bremia lactucae* Regel. Physiol. Plant Pathol. 14: 291-312.

MAYAMA, S., and A.J. PAPPELIS. 1977. Application of interference microscopy to the study of fungal penetration of epidermal cells. Phytopathology, 67: 1300-1302.

OWEN, J.H., J.C. WALKER, and M.A. STAHMAN. 1950. Pungency, color, and moisture supply in relation to disease resistance in onion. Phytopathology, 40: 292-297.

POHLMANN, M.M., and A.J. PAPPELIS. 1977. Improving process skills among college non-science majors with Science-A Process Approach materials. J. College Sci. Teaching. 6: 167-169.

PAPPELIS, A.,J., K.V. SOMASEKHARA, J.N. BEMILLER, and JD. WILNER. 1976. Increased resistance to spread of *Botrytis allii* in onion bulbs and induced chlorogenic acid synthesis. proc. Am Phytopathol. Soc. 3: 232 (Abstr.).

PAPPELIS, C.K., M.M. POHLMANN, and A.J. PAPPELIS. 1980. Can instruction improve science process skills of premedical and predental students? J. Rsch. Sci. Teaching. 1: 25-29.

SOMASEKHARA, K.V. 1974. Response of onion bulb scale epidermal nuclei to *Botrytis allii* as studied with quantitative interference microscopy and cytochemistry. Ph.D. Dissertation, Southern Illinois University/Carbondale, Carbondale, Illinois.

TICHELAAR, G.M., 1967. Studies on the biology of *Botrytis allii* on *Allium cepa*. Neth. J. Plant Pathol. 73: 157-160.

VENATESWARHU, B., and G.S. SIROHI. 1974. Photoperiodic studies on disease intensity, sugars, starch and phenol content of brown rust *(Puccinia recondita)* on wheat. Indian J. Agric. Sci. 44: 549-554.

WALKER, J.C., and C.C. LINDEGREN. 1924. Further studies on the relation on onion scale pigmentation to disease resistance. J. Agric. Res. 29: 507-514.

WALKER, J.C. and M.A. STAHMAN. 1955. Chemical nature of disease resistance in plants. Ann. Rev. Plant Physiol. 6: 351-366.