Nuclear Orientation in Onion Epidermal Cells in Relation to Wounding and Infection

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ABSTRACT

Nuclei of onion bulb scale inner epidermal cells moved toward wounds or penetration sites of *Botrytis allii* or *Aspergillus niger*, the orientation effect being appreciably greater with infection. Nuclei 2 cm or more from these influences were oriented as those in untreated tissue.

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Additional key words: Allium cepa, Botrytis allii, Aspergillus niger, cell killing, nuclear movement.

Black and gray molds of onion incited by *Aspergillus niger* van Tiegh. and *Botrytis allii* Munn, respectively, are two of the most important transit and storage diseases of harvested bulbs (2, 3, 10). Both *B. allii* and *A. niger* produce cellulolytic and pectinolytic enzymes (1, 9, 12). In addition, *A. niger* produces a toxic, thermostable metabolite which inhibits seed germination, disorganizes fleshy scales and green leaf tissue, and acts as a protoplasmic poison to host tissue (7).

In natural conditions, *B. allii* invades the onion bulb primarily through dead or senescent tissue in the neck, or through wounds in the bulb. After penetration, its spread is restricted, presumably by the pungent compounds of the fleshy bulb scales (3). The infected area appears watersoaked and is more acidic than normal (pH 4.2, compared to 6.6, respectively); these two areas being separated by a mycelium-free area about 10 mm in width (10). Nuclei of inner epidermal cells adjacent to *B. allii* or *A. niger* were shown to decrease in size (5, 6) and dry mass (6). *B. allii* releases toxic substances that reduce the nuclear network, result in an empty zone around the nucleolus, and lead to nuclear degeneration (4). Cell death occurs in advance of hyphal penetration (4, 5, 6). Nuclei in epidermal cells of inoculated bulbs respond as do those in naturally infected bulbs (6).

This report presents data on changes in the orientation of nuclei in epidermal tissue of white onion bulbs following wounding and inoculation with either *B. allii* or *A. niger.*

MATERIALS AND METHODS.–Experiments were conducted using *A. niger* and *B. allii* (both isolated from onions) as pathogens. Nuclear orientation following wounding and wounding plus inoculation was compared to that in untreated tissue removed at the time of wounding. Inoculation was followed by 48 hr incubation in the dark at 25C with high humidity. Nuclear location was recorded as being along the radial wall nearest or furthest from the wound (or inoculation) site or in the central part of the cell against a tangential wall. Five replications were used in each study.

In the first experiment, the outer dry scales and two or three turgid scales were removed from 10 bulbs, a tissue square (5 mm on a side) was removed from the equatorial region of the exposed scale, and five of the wounded bulbs were inoculated at the site of the wound with spores of *A niger.* For convenience, the initial wound location was called L0 and subsequent successive 5 mm-squares around the equatorial plane, L1 through L4. The inner (adaxial) epidermis was removed from L0, L1 and L4 of wounded controls and inoculated bulbs, mounted in 1% methylene blue, and observed for nuclear orientation. A sequence of 40 cells was studied for nuclear location in each of three horizontal ranks (upper-, central-, and lower-third of the tissue square) beginning at the lateral edge closest to the wound. In L0, right and left radial walls were arbitrarily called "toward" and "away" from the wound, respectively.

In the second experiment, 10 onions were wounded at L0 and then five were inoculated with *B. allii.* Initial nuclear orientation in L0 was compared with that in L1, L2, L3, and L7 48 h after inoculation and incubation as above.

RESULTS.–Nuclei of host cells tended to migrate to that face of the cell closest to the wound or infection. Mycelium of *A. niger* penetrated about 15 rows of cells and the nuclei in these cells were often completely disintegrated (Table 1). Tissue maceration commonly included the first 10 cells along the inoculated surface. Nuclei in the first 15 rows stained intensely with methylene blue, those in the next 10 rows stained less intensely, and most of the nuclei more distant from the infection did not stain. The cytoplasm was granular in appearance in the first 15 cells and vacuole boundaries could not be disinguished. In the region containing mycelium, nuclear orientations were mostly in the centers of the cells. Of the remainder, more were toward than away from the wound and inoculation site. In tissue adjacent to the infection, most nuclei were oriented toward the wound. In L4, nuclei were found mostly in the center with about equal numbers on either side of the cell. In general, wounding alone induced a

moderate degree of nuclear movement toward the wound, wounding and inoculation caused a great degree of movement toward the mycelium, and distant cells remained like those in L0.

Forty-eight hours after wounding, approximately 50% of the nuclei in the first three horizontal rows of cells above the wound moved downward toward the wound (into the lower third of the cell). Beyond these three cell rows, the nuclei were almost always in the central third of the cell.

Most nuclei (64%) in L0 of bulbs to be inoculated with *B. allii* were in the center area of the cells with about equal numbers along the radial walls. The mycelium penetrated into about 35 vertical cell rows after 48 h of incubation and macerated tissue in the first 20 rows. Methylene blue staining of nuclei was most prominent in cells in the infected area of L1 and least in L3 and L7. About 60% of the nuclei in L1 and L2 were along the wall nearest the infection. The greatest number of nuclei to move toward the mycelium (71%) were in the last 10 cells of L1 in proximity to the hyphal tips. The distribution of nuclei in L3 and L7 were more like those in L0 (Table 2).

DISCUSSION.—Nuclear migration toward the site of infection (*B. allii* or *A. niger*) was greater than toward uninoculated wounds. From the results of methylene blue staining, we conclude that cell death in the infected area and cellular injury in advance of the pathogens were caused by substances secreted by the pathogens. Since few nuclei in the first ten rows of cells moved toward the wound and inoculation site, we suggest that the time required to induce nuclear movement is greater than the time required for the pathogen to kill these cells. Nuclei in cells more distant from the wound moved toward the mycelium before the cells were killed and remained in their new locations after death of the cells.

Pearson (8) observed that as *Gibberella saubinetii* grew into cortical tissue of roots, the nuclei and most of the cytoplasm in adjacent cells became oriented toward the infection. She observed that positive nuclear orientation occurred toward hyphae, needle wounds, and ruptures in the root tissue resulting from emerging adventitious roots and concluded that the nuclear and cytoplasmic movements were probably the first symptoms in response to these factors.

Since nuclei of epidermal cells of *Allium cepa* become oriented toward wounds and pathogens (8, 11), this phenomenon may prove to be a valuable bioassay system for both pathological and physiological studies of fungal toxins.

TABLE 1. Nuclear movement means (percentages) for inner epidermal tissue of control and *Aspergillus niger*-inoculated white onion bulbs. Forty cells (1-40) were scored in each of three horizontal ranks to the right of each inoculation site in five bulbs (replications)

Location[a]		Treatment[b]					
		Wounded			*A. niger*		
Tissue	Cells	T	C	A	T	C	A
L0	1-40	14	73	13	...	...	...
L1	1-10	24	67	9	29	67	4
	11-20	29	63	8	53	43	4
	21-30	28	61	11	66	29	5
	31-40	24	61	15	73	23	4
	1-40	26	63	11	55	41	4
L4	1-10	15	68	17	20	63	17
	11-20	8	77	15	22	57	21
	21-30	17	74	9	22	59	19
	31-40	13	67	20	17	61	22
	1-40	13	72	15	20	60	20

[a]L0 = initial wound location. Consecutive pieces (5 mm on a side) around the equatorial plane of the third turgid leaf base are referred to as L1 through L4.

[b]T = nucleus along the radial wall nearest the wound or inoculation site; A = opposite of T; and C = nucleus in central part of cell against a tangential wall.

TABLE 2. Nuclear movement means (percentages) for inner epidermal tissue of white onion bulb tissue inoculated with *Botrytis allii*. Forty cells (1-40) were scored in each of three horizontal ranks to the right of each inoculation site in five bulbs (replications)

Location[a]		*B. allii*[b]		
Tissue	Cells	T	C	A
L0	1-40	16	64	20
L1	1-10	39	53	8
	11-20	60	35	5
	21-30	59	37	4
	31-40	71	24	5
	1-40	57	37	6
L2	1-10	67	27	6
	11-20	64	27	9
	21-30	64	27	9
	31-40	48	41	11
	1-40	61	31	8
L3	1-40	31	44	25
L7	1-40	24	40	36

[a]L0 = initial wound location. Consecutive square pieces (5 mm on a side) around the equatorial plane of the third turgid leaf base are referred to as L1 through L7.

[b]T = nucleus along the radial wall nearest the wound or inoculation site; A = opposite of T; and C = nucleus in central part of cell against a tangential wall.

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