Inheritance of Resistance to Neck-rot Disease Incited by *Botrytis allii* in Bulb Onions

Mau-Wei Lin¹, James F. Watson², and James R. Baggett³

Department of Horticulture Oregon State University, Agricultural and Life Sciences, Building 4017, Corvallis, OR 97331-7304

Additional index words. vegetable breeding, *Allium cepa*

Abstract. The inheritance of resistance to neck rot, incited by *Botrytis allii* Munn, was studied in four crosses between resistant and susceptible bulb onion (*Allium cepa* L.) lines, one cross between two resistant lines, and one cross between two susceptible lines. Using tests of excised scale pieces inoculated with a spore suspension and incubated in plastic boxes at 20C for 6 days, parents, F₁, F₂, and backcrosses were assigned disease indices (DI) derived from infection severity scores of individual bulbs. Generation means and frequency distribution of DI indicated that inheritance was quantitative and mostly additive with a small amount of dominance for susceptibility. Estimates of gene effects using a three-parameter model also indicated that additive effects predominated, with some dominance and no epistasis. Broad-sense heritability estimates ranged from 42% to 63% in the resistant x susceptible crosses.

Neck rot, incited by *Botrytis allii*, causes serious storage losses in bulb onions. Infection by *B. allii* is favored by harvesting before bulbs mature fully, inadequate curing of the bulbs, and the occurrence of cool, moist weather before find after harvest. Development of decay in storage is favored by temperatures of 15 to 20C and high humidity (Munn, 1917; Owen et al., 1950; Walker, 1926).

Munn (1917) and Groenendijk and Petiet (1963) reported that there are conspicuous differences in susceptibility to *B. allii* among onion cultivars and that white onions are more susceptible than yellow or red onions. Van Der Meer and Van Bennekom (1970) developed a test method for differentiating levels of neck-rot resistance. Vik and Astvei (1984), applying Van Der Meer's methods in an onion breeding program, found that there was no clear-cut or specific resistance among cultivars. They concluded that resistance is a quantitative character and that inbreeding and selection can result in marked reduction in disease incidence. Miyaura et al. (1985) divided onion bulbs into resistant and susceptible groups after scale tests and compared their offspring performance. The mean disease index of selfed progeny from resistant bulbs was lower than the mean disease index from susceptible bulbs. They suggested that heritable resistance to neck rot can be obtained by selection.

The availability of onion inbred lines with pronounced levels of resistance or susceptibility has made breeding for neck-rot resistance a distinct possibility. The purpose of this study was to investigate the inheritance of neck-rot resistance.

Materials and Methods

Four crosses were made between distinctly neck-rot susceptible and resistant onion inbreds. Additionally, a resistant (W420) x intermediate (B6693) and a susceptible x susceptible cross were made. The most resistant parents, W420 and W202, and susceptible parent W407, were lines developed and released by the Univ.

of Wisconsin. B6693 is a line of intermediate resistance released by the U.S. Dept. of Agriculture, Univ. of Wisconsin, and Michigan State Univ. Neck-rot susceptible parents Pg- 1b (Pg) and D10- 1b (D10) are from the Univ. of Idaho and Sunseeds Co., respectively. All of the parents are yellow long-day onion lines except D10, which is a white long-day onion line. W420 and B6693 are high pungency, high soluble solids lines; W202 is a medium pungency, medium soluble solids line; and Pg and D10 are low pungency, low soluble solids lines.

Crosses were made by hand emasculation of female parents before stamen dehiscence and pollination by hand. To obtain F₁ and backcross seed, F₁ and parent bulbs were planted in the greenhouse in September 1986, vernalized in pots, and flowered in June and July 1987. Selfing of the F₁ and parent plants was done on an individual plant basis using flies as pollinators in small cages. Additional F₁ seeds and backcrosses were obtained by hand emasculation and pollination by hand or flies. To avoid contamination, umbels of female plants were covered with paper bags before pollination.

Because of difficulty in obtaining seed from hand emasculated onion flowers, reciprocal crosses were not made. We have not observed reciprocal differences in onion crosses and are not aware of reports of reciprocal differences.

Seeds for production of bulbs for neck-rot susceptibility tests of all generations were planted in the greenhouse on 19 Mar. 1988. Plants were transplanted to the field after 6 weeks into rows 51 cm apart with 9 cm between plants, arranged in a randomized complete block with four replications. In each replication, the six generations of a single cross were randomized in one row. Cultural methods were typical of those used for commercial production on mineral soils in western Oregon. Bulbs were dug 15 Sept., field-cured for 3 weeks, cleaned, and stored at 5 to 10C.

Neck-rot tests were conducted during October to January using modifications of the method of Miyaura et al. (1985). A culture of *B. allii*, provided by the Sunseeds Co. plant pathology lab, was maintained on potato-dextrose agar. New cultures were started every 2 months and incubated at 20C. Spores were obtained from a new heavily sporulated culture by adding 20 ml sterilized water to the petri dish and loosening spores by slight rubbing with a flamed scalpel. Mycelium and spores were blended for 30 sec to get an even spore suspension. Spore concentration was adjusted to 10⁶ to 10⁸ spores/ml. Then 50 mg of the antibiotic Cabenecilin was added to the pathogen suspension to suppress bacterial contamination.

Received for publication 22 Mar. 1994. Accepted for publication 7 Feb. 1994. Oregon Agricultural Experiment Station technical paper no. 10,438. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Former graduate research assistant. Current address: Horticulture Dept., National Ping-Tung Institute of Agriculture, Ping-Tung, Taiwan, R.O.C. 900.

²Courtesy assistant professor and plant breeder, Sunseeds Co., Brooks, OR 97305.

³Professor.

Table 1. Mean onion neck rot disease indices (DI) in generations of crosses between neck-rot resistant and susceptible lines.

Cross	Generation ^z	No. bulbs ^y	Mean DI
W420 (PR) x Pg (PS)	PR	64	0.5 ± 0.08
	PS	54	3.4 ± 0.10
	F ₁	49	2.8 ± 0.17
	F ₂	98	2.2 ± 0.13
	BCR	64	1.0 ± 0.13
	BCS	45	2.5 ± 0.17
	LSD _x		1.0
W202 (PR) x D10 (PS)	PR	64	0.7 ± 0.12
	PS	63	3.3 ± 0.10
	F ₁	52	3.1 ± 0.12
	F ₂	95	2.7 ± 0.13
	BCR	59	2.3 ± 0.15
	BCS	29	3.6 ± 0.10
	LSD		0.7
W420 (PR) x D10 (PS)	PR	64	0.8 ± 0.11
	PS	64	3.1 ± 0.12
	F ₁	12	2.1 ± 0.37
	F ₂	95	1.9 ± 0.17
	BCR	64	1.9 ± 0.14
	BCS	55	1.8 ± 0.18
	LSD		0.9
B6693 (PR) x Pg (PS)	PR	63	1.1 ± 0.11
	PS	64	3.0 ± 0.12
	F ₁	25	1.5 ± 0.13
	F ₂	116	2.3 ± 0.12
	BCR	64	1.6 ± 0.12
	BCS	64	2.2 ± 0.12
	LSD		0.6
W420 (PR) x B6693 (PS)	PR	60	0.8 ± 0.12
	PS	46	2.0 ± 0.17
	F ₁	62	0.9 ± 0.13
	F ₂	107	0.7 ± 0.08
	BCR	62	0.4 ± 0.07
	BCS	62	1.0 ± 0.11
	LSD		0.6
W407 (P1) x D10 (P2)	P ₁	60	3.5 ± 0.09
	P ₂	49	3.4 ± 0.10
	F ₁	39	3.2 ± 0.09
	F ₂	95	3.3 ± 0.08
	BC ₁	59	3.2 ± 0.12
	BC ₂	26	3.6 ± 0.12
	LSD		NS

^yPR = most resistant parent, PS = most susceptible parent; BCR = backcross F₁ x PR; BCS = backcross F₁ x PS.

^zTotal bulbs tested in four replications.

^xLSD at 0.05 probability shown when analysis of variance indicated significant generation differences.

^{NS}Nonsignificant.

Before scale pieces were cut from onion bulbs, the dry scales were removed and the bulb surface was sterilized with 75% ethyl alcohol. Two rectangular scale pieces (1.5 x 2 cm) were cut from each of the second and third scale layers of each bulb. The inner epidermis was removed from the scale pieces.

Scale pieces were placed on blue blotter paper in a 32 x 24 x 10-cm plastic alcohol-sterilized box. The paper was moistened by adding 55 ml of sterilized water to each box to provide high humidity.

Each piece of scale was inoculated with one drop (≈ 0.04 ml) of

Table 2. Deviation in mean neck-rot disease index of the F₁ generation and backcrosses from their mid-parent values.^y

Cross	Deviation in disease index		
	F ₁ -MP	BC ₁ -MP	BC ₂ -MP
W420 x Pg	0.81 ^{NS}	-0.58 ^{NS}	-0.56 ^{NS}
W202 x D10	1.12*	0.39 ^{NS}	0.46 ^{NS}
W420 x D10	0.11 ^{NS}	-0.42 ^{NS}	-0.74 ^{NS}
B6693 x Pg	-0.55 ^{NS}	0.30 ^{NS}	-0.10 ^{NS}
W420 x B6693	-0.55 ^{NS}	-0.41 ^{NS}	-0.49 ^{NS}
W407 x D10	-0.24 ^{NS}	-0.15 ^{NS}	-0.34 ^{NS}

^yAfter the methods of Kim and Brewbaker (1977).

^{NS},*Nonsignificant or significant at P = 0.05.

pathogen suspension on the center of the scale surface. One drop of sterilized water was added. After inoculation, the box was covered and incubated at 20C for 6 days (144 h). Each individual scale piece was evaluated using a 0 to 4 scoring system where 0 = no visible mycelium growth; 1 = diameter of mycelium <0.5 cm; 2 = diameter of mycelium >0.5 cm, no sporulation; 3 = heavy mycelium growth, little sporulation; 4 = heavy sporulation. The disease index (DI) for an individual bulb was the average of scores assigned to four scale pieces.

Broad-sense heritability estimates were calculated from the variances of parents and F₁ and F₂ generations using the method of Mahmud and Kramer (1951). Estimates of gene effects were made using the models of Hayman (1958).

Results and Discussion

Generation means and frequency distributions of DI demonstrated the quantitative nature of differences in neck-rot resistance. Generation means (Table 1) generally indicated that inheritance of resistance is additive, but some individual generations in the crosses varied from this, usually suggesting dominance of resistance. In W420 x Pg, generation means displayed an additive relationship except for the F₁, which suggested dominance of susceptibility (this deviation from the F₂ was nonsignificant at P = 0.5). All generation means of W202 x D10 indicated slight dominance of susceptibility. In W420 x D10, BCS showed dominance of resistance while the F₁ and F₂ indicated additive inheritance. As expected, generation means of susceptible x susceptible cross W407 x D10 did not differ. Except for the F₁ of W202 x D10, deviations of F₁ and backcross means from their mid-parent values were nonsignificant (Table 2).

DI for each generation, including parents, was distributed over several score classes, indicating variation of infection severity independent of genotype. DI of resistant and susceptible parents overlapped, but there were very few plants of the resistant parents (PR) in score class 4 and very few susceptible parent (PS) plants in score class 0 or 1. Frequency distributions of crosses W420 x Pg and W420 x D10, which had strong parental differences, are shown in Fig. 1A and B. F₁ and F₂ generations in these crosses were somewhat skewed toward PS, indicating some dominance. The distributions for the backcrosses in W420 x Pg were distinctly skewed toward their respective parents, but in W420 x D10 the backcrosses were broadly distributed. Distribution for W202 x D10 (not shown) was similar to that of W420 x Pg. B6693 x Pg (not shown) was similar to W420 x Pg except that parent values were not as well separated. Cross W420 x B6693 (Fig. 1C), between a highly resistant and moderately resistant parent, and W407 x D10, between two susceptible parents, produced expected distributions and demonstrated the range of DI values obtained even in a

Fig. 1. Frequency distributions of disease indices (DI) in generations of onion crosses (A) W420 x Pg, (B) W420 x D10, and (C) W420 x B6693.

predominately resistant or susceptible population.

Estimates of gene effects. Estimates of gene effects were made using the three parameter models of Hayman (1958). The small and nonsignificant chi-square values (Table 3) due to deviations from the model for DI in all of the crosses indicate that the additive-dominance model adequately describes these data and provides no evidence for epistatic effects. The estimates of additive effects (a) were significant in all of the resistant x susceptible crosses and were always greater in these crosses than the estimates for dominance effects (d), which were not significant in any cross. Estimates of additive effects in the resistant x susceptible crosses were fairly large relative to the mean effects, suggesting that genes with additive effects for DI were highly associated, most increasing alleles being in the susceptible parents and decreasing alleles in the resistant parents. This was especially true

Table 3. Estimates of gene effects for neck-rot disease index from generations means using a three-parameter model.

Cross	Gene effects			
	Mean	Additive	Dominance	χ^2
W420 x Pg	2.1**	-1.5**	0.5	3.41 ^{NS}
W202 x D10	2.7**	-1.3*	1.1	2.2 ^{NS}
W420 x D10	3.4**	-1.0*	0.1	0.27 ^{NS}
B6693 x Pg	1.5**	0.9*	-0.6	1.47 ^{NS}
W420 x B6693	0.9*	-0.2	-0.8	2.21 ^{NS}
W407 x D10	3.3**	-0.1	0.2	0.02 ^{NS}

***Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

in the cross W420 x Pg- 1b.

Neither additive nor dominance effects were significant in the resistant x resistant cross (W420 x B6693) or the susceptible cross (W407 x D10-1b). This supports the indications from the generation means and relatively small variances (Table 1) that there was little segregation for DI in these crosses.

Heritability. Broad-sense heritability estimates for the four resistant x susceptible crosses were in the moderate range; 63% for the cross W420 x Pg, 54% for W202 x D10, 42% for W420 x D10, and 56% for B6693 x Pg. In the two crosses where the parents did not differ as much, the heritability estimates were lower. For the susceptible x susceptible cross (W407 x D10), the broad-sense heritability estimate was 15%. In the resistant x intermediate cross W420 x B6693, relatively large variances of the parents and F_2 compared to the smaller variance of the F_2 resulted in a calculated broad-sense heritability of -62%. Because broad-sense heritability values are in the range 0 to 1 by definition, this result is interpreted to estimate a broad-sense heritability of 0% or very low for this cross.

Differences in neck-rot resistance among the parents used in this study were heritable and quantitative. The primary importance of additive gene action was demonstrated by the generation means, the large additive gene effects relative to dominance effects, the absence of epistatic gene effects, and the moderate broad-sense heritability estimates. Selection for neck-rot resistance should be effective using methods appropriate for quantitative traits.

Literature Cited

- Groenendijk, J. and J. Petiet. 1963. A method to test onions on their sensitivity to *Botrytis allii*. Euphytica 12:94-96.
- Hayman, B. I. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12:371-390.
- Mahmud, I. and H. H. Kramer. 1951. Segregation for yield, height, and maturity following a soybean cross. Agron. J. 43:605-609.
- Miyaura, K., Y. Shinada, and W. H. Gabelman. 1985. Selection for resistance of onion to *Botrytis allii* by scale inoculation method. HortScience 20:769-770.
- Munn, M. T., 1917. Neck-rot disease of onions. New York Agr. Expt. Sta. 437:363-454.
- Owen, J. H., J. C. Walker, and M. A. Stahmann. 1950. Pungency, color and moisture supply in relation to disease resistance in the onion. Phytopathology 40:292-297.
- Van Der Meer, J. L. and J. L. Van Bennekom. 1970. Testing onions and other *Allium* species for resistance to *Botrytis allii* Munn. Euphytica 19:152-162.
- Vik, J. and K. Astvei. 1984. Breeding for resistance to neck-rot in onion. Euphytica 33:257-262.
- Walker, J. C. 1926. Botrytis neck-rot of onions. J. Agr. Res. 33(10):893-927.