Genetic Control of Reaction to Common Blight Bacterium in Bean (*Phaseolus vulgaris*) as Influenced by Plant Age and Bacterial Multiplication[1]

D. P. Coyne, M. L. Schuster, and K. Hill[2]
University of Nebraska, Lincoln

Abstract. Reaction to *Xanthomonas phaseoli* (E.F. Smith) Dowson, cause of common blight disease in beans, *Phaseolus vulgaris* L., was inherited quantitatively in the cross early flowering susceptible cv. Great Northern (GN) 1140 x late flowering tolerant line 'GN Nebr. #1, sel. 27'. F_1 populations showed partial dominance for susceptibility at 25 days and nearly complete dominance at 43 days after inoculation. Genes controlling delayed flowering, under long photoperiod and high temp, and tolerant reaction were linked. Early, blight tolerant advanced lines were not obtained by pedigree selection. They were obtained, however, by the backcross method, using 'GN Nebr. #1, sel. 27' as the recurrent parent. Different photoperiod x temp regimes in growth chambers induced flowering at the same or different times in nearly-isogenic lines of the same age. Vegetative plants exhibited higher levels of tolerance and lower bacterial populations than did plants in the pod stage. The bacterial population in susceptible 'GN 1140' was higher than in early and late tolerant lines.

Common blight, caused by the bacterium *Xanthomonas phaseoli* is considered one of the most serious seed-borne bacterial diseases of dry edible and green beans in many production areas in the world[3,4]. (12, 17, 23, 25). Nebraska has a successful dry bean industry, and for many years about 90,000 acres of beans have been grown. Most of this acreage is devoted to Great Northern (GN) and the remainder to Pinto beans. Most of the bean seed planted in Nebraska is produced in disease-free, semi-arid areas of Idaho to eliminate seed-borne infection. Occasionally, however, crop losses have occurred when environmental conditions were favorable for bacterial multiplication and spread. The causal bacteria are known to survive the winter on infected bean straw and can infect the new bean crop (20, 21).

There are no satisfactory chemical control measures for common blight (16), and tolerant commercial cultivars were only recently made available with the introductions of the late maturing 'G.N. Tara' and 'G.N. Jules' in Nebraska (2, 6, 7). Although these new varieties have performed well, earlier maturing common blight-tolerant varieties would be less likely to be injured by early frost. Bacterial disease-tolerant varieties could also lead to the establishment of new bean seed industries in regions where the diseases occur.

One objective in our genetic improvement program for beans is the development of early maturing G.N. dry bean cultivars tolerant to *X. phaseoli*. Although tolerant germplasm was reported by Rands and Brotherton (15) in 1925, this material is now unavailable. Honma (11) reported that the Tepary bean *P. acutifolius* Gray was highly tolerant to *X. phaseoli* and he succeeded in making the interspecific cross *P. vulgaris* x *P. acutifolius*. Disease reaction to *X. phaseoli* was quantitatively inherited in his cross. The moderately-tolerant cv. G.N. Nebraska #1[5] derived from this interspecific cross, released in 1961, has performed well in western Nebraska, but it can develop moderate blight under heavy inoculum levels. Several late maturing, vigorous, highly common blight-tolerant rogue plants were isolated in diseased fields of this cultivar. Their tolerance was confirmed in subsequent tests and was found to be associated with late maturity (8, 9). Using this new germplasm source, Coyne et al., (9) reported quantitative inheritance of disease reaction and low heritability, estimated by parent offspring regression under field conditions. Pompeu[6] has reported a similar pattern of inheritance. Coyne et al., (9) observed low negative correlation in the F_2 between tolerance and days to flowering which suggested that earliness and tolerance could be combined.

The possibility that age of plant at inoculation may have affected disease reaction was not considered in the earlier studies. Pompeu[6] and Honma (11) utilized seedling populations and recorded the disease reaction within a relatively short period after inoculation. We studied the sequential development of disease reaction in tolerant and susceptible plants inoculated in both vegetative and reproductive stages, to clarify the interrelationships between disease reaction and plant development.

Materials and Methods

Genetic investigations in field - 1963 to 1971. F_1 plants from the cross early-maturing, common blight-susceptible 'GN 1140' x late-maturing, common blight-tolerant 'GN Nebraska #1, sel. 27' were grown in the greenhouse to produce F_2 seed. Two rows of each parent, and 9 rows of the F_2 generation, were planted at Scottsbluff, Nebraska, on June 4, 1963, in a randomized complete block design, with 3 replications of each entry. Only 1 row of the F_1 generation was used in 2 replications, due to limited seed. Seeds were planted 18-inches apart in rows 20-ft long and 22-inches apart. The date of first bloom was recorded for each plant.

The plants were inoculated with *X. phaseoli* on July 15 and again on July 20. 'GN 1140' was starting to bloom on the former date and most plants of 'GN Nebraska # sel 27' flowered 1 week after the second inoculation. We used the leaf watersoaking method of inoculation (19). The bacterial

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[2]Professor, Department of Horticulture and Forestry; Professor, Department of Plant Pathology; Technician, Department of Horticulture and Forestry. The effect of bacterial concn on disease reaction was studied at Cornell University, Ithaca, N. Y. Appreciation is expressed for the assistance of Robert Dickey, Department of Plant Pathology and D. H. Wallace, Department of Plant Breeding and Biometry.

[3]Leakey, Colin L. A. 1972. Background to a programme of breeding for resistance to bacterial blight of beans caused by *Xanthomonas phaseoli.* Makerere University, Kampala, Africa.

[4]Personal correspondence - Dr. Luis Camacho, ICA, Palmira, Colombia, S. A.

[5]Description and performance of the G. N. Nebraska #1, dry bean. 1961. Department of Horticulture and Forestry Report, University of Nebraska, Lincoln.

[6]Pompeu, Antonio Sidney. 1971. Inheritance of resistance of *Phaseolus vulgaris* L. (dry beans) to *Xanthomonas phaseoli* Dows. (common blight). Ph.D. Thesis. Cornell Univ., Ithaca, N. Y.

Table 1. Frequency distribution in number of plants in days to first bloom classes and common blight classes, 2 weeks after inoculation in parental, F_1, and F_2 generations of the cross 'GN 1140' (early and susceptible) x 'GN Nebraska #1, sel. 27' (late and tolerant).

Population	Disease rating[z] (8/9)	Days to first bloom 30 34	35 39	40 44	45 49	50 54	55 59	60 64	Row Totals
					No. of plants				
GN 1140	1								
	2		3	3	3				9
	3		13	41	8				62
	4	1	31	57	12				101
	5								
	Column totals	1	47	101	23				
GN Nebr. #1, sel. 27	1				1	3	60	89	153
	2								
	3								
	4								
	5								
	Column totals				1	3	60	89	
F_1 GN 1140 x GN Nebr. #1, sel. 27	1				1				1
	2		2	6	3				11
	3		3	11	8				22
	4		1	3	3				7
	5								
	Column totals		6	20	15				
F_2 GN 1140 x GN Nebr. #1, sel. 27	1	1	13	22	70	12	20	2	140
	2	0	34	73	96	13	13	3	232
	3	1	74	75	50	10	4	0	214
	4	0	45	29	17	0	0	0	91
	5	0	1	0	1	0	0	0	2
	Column totals	2	167	199	234	35	37	5	

[z]Disease rating classes: 1) no visible symptoms; 2) slight, small lesions on about 1-5% of leaves; 3) moderate lesions of various sizes, some leaves chlorotic; 4) severe, many large lesions on most leaves, pronounced chlorosis and necrosis; 5) very severe, plants chlorotic, necrotic, and largely defoliated.

suspension, prepared by washing 100 two-day-old petri dish cultures of Nebr. isolate *Xps.* into 50 gallons of water, was sprayed on plants at 150 p.s.i., to cause water soaking of leaves.

The plants were rated for disease reaction on August 9 and 27 (Table 1). The frequency distributions of numbers of plants in disease reaction classes and days to first bloom classes were recorded for each date.

Early maturing F_2 segregates in the crosses 'GN Nebraska #1 sel. 27' x 'GN 1140' and 'GN Nebraska #1, sel. 27' x Plant Introduction 165078 were backcrossed to transfer genes for early flowering into the recurrent parent 'GN Nebraska #1, sel. 27'. Six and 5 backcrosses were used, respectively, to develop 2 early-maturing, tolerant, nearly-isogenic 'GN Nebraska #1, sel. 27' lines. Those 2 lines, 'GN 1140', 'Dark Red Kidney', and Plant Introduction 165078 were grown in a randomized complete block design, with 3 replications, at Lincoln and at Scottsbluff in 1971. Seed was spaced at 18-inches in single-row plots, 15-ft long and 22-inches apart. Each plot was bordered by a row of susceptible 'GN UI #59'. Each test was inoculated when 'GN 1140' started to flower. Disease reaction ratings and the stages of development of the plants were recorded at 3 intervals at the Lincoln test and once at Scottsbluff.

Cell concn inoculation experiment. 'GN Nebraska #1, sel. 27', its early nearly-isogenic line, and 'GN 1140' were grown separately in each of four 8-inch pots, 2 plants per pot, in growth chambers replicated twice and programmed at photoperiod 14 hr, and 26.6° day and 21.1°C night temp. Under these conditions, 'GN Nebraska #1, sel. 27' flowered much later than the 2 other lines. The development stage of each line at inoculation was recorded. Trifoliolates at 6 to 7th node were inoculated on plants in 3 pots of each line in each chamber. Noninoculated plants of each line served as controls. We used the multiple needle puncture method described by Andrus (1). The terminal leaflet of the trifoliolate was inoculated with 5.4 x 10^7 cells per ml and 1 lateral leaflet with 2.0 x 10^9 cells per ml of Nebr. isolate *Xps.* The reactions were recorded 10 days later on a numerical scale.

Bacterial multiplication. The same 3 lines were grown separately in each of 2 six-inch pots, 2 plants per pot, replicated twice, in a growth chamber, programmed at 9-hr photoperiod and temp 26.6° day and 21.1°C night. This treatment induced flowering of the lines at the same time. All leaves were inoculated at the white flower bud stage using the watersoaking method (19) with 2 x 10^4 cells per ml of Nebr. isolate *Xps.* The lower cell concn was employed because Scharen (18) found little difference in the population of bacteria between tolerant and susceptible lines when a high cell concn, 10^6 cells per ml, was used. Leaf disc samples were punched at 1 hr, and 2, 4, 6, 10, and 14 days after inoculation. The leaves were washed with sterile distilled water before punching out the discs. Eighteen 6-mm diam leaf discs per replicate were obtained with a cork borer. Six discs were obtained from each of 3 leaflets situated in the top, middle, and bottom areas of the plant to form a sample. The discs were placed in 5 ml of sterile, distilled water and thoroughly comminuted in a leaf crusher. After the comminuted sample stood for 1 hr in an iced container, 3 serial dilutions were prepared in sterile distilled water. One ml aliquots of each dilution were then spread over the surface of potato-peptone-agar plates and incubated for 48 hr at 23.9°C. Colony counts were made on a Quebec counter and automatic register made by the American Optical Corporation. Disease reaction ratings were recorded at the 14 days sampling time.

In another experiment, 2 pots containing 2 plants, replicated twice, of the same 3 lines, were grown in a growth chamber, programmed for 14 hr light and 29.3°C day and 23.9°C night. This treatment induced early flowering of 'GN 1140', the early nearly-isogenic 'GN Nebraska #1, sel. 27'. In addition, 2 pots containing 2 plants, replicated twice, of 'GN Nebraska #1, sel. 27' were grown in another growth chamber programmed at 9-hr photoperiod and temp 26.6°C day and 21.1°C night. This

Table 2. Frequency distribution in number of plants in days to first bloom classes and common blight classes, 32 days after inoculation in parental, F_1 and F_2 generations of the cross 'GN 1140' (early and susceptible) x GN Nebraska #1, sel. 27' (late and tolerant).

Population	Disease rating[z] (8/27)	Days to first bloom 30 34	35 39	40 44	45 49	50 54	55 59	60 64	Row Totals
						No. of plants			
GN 1140	1								
	2								
	3		6	4	4				14
	4	1	35	92	17				145
	5								
	Column totals	1	41	96	21				
GN Nebr. #1, sel. 27	1				1		42	77	120
	2					1	18	12	31
	3								
	4								
	5								
	Column totals				1	1	60	89	
F_1 GN 1140 x GN Nebr. #1, sel. 27	1								
	2		1		1				2
	3		3	14	9				26
	4		2	5	4				11
	5			1	1				2
	Column totals		6	20	15				
F_2 GN 1140 x GN Nebr. #1, sel. 27	1	0	0	0	2	2	0	0	4
	2	1	20	41	91	13	23	2	191
	3	0	67	105	107	14	13	1	307
	4	1	77	50	29	4	1	0	162
	5	0	3	3	5	0	0	0	11
	Column totals	2	167	199	234	33	37	3	

[z]Disease rating scale: see footnote in Table 1.

treatment induced early flowering in this line similar to the same age early lines in the other chamber. When most of the pods of this line reached the plump green pod stage the pots were moved into the other chamber, 14 hr photoperiod. The temp of this chamber was now programmed at 26.6°C day and 21.1°C night. In this chamber 'GN 1140' and the early nearly-isogenic 'GN Nebraska #1, sel. 27' had many large flat green pods while 'GN Nebraska #1, sel. 27' was vegetative. Leaf inoculation with 2 x 10^4 cells per ml, leaf sampling and bacterial population estimation procedures were the same as

Table 3. Common blight disease reaction (D.R.) ratings[z] of cultivar and breeding lines recorded on 3 dates at Lincoln and 1 date at Scottsbluff.

Entry	Lincoln (inoculated 6/24) 7/21 D.R.	7/21 (Dev. stage)	8/6 D.R.	8/6 (Dev. stage)	8/20 D.R.	8/20 (Dev. stage)	Scottsbluff (inoculated 7/18) 8/16 D.R.	8/16 (Dev. stage)
1. GN Nebr. #1, sel. 27 (late)	1.0 i[y]	starting to flower	1.0 i	some small pods	1.2 hi	lower pods filled - some flowers	1.0 d	still in flower - lower pods still flat.
2. [w]GN Nebr. #1, sel. 27 (early - near isogenic)	1.3 hi	many lower pods filled -some flowers	1.7 gh	some lower pods yellow	H[x]		1.8 c	lower pods filled
3. GN Nebr. #1, sel. 27 (early - near isogenic)	2.0 fg	many lower pods filled -some flowers	2.0 fg	ready for harvest	H		1.8 c	lower pods filled
4. GN 1140	4.0 b	all pods filled - no flowers	5.0 a	pods nearly dry	H		3.5 a	some lower pods yellow
5. Dark Red Kidney	2.7 de	many lower pods filled -some flowers	4.0 b	some pods starting to fill	4.0 b	ready for harvest	3.7 a	lower pods filled
6. PI 165078	2.3 ef	starting to flower	3.3 c	some small pods	4.3 b	lower pods nearly filled -some flowers	2.5 b	still flowers - lower pods flat
Date means	2.2 b		2.8 a		3.2 a			

[z]See footnote of Table 1 for explanation of disease reaction ratings.

[y]Mean separation, within locations, by Duncan's multiple range test, 5% level.

[x]H - plants already harvested.

[w]Entries 2 and 3 derived from 6 and 5 backcrosses to 'GN Nebr. #1, sel. 27', respectively.

described for the other bacterial population experiment. Two samples of eighteen 6-mm leaf discs per replicate were obtained at 1 hr, and at 3, 6, 10, 14, and 18 days after inoculation. Fresh wt of the discs were recorded.

Results

Genetic investigations in field - 1963-1971. Disease reactions under field conditions 2 weeks after inoculation indicated partial dominance for susceptibility in F_1 (Table 1). A continuous distribution of disease ratings was recorded for the F_2, at which time segregates apparently combining early flowering and tolerant disease reaction were observed. Thirty-two days after inoculation a significant change in the distribution of disease ratings was observed (Table 2). The F_1 now showed nearly-complete dominance for susceptibility. Only 4 plants, out of 675, combining no disease symptoms and moderately early flowering dates were found in F_2, suggesting that several genes controlled the tolerant reaction. In the F_2, numerous plants with a disease rating of 2 and 3 within the range of flowering of 'GN 1140' were detected. Pedigree selection was adopted to select for earliness and blight tolerance. Two F_2 plants with a disease reaction of 1 and 45 plants with a disease index of about 2, all of which flowered within the range of 'GN 1140', were selected. The progeny from the selected plants, F_3 to F_6, were grown in a blight nursery in the field in subsequent years and inoculated with *X. phaseoli* Nebraska isolate *Xps.* Heritability for the disease reaction was found to be low under field conditions. None of the derived lines combined disease reaction rating of 2 or less with the early maturity of 'GN 1140'. Many advanced lines developed little blight, (disease rating of 2), combined with maturity intermediate between the 2 parents. The later maturing lines had a higher level of tolerance. This suggested that linkage existed between some genes controlling tolerance and some genes for late flowering. Delayed flowering in beans is due to the response to long photoperiod and high temp and is under the control of relatively few genes (3, 4). A slight bimodal distribution for days to flowering was observed in the F_2 'GN 1140' x 'GN Nebraska #1, sel. 27' indicating that few genes were involved in controlling this trait. The bimodal distribution would be more pronounced if a higher temp occurred to induce a greater delay of flowering of the late parent (3, 4).

While pedigree selection was in progress, a backcross program using 'GN Nebraska #1, sel. 27' as the recurrent parent was conducted to transfer genes controlling early flowering and to break the linkage between lateness and blight tolerance. The resulting early maturing nearly-isogenic lines were only slightly later than the early blight-susceptible 'GN 1140' (Table 3). A line derived from 6 backcrosses was as tolerant as the recurrent parent but a second early line derived from 5 backcrosses was slightly less tolerant. Disease severity increased during the course of development at Lincoln (Table 3). At Scottsbluff, however, where temp were less favorable for the bacteria, there were no differences in disease ratings between the 2 nearly-isogenic lines. Disease ratings of the susceptible cultivars were lower at Scottsbluff than at Lincoln. This effect was more pronounced in the susceptible 'GN 1140', 'Dark Red Kidney', and Plant Introduction 165078, than in 'GN Nebraska #1, sel. 27' and its nearly-isogenic lines. Plants in the late pod stage of development were more susceptible than plants in the flowering stage. In comparing the disease reactions of cultivars, it is important to make comparisons between lines at the same stage of development (Table 3). The late maturing Plant Introduction 165078, however, was very susceptible to blight.

Cell concn inoculation experiment. At the time of inoculation 'GN 1140', had well-filled plump pods and the nearly-isogenic line had large flat pods; 'GN Nebraska #1, sel. 27' was still in a vegetative state. Disease development was usually more severe if the inoculum contained 2 x 10^9 bacterial cells per ml than if it contained 5.4 x 10^7 cells per ml (Table 4). 'GN 1140' developed a susceptible reaction with both cell concn. 'GN Nebraska #1, sel. 27' and the nearly-isogenic line developed tolerant reactions with the low cell concn but the

Table 4. Disease reaction ratings[z] of same age trifoliolate leaflets on 3 bean lines inoculated with 5.41 x 10^7 and 2 x 10^9 cells per ml.

Line	5.41 x 10^7 cells per ml	2 x 10^9 cells per ml	Stage of plant growth[y] at inoculation
GN 1140 (early)	3.6 b[x]	4.7 a	Plump pods
GN Nebr. #1, sel. 27 (late)	1.4 d	2.6 c	Vegetative
GN Nebr. #1, sel. 27 nearly-isogenic (early)	1.9 d	3.8 b	Most pods large flat

[z]Rating scale used by Pompeu[6]; 1) no visual symptoms; 2) up to 5% yellowing of area around needle marks; 3) from 5 up to 25% ditto; 4) 25 up to 50% ditto; 5) 50 to 75% ditto; 6) 75 to 100% ditto.

[y]Plants were grown under 14-hr photoperiod, 26.6 and 21.1°C, day and night, respectively, to delay flowering of 'GN Nebraska #1, sel. 27'.

[x]Mean separation by Duncan's multiple range test, 5% level.

Table 5. Bacterial populations[z] in leaves of 3 bean lines at different intervals after inoculation with 2 x 10^4 cells per ml.

Interval after inoculation	Bean line: GN 1140 (sus)	GN Nebr. #1 sel. 27[y] (tol)	Nearly-isogenic GN Nebr. #1 sel. 27 (tol)
	Bacterial population (x 10^3)		
1 hr	7.8 d[x]	3.9 d	4.8 d
2 days	19.9 a	6.2 d	11.6 b
4 days	30.2 a	11.4 b	13.1 b
6 days	33.8 a	13.2 b	16.7 b
10 days	30.4 a	9.9 b	13.5 b
14 days	30.3 a	11.8 b	16.8 b
Disease symptoms 14 days	Moderate symptoms	No symptoms	No symptoms

[z]Expressed as no. bacterial cells per 18 six-mm diam leaf discs.

[y]Grown under 9-hr photoperiod, 26.6 day and 21.1°C night to induce flowering at same time in all 3 lines. Plants inoculated at the white flower bud stage.

[x]Mean separation by Duncan's multiple range test, 5% level.

Table 6. Bacterial populations[z] in leaves of bean lines at different intervals after inoculation with 2 x 10^4 cells per ml.

Time interval after inoculation	Bacterial population (x 10^3): GN 1140[y]	Nearly-isogenic GN Nebr. #1 (tol)[y]	GN Nebr. #1 sel. 27 (tol)[x]	GN Nebr. #1 sel. 27 (tol)[y]
1 hr	5.9 i[w]	3.3 k	4.6 j	1.6 m
3 days	10.0 g	5.8 i	9.0 gh	3.6 jk
6 days	15.9 d	8.2 h	11.7 f	6.1 i
10 days	20.1 c	11.6 f	14.6 e	9.2 gh
14 days	22.9 b	16.6 d	19.3 c	12.6 f
18 days	26.8 a	19.2 c	22.9 b	15.5 de
Stage of growth at inoculation	flat green pod	flat green pod	plump green pod	vegetative
Fresh wt. of discs, g	0.067 a	0.071 a	0.068 a	0.067 a

[z]Expressed as no. bacterial cells per 18 six-mm diam leaf discs.

[y]Grown with 14 hr photoperiod at 29.3°C day and 23.9°C night.

[x]Grown until inoculated with 9 hr photoperiod at 26.6°C day and 21.1°C night, and then under conditions mentioned above in [y].

[w]Mean separation by Duncan's multiple range test, 5% level.

nearly-isogenic line was slightly less tolerant. With the high cell concn, the isogenic line developed severe symptoms and 'GN Nebraska #1, sel. 27' developed moderate symptoms.

Bacterial multiplication. In the first experiment all 3 lines were in the white flower bud stage at inoculation. The bacterial population nearly doubled between 1 and 48 hr (Table 5). It reached a plateau at 4 days and remained stable to 14 days. Leaves of susceptible 'GN 1140' contained about double the bacterial population as leaves of the tolerant lines; there was no significant difference in bacterial populations in 'GN Nebraska #1, sel. 27' and in the nearly-isogenic line. On the 14th day, moderate susceptible symptoms were observed in 'GN 1140'. No symptoms were observed on the 2 tolerant lines, even though the leaves contained high populations of common blight bacteria.

In the second experiment, at the time of inoculation, 1 lot of the tolerant 'GN Nebraska #1, sel. 27' was in the vegetative stage of growth. Another lot of this line, 'GN 1140', and the early nearly-isogenic line were in the pod stage (Table 6). The bacterial population of the leaves of vegetative 'GN Nebraska #1, sel. 27' was lower than that in all the other lots (Table 6). The bacterial population of the susceptible pod stage inoculated 'GN 1140' was higher than in the pod stage inoculated tolerant lines. The bacterial population of podded 'GN Nebraska #1, sel. 27' was higher than in the nearly-isogenic line and this is attributed to more advanced maturity of the former line. The bacterial populations in all lines nearly doubled between 1 and 72 hr and then showed a smaller increase at each sampling time after the third day. There was no difference in the fresh wt of the leaf disc samples of the lines (Table 6). Fourteen days after inoculation the pods of 'GN 1140' and 1 lot of 'GN Nebraska #1, sel. 27' were ripening while the nearly-isogenic line had green plump pods and green foliage. At this time the other lot of 'GN Nebraska #1, sel. 27' started to flower.

Discussion

Susceptibility or tolerance of beans to common bacterial blight, caused by *X. phaseoli,* depends greatly upon the stage of development of the plant; plants are more susceptible when in the reproductive stage than when in the vegetative stage. In previous investigations, the effects of leaf age on susceptibility were compared and conflicting results reported. Goss (10) found that the older leaves were more susceptible, while Patel and Walker (14) reported that the younger leaves were more susceptible. Neither author gave attention to stage of plant development as related to disease reaction. Our results have important implications in establishing the disease reactions of breeding lines and cultivars which possess different maturities, and also in studying the inheritance of disease reaction in populations which are segregating for days to flowering and maturity. Several workers[4] (11) have reported on the inheritance of disease reaction in inoculated seedlings and young plants and have recorded the disease reaction up to 2 weeks after inoculation. Our experiments emphasize the importance of studying the reaction of the plants at different stages of development and at different intervals after inoculation. Many lines may have a moderate level of tolerance in the vegetative stage but become susceptible during the reproductive or pod development stage. From a breeding standpoint, it is important to select plants which exhibit high tolerance during pod development, which is the more susceptible stage of plant growth.

Increased selection pressure is imposed by *X. phaseoli* on early maturing bean lines in Nebraska, because the more susceptible pod stage of growth occurs during a period of higher temp than in the case of the late maturing lines. A higher level of tolerance is, therefore, required in early lines compared to later maturing lines. The mean Western Nebraska temp in the field in early August is close to 26.6°C while in early September it is close to 21.1°C. Goss (10) reported that common blight developed rapidly at a high temp of 32°C but slowly at 20°C and 16°C.

In screening germplasm, selections, and breeding lines for reaction to *X. phaseoli,* it is important to use a suitable, standardized concn of bacteria. Our results indicate that 2×10^9 cells per ml is too high a concn to use, since 1 line showing tolerance at a bacterial concn of 5.41×10^7 cells per ml showed susceptibility at this high concn. We believe that the higher concn of cells probably does not simulate natural conditions of field infection.

Linkage was found between some of the genes controlling tolerance and genes controlling delayed flowering, a response of the genotype to long photoperiod x high temp (3, 4). The flowering response is under the control of relatively few genes (3, 4, 5). Considerable environmental variation may affect the expression of the flowering trait. Also, the seeds may not emerge uniformly in the field. If the temp is not at a high enough level under a long photoperiod, the delay in flowering of the late parent may not be so pronounced. Overlapping of the distributions of days to flowering of the early and late parent may then occur. These variation factors make it difficult to detect qualitative gene effects in some situations. In view of the nature of the genetic control of disease reaction and days to flowering, the backcross method, with 'GN Nebraska #1, sel. 27' as the recurrent parent, was used successfully in transferring genes for early flowering into the highly blight-tolerant 'GN Nebraska #1, sel. 27'. Both late and early, nearly-isogenic, common blight-tolerant lines were tested for reaction in disease nurseries. The early, tolerant, nearly-isogenic line showed promise of introduction as a new 'GN' cultivar and may be valuable in expanding the bean seed production industry in Nebraska. The later-maturing common blight-tolerant cvs. GN Tara and GN Jules have performed satisfactorily in Nebraska and other states. However, there is a need for an earlier, less-viny 'GN' type to reduce the potential risk of crop loss due to early fall freeze.

Large populations of *X. phaseoli* develop in inoculated leaves of tolerant lines and this occurs even when the leaves are inoculated with a low cell concn 2×10^4 cells per ml. This has serious implications for the breeders and seed producers of tolerant cultivars. Wellhausen (24) and Lincoln (13) found that the virulence of a bacterial population increased through mutation and selection during passage through a tolerant host. Thus, in order to reduce the possibility of breakdown of tolerance due to the emergence of more virulent strains and races, seed of tolerant varieties should be saved from plants which are free of bacteria.

Variation in virulence of *X. phaseoli* has recently been reported by Schuster and Coyne (22). 'GN Nebraska #1, sel. 27', the tolerant selection used in our breeding program, was moderately susceptible to isolates originating from Colombia, South America. Whether such differences in virulence also occur in isolates from states other than Nebraska where beans are grown is not known. Efforts should therefore now be directed to locating new germplasm sources with tolerance to a wide range of *X. phaseoli* strains.

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Characterization of Anthocyanins During Ripening of Fruit of *Vaccinium corymbosum*, L. Cv. Wolcott[1]

Donald J. Makus[2] and Walter E. Ballinger[3]
North Carolina State University, Raleigh

Abstract. Anthocyanins (Acy) in ripening blueberry fruit of the cv. Wolcott were identified and their amounts estimated spectrophotometrically. Fifteen Acy, all combinations of Mv, Pt, Pn, Dp, and Cy with glu, gal, or arab[4] in the 3-position were found in unripe, red fruit. Ripe and overripe fruit contained all these Acy except Pn-gal. Cy-glu and Cy-gal comprised about 40% of the total Acy in unripe fruit, while Mv-glu and Mv-gal constituted about 60% in the overripe fruit. The relative proportions of 3-glucosides, 3-galactosides, and 3-arabinosides remained fairly constant throughout fruit ripening.

The pigments of *Vaccinium corymbosum* and *V. angustifolium* Ait. have been studied in detail (6, 11). Anthocyanins (Acy) of fruit of plants of the genus *Vaccinium* studied thus far are non-acylated, 3-substituted monoglycosides (6, 11, 16, 18). They include all possible combinations of 5 aglycones (Cy, Dp, Mv, Pn, and Pt)[4] and 3 sugars (arab, gal, and glu)[4]. Traces of diglycosides have been reported, but these have never been isolated in sufficient amounts to permit their complete characterization (6, 11).

Quantitative changes in Acy pigments during ripening of Montmorency cherries, *Prunus cerasus* L., have been found by Dekazos (9). Dekazos (8) did not find any qualitative changes in Acy due to ripening in the 7 Acy he identified in these cherries. Von Elbe et al. (22) obtained similar qualitative results with 8 cultivars of tart cherries *(P. cerasus).* Ashtakala and Forward (3) examining developing flower buds of 6 cultivars of *Iris germanica* L. found a similar pattern of pigment increase without accompanied qualitative changes.

Since no work has been reported on changes in pigments of *V. corymbosum* during fruit ripening, the present work was undertaken to delineate any qualitative or quantitative differences (mainly qualitative) in Acy content which may occur during blueberry fruit ripening; and to accrue basic pigment information which might be an aid to plant breeders, plant physiologists and food technologists.

In a study of pigment systems as complex as blueberry fruit, paper chromatography is the technique of necessity. However, since the long (3 weeks) chromatographic purification process imposed by this study is known to present many recovery difficulties, all quantitatives results should be regarded as estimates.

Materials and Methods

Fruits of all stages of ripeness of the widely planted blueberry cv. Wolcott [Weymouth x (Stanley x Crabbe 4)] were harvested in 1969 and 1970 in eastern North Carolina and returned to Raleigh in an ice chest. The berries were sorted

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[2]Graduate Assistant, Department of Horticultural Science.

[3]Professor, Department of Horticultural Science.

[4]Abbreviations used are as follows: Cy = cyanidin, Dp = delphinidin, Mv = malvidin, Pn = peonidin, Pt = petunidin, arab = 3-arabinose, gal = 3-galactose, glu = 3-glucose, gly = 3-glycosides, Acy = anthocyanin(s).

[5]Mention of trademark name or proprietary product does not imply approval to the exclusion of other products that may also be available.

[6]A = absorbance; a = molar absorptivity coefficient (at a given wavelength) b = cell path (1 cm); c = concn (M/1). Terms used are further defined in *Analytical Chemistry*.