Response of *Brassica oleracea* var. *capitata* to Wound and Spray Inoculations with *Xanthomonas campestris* pv. *campestris*

Phillip D. Griffiths[1] and Cathy Roe
Department of Horticultural Sciences, Cornell University New York State Agricultural Experiment Station, Geneva, NY 14456

Additional index words. black rot, cabbage, host-plant resistance, breeding

***Abstract*. Eighteen cabbage breeding lines and cultivars were evaluated for resistance to black rot caused by *Xanthomonas campestris* pv. *campestris* following wound and spray inoculations at the juvenile and mature stages. Plants were evaluated using four inoculation procedures (juvenile wound, juvenile spray, mature wound, and mature spray) in replicated greenhouse and field experiments. The breeding lines Badger #16, Cornell 101, Cornell 102, NY 4002 and accession PI 426606 exhibited high levels of resistance following all inoculation procedures. 'Silver Dynasty' was the most resistant commercial cultivar based on the four tests, yet ranked 12th following the juvenile wound inoculation. The juvenile spray inoculation had a high correlation with both wound and spray inoculations in field experiments (0.89 and 0.86, respectively); however, the juvenile wound inoculation did not correlate well with mature wound and spray inoculations (0.58 and 0.51, respectively). The results indicate that the juvenile wound inoculation is not the most appropriate approach for determining field resistance in *Brassica oleracea*, and that resistant material could be selected against using this approach. A high correlation between juvenile spray inoculation disease severity ratings and mature plant resistance indicates that plants can be evaluated effectively at the juvenile stage for mature plant resistance to black rot.**

Black rot is a bacterial disease of cabbage (*Brassica oleracea* var. *capitata*) caused by *Xanthomonas campestris* pv. *campestris* (Xcc). Xcc is a seedborne pathogen (Cook et al., 1952) that can overwinter on cruciferous weeds and wild relatives of cultivated *Brassica* crops (Schaad and Dianese, 1981). Xcc does not have an active penetration mechanism for infection, and the primary means of entry is through the hydathodes, stomates and wounds. Infection is typically characterized by V-shaped chlorotic regions at the leaf margins that appear 10 to14 d after Xcc infection through the hydathodes (Williams, 1980). Infected seeds are a primary source of inoculum from which epidemics can begin, consequently, it is important to breed cabbage cultivars that have resistance to the disease at both the juvenile and mature stages.

Black rot resistance has been derived from several *B. oleracea* sources including the cabbage cultivar 'Early Fuji' (Bain, 1952) and the cabbage plant introduction (PI) 436606 (Hunter et al., 1987). These sources have resulted in resistant breeding lines including Badger #16 (Williams, University of Wisconsin) and NY 4002 (Dickson, Cornell University) that have been used in the development of black rot resistant cabbage cultivars. The inheritance of black rot resistance from *B. oleracea* is complicated and has been associated with at least two genes (Camargo et al., 1995; Williams et al., 1972). Hunter et al. (1987) also suggested that juvenile and mature resistance to black rot may be under separate genetic control mechanisms. These issues complicate approaches to introgressing black rot resistance, and a better understanding of the response of *B. oleracea* to inoculation will help improve the selection of breeding lines for development of resistant cultivars.

The objectives of this study were to evaluate cabbage cultivars and breeding lines for resistance to New York isolates of black rot at the juvenile and mature stages using both wound and spray inoculation procedures, and to study correlations between the inoculation techniques to evaluate the effectiveness of juvenile screening in determining mature plant resistance.

Received for publication 12 May 2004. Accepted for publication 4 Aug. 2004.
[1]To whom reprint requests should be addressed; e-mail pdg8@cornell.edu.

Materials and Methods

Plant material. Ten susceptible cultivars of cabbage ('Atria', 'Azan', 'Bobcat', 'Fresco', 'Genesee', 'King Cole', 'Morris', 'Rinda', 'Superdane', and 'Transam'), four black rot resistant cabbage breeding lines (Badger # 16, Cornell 101, Cornell 102, and NY 4002), three cabbage cultivars with high levels of black rot resistance ('Silver Dynasty', 'Matsumo', and 'Tenacity') and a black rot resistant plant introduction (PI 436606) were evaluated in greenhouse and field experiments between May and August 2003 (Table 1). All seeds for the greenhouse inoculations were planted in 'Cornell Mix' (Boodley and Sheldrake, 1972) in 32-cell (125-cm^3) Styrofoam trays (Speedling, Sun City, Fla.) with one seed per cell, and four replications with eight plants per replication. Greenhouse replications represented separate inoculations and mist treatments in 4 consecutive weeks. For the field inoculation of mature plants, genotypes were seeded in 128-cell (15.6-cm^3) trays and were greenhouse grown for 3 weeks. Plants were moved to cold frames for 7 d before field planting, and were transplanted in four replications with 10 plants per plot at a distance of 45 cm between plants with 90 cm row spacing in randomized plots with overhead irrigation set at 8 m spacing every 6 m.

Inoculation. Plants were grown in a 90-m^2 greenhouse set at 27/24 °C (day/night) with a 14-h photoperiod under 1000-W metal halide lamps (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in preparation for four inoculation approaches: juvenile wound (greenhouse inoculation of juvenile plants by wound), juvenile spray (greenhouse inoculation of juvenile plants by spray), mature wound (field inoculation of mature plants by wound), and mature spray (field inoculation of mature plants by spray). For the juvenile plant inoculations, temperatures were set to represent the optimal conditions for Xcc growth suggested by Staub and Williams (1972). Four isolates of Xcc isolated from infected cabbages in New York were obtained from H. Dillard (Plant Pathology, N.Y. State Agricultural Experiment Station, Cornell Univ., Geneva). These four Xcc isolates were grown on YDCP medium (Shelton and Hunter, 1985) for 3 d in preparation for inoculations. For the juvenile

Table 1. Sources of cultivars/breeding lines evaluated for black rot resistance in field and greenhouse trials.

Genotype	Source
Badger	Breeding Line, Paul Williams, University of Wisconsin, Madison
PI436606	Accession, USDA NE-9, Geneva, N.Y.
Cornell 102	Breeding Line, Cornell University, Ithaca, N.Y.
Cornell 101	Breeding Line, Cornell University
NY4002	Breeding Line, Cornell University
Silver Dynasty	Seminis Vegetable Seeds, Woodland, Calif.
Tenacity	Sakata Vegetable Seeds, Woodland, Calif.
Bobcat	Reeds Seeds, Cortland, N.Y.
Matsumo	Bejo Seeds,Warmenhuizen, Netherlands
Fresco	Bejo Seeds
Azan	Seminis Vegetable Seeds
Transam	Bejo Seeds,Warmenhuizen
Atria	Seminis Vegetable Seeds
Genesee	Seminis Vegetable Seeds
Rinda	Seminis Vegetable Seeds
Superdane	Reeds Seeds
Morris	Bejo Seeds
King Cole	Harris Moran, Modesto, Calif.

Table 2. Mean disease severity ratings of juvenile (juv) and mature cabbage cultivars/breeding lines inoculated using the wound and spray procedures in greenhouse and field studies at New York State Agricultural Experiment Station, Geneva, Summer 2003. Mature wound and spray inoculations are the mean of two ratings 8 and 12 weeks after transplanting.

Genotype	No. tested	Juvenile wound	Juvenile spray	Mature wound	Mature spray	Total mean
Badger	142	2.72 gh[z]	1.56 h	1.34 ij	1.18 h	1.65 j
PI436606	135	2.47 h	1.50 h	1.18 j	2.06 e–g	1.77 j
Cornell 102	124	3.06 f–h	1.59 h	1.44 h–j	2.11 ef	2.05 i
Cornell 101	128	3.31 d–g	1.84 gh	1.52 g–j	1.88 fg	2.11 hi
NY4002	138	3.50 c–f	1.84 gh	1.65 g–i	1.68 g	2.13 hi
Silver Dynasty	130	3.81 b–f	2.22 f–h	1.56 g–j	1.09 h	2.16 hi
Tenacity	142	3.53 c–f	2.41 fg	1.66 g–i	1.78 fg	2.28 g–i
Bobcat	142	3.16 e–h	2.13 f–h	1.79 g–i	2.46 e	2.36 f–h
Matsumo	141	4.09 a–d	2.78 ef	1.58 g–j	2.00 fg	2.53 fg
Fresco	137	4.03 a–d	3.13 de	1.94 g	3.24 d	3.06 e
Azan	137	3.10 e–h	3.63 cd	3.63 de	4.00 c	3.62 d
Transam	138	3.59 c–f	4.45 ab	3.72 d	3.69 c	3.85 cd
Atria	134	3.16 e–h	4.41 ab	3.27 e	4.49 b	3.85 cd
Genesee	125	3.87 b–d	4.13 a–c	4.21 bc	3.97 c	4.04 bc
Rinda	138	4.23 a–c	3.84 b–d	4.03 cd	4.53 ab	4.17 b
Superdane	143	3.75 b–f	4.59 a	4.67 a	4.93 a	4.52 a
Morris	137	4.39 ab	4.77 a	4.45 ab	4.84 ab	4.62 a
King Cole	135	4.66 a	4.59 a	4.46 ab	4.87 ab	4.65 a

[z]Means groupings separated according to Duncan's multiple range test ($p \leq 0.05$).

Table 3. Rankings of cabbage cultivars and breeding lines in four inoculation trials at New York State Agricultural Experiment Station, Geneva, Summer 2003. Rankings for mature plants in field tests are the mean of two ratings 8 and 12 weeks after transplanting.

Genotype	Juvenile wound	Juvenile spray	Mature wound	Mature spray	Total mean rank
Badger	2	2	2	2	1
PI436606	1	1	1	7	2
Cornell 102	3	3	3	8	3
Cornell 101	7	4	4	5	4
NY4002	8	4	7	3	5
Silver Dynasty	12	7	5	1	6
Tenacity	9	8	8	4	7
Bobcat	6	6	9	9	8
Matsumo	15	9	6	6	9
Fresco	14	10	10	10	10
Azan	4	11	12	13	11
Transam	10	15	13	11	12
Atria	5	14	11	14	12
Genesee	13	13	15	12	14
Rinda	16	12	14	15	15
Superdane	11	16	18	18	16
Morris	17	18	16	16	17
King Cole	18	16	17	17	18

inoculations plants were wound inoculated at the 21-d stage, by piercing the leaves either side of the midrib with two Xcc infected needles (Tonguc and Griffiths, 2004a and 2004b). This was performed for each of the four Xcc isolates (Shaw and Kado, 1988) and the plants were transferred to a 100% humidity chamber for 72 h following the inoculation. For the juvenile spray inoculation, the four isolates were blended into a slurry and sprayed onto 21-d-old plants before placing in the 100% humidity chamber for 72 h. For the field wound inoculation, plants were inoculated using the same approach as the juvenile wound 21 and 42 d after transplanting. For the field spray inoculation, plants were spray inoculated using a slurry of the four Xcc isolates. The isolates were blended with water, strained, and applied using a backpack mistblower (model 444; Solo, Newport News, Va.) 21 and 42 d after transplanting. Field inoculations were performed following irrigation of the plots to increase turgor pressure within the plants, and an overhead irrigation system was used to irrigate the field plots for 30 min daily for 2 weeks after each inoculation.

Evaluation. Greenhouse inoculated plants were evaluated 10 to14 d after infection using a rating scale of 1 to 5 (1 = highly resistant, 5 = highly susceptible) as described by Tonguc and Griffiths (2004a), the juvenile spray inoculation had a slower response than the juvenile wound approach, and was rated at 21 d. Field inoculated plants were evaluated using a similar scale modified for mature plants 8 and 12 weeks after transplanting to the field, the means of the two ratings were used for evaluating field resistance as they were very highly correlated (data not shown). Disease severity ratings were analyzed to evaluate significant mean groupings using Duncan's multiple range test (SAS, 1997). Correlations among the four tests were calculated using Spearman's rank correlation.

Results

The symptom response to juvenile spray inoculations was slower and less severe than the wound inoculations, and ratings were made after 21 d instead of the 14 d rating of wound inoculated juvenile plants. All breeding lines showed high levels of resistance to black rot following the four inoculation procedures; however, Cornell 101 and NY 4002 were more susceptible in response to the juvenile wound inoculation (ranking 7th and 8th, respectively) when compared to their response to the three other inoculation tests (Tables 2 and 3). Badger #16 was ranked highest overall (Table 3) when mean rankings of the two ratings were used for the evaluation of plants in the field tests, but was significantly different from the cultivar 'Silver Dynasty' in the field spray inoculation trial (Table 3). Evaluation of the mortality rates in the juvenile inoculation tests (Table 4) also indicated that susceptible cultivars exhibited a high mortality rate following infection with Xcc ('Azan', 'Transam', 'Atria', 'Genessee', 'Rinda', 'Superdane', 'Morris', and 'King Cole'), whereas the Xcc resistant lines or cultivars grew out of the infection and remained viable plants. The reduced level of systemic infection of Xcc was also observed in cultivars 'Bobcat' and 'Fresco'.

Differences were observed among the inoculation procedures based on rank correlations over the four tests (Table 5). As with NY 4002 and Cornell 101 additional anomalies were observed with respect to the juvenile wound inoculation including 'Silver Dynasty', which ranked 12th, but 1st in the field mature spray test. The correlations between the juvenile spay inoculation and both the mature wound and spray procedures applied to field plants were also high (0.89 and 0.86, respectively). These results suggest that the juvenile wound approach, may not correlate accurately with field resistance in black rot resistant *B. oleracea*, even though it may overcome susceptible plants, it can also overcome genotypes exhibiting high levels of field resistance.

Discussion

All cultivars and breeding lines in these inoculation tests showed symptoms of black rot in at least some plants. Xcc resistance is controlled by multiple genes in the *B. oleracea* cultivars and breeding lines evaluated in this study. The results suggest that the inoculation approach may affect the response of cultivars to Xcc infection, while the juvenile spray inoculation has a high correlation with field resistance, the juvenile wound approach does not. The high correlation of the juvenile spray approach with field resistance indicates that breeding lines can be selected for resistance to Xcc at the juvenile stage, and that resistance in juvenile plants is under similar genetic control to resistance in mature plants.

The inoculation procedures tested have concentrated on *B. oleracea* derived sources of black rot resistance that have quantitative genetic control. Alternative sources of resistance to black rot are now being developed from related *Brassica* species, including *B. carinata* Braun (Tonguc et al., 2003) and *B. juncea* (L.) Czern. (Tonguc and Griffiths, 2004b) that are controlled by a single gene. The wound inocula-

Table 4. Mortality responses of juvenile seedlings in response to systemic infection following inoculation with four isolates of *Xanthomonas campestris* pv. *campestris.*

	Mortality (%)	
Genotype	Juvenile wound	Juvenile spray
Badger	0	0
PI436606	0	0
Cornell 102	0	0
Cornell 101	3	0
NY4002	0	0
Silver Dynasty	0	0
Tenacity	3	0
Bobcat	3	0
Matsumo	0	0
Fresco	9	6
Azan	21	9
Transam	50	26
Atria	41	25
Genesee	59	50
Rinda	55	22
Superdane	59	59
Morris	76	52
King Cole	87	59

Table 5. Correlations of black rot disease severity ratings in response to inoculations of juvenile and mature plants using wound and spray approaches calculated using Spearman's rank correlation.

Comparison	Spearman's rank correlation
Juvenile wound vs. juvenile spray	0.75
Juvenile wound vs. mature wound	0.58
Juvenile wound vs. mature spray	0.51
Juvenile spray vs. mature wound	0.89
Juvenile spray vs. mature spray	0.86
Mature spray vs. mature wound	0.89

tion is appropriate when single gene resistance is being screened because it has a gene for gene response to the black rot race. In *B. oleracea* derived black rot resistant genotypes it is possible that the resistance mechanisms include reduction of infection through leaf hydathodes, a mechanism that would be directly overcome using the wound inoculation approach. In mature wound inoculation studies, plants also become infected through the hydatodes in the field from susceptible plants, hence, similar responses are observed from wound and spray inoculations in the field. The results presented will enable a more effective approach to selecting *B. oleracea* for resistance to Xcc by using the correlation of juvenile spray resistance with mature plant resistance in the development of black rot resistant cultivars.

Literature Cited

Bain, D.C. 1952. Reaction of brassica seedlings to black rot. Phytopathology 42:497–500.

Boodley, J.W. and R. Sheldrake, Jr. 1972. Cornell peat-lite mixes for commercial plant growing. Cornell Info. Bul. 43:1–8.

Camargo, L.E.A., P.H. Williams, and T.C. Osborn. 1995. Mapping of quantitative loci controlling resistance to *Brassica oleracea* to *Xanthomonas campestris* pv. *campestris* in the field and greenhouse. Phytopathology 85:1296–1300.

Cook, A.A., R.H. Larson, and J.C. Walker. 1952. Relation of the black rot pathogen to cabbage seed. Phytopathology 42:316–320.

Hunter, J.E., M.H. Dickson, and J.W. Ludwig. 1987. Sources of resistance to black rot of cabbage expressed in seedlings and adult plants. Plant Dis. 71:263–266.

SAS Institute. 1997. SAS user's guide. SAS Inst., Cary, N.C.

Schaad, N.W. and J.C. Dianese. 1981. Cruciferous weeds as sources of inoculum of *Xanthomonas campestris* in black rot of crucifers. Phytopathology 71:1215–1220.

Shaw, J.J. and C.I. Kado. 1988. Whole plant wound inoculation for consistent reproduction of black rot of crucifers. Phytopathology 78:981–986.

Shelton, A.M. and J.E. Hunter. 1985. Evaluation of the potential of the flea bettle *Phyllotreta cruciferae* to transmit *Xanthomonas campestris* pv. *campestris,* casual agent of black rot of crucifers. Can. J. Plant Path. 7:308-310.

Staub, T. and P. H. Williams. 1972. Factors influencing black rot lesion development in resistant and susceptible cabbage. Phytopath. 62:722-728.

Tonguc, M., E. Earle and P. D. Griffiths. 2003. Segregation distortion of *Brassica carinata* derived black rot resistance in *Brassica oleracea*. Euphytica 134:269-276.

Tonguc, M. and P. D. Griffiths. 2004a. Evaluation of *Brassica carinata* accessions for resistance to black rot (*Xanthomonas campestris* pv. *campestris*). HortScience 39(5):952–954.

Tonguc, M. and P. D. Griffiths. 2004b. Development of black rot resistant interspecific hybrids between *B. oleracea* L. cultivars and A 19182. Euphytica (in press).

Williams, P.H., T. Staub, and J.C. Sutton. 1972. Inheritance of resistance in cabbage to black rot. Phytopathology 62:247–252.

Williams, P.H. 1980. Black rot: A continuing threat to world crucifers. Plant Dis. 64:736–742.