"Final Project Report to the NYS IPM Program, Agricultural IPM 2003-2004"

Title: **Breeding Cabbage for Resistance to Black Rot (*Xanthomonas campestris*).**

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Cooperator(s): **Lisa Earle**

Type of grant: **Pest-resistant crops; allelopaths**

Project location(s): **All of NY**

Abstract:

Fresh-market cabbage is the highest value vegetable crop in NY State. Black rot is a serious disease of cabbage especially during warm, damp seasons in the northeast. Breeding for improved resistance to black rot will be important for the future protection of this crop in NY State, through development of new varieties with enhanced resistance to this disease.

Background and Justification:

Black rot is a serious disease of cole crops (including cabbage, broccoli and cauliflower) and is easily spread from contaminated seeds in nurseries, and through mechanical transmission in the fields. Symptoms of the disease include V-shaped lesions originating from the margin, and as the lesions enlarge the plant wilts and eventually rots. The most effective approaches to controlling black rot are through good farm management practices, hot water treatment of seeds and the use of cultivars with resistance to the disease. Current sources of host plant resistance while partially effective, are not complete, are difficult to breed into varieties (multiple genes) and still result in spread of the disease throughout plantings. Incorporation of more effective resistance to black rot in cole crops will benefit growers economically, and environmentally, by reducing the need for chemical management, especially as it has limited effect on this disease.

Some cabbage varieties have limited resistance to black rot, but this is inadequate, particularly when there is high disease pressure. The major races to black rot are race 1 and race 4 which account for approximately 95% of the pathogen worldwide and 100% of NY isolates. Complete resistance to race 1 has not been found in *B. oleracea*, however, resistance to races 1 and 4 (apparently race non-specific resistance) has been identified in some of the mustard species (*B. carinata*, *B. nigra* and *B. juncea*). A source of resistance identified in Ethiopian mustard (*B. carinata* PI 199947) has been introgressed to broccoli using protoplast fusion and backcrossing. This source gives the plant complete resistance at the juvenile and mature stages in greenhouse trials, and has been been introgressed to broccoli by Lisa Earle using PI 199947. These lines have been used to derive breeding lines for evaluation of black rot resistance.

Crosses of this material were been made to cabbage, cauliflower, broccoli and Brussel's sprouts between 2000-2002 and the germplasm was advanced and field tested during the summers of 2001 and 2002. The resistance is superior to that available in any current germplasm and if successfully incorporated could fulfill a great need by the seed industry (where seed-borne contamination is a problem) and the NY growing community. Considerable greenhouse and field screening of breeding lines will enable introgression of this resistance to commercial types, and evaluation of the material relative to resistance derived from *B. oleracea*.

Additional embryo rescue work has resulted in interspecific crosses between *B. oleracea* and an additional black rot resistant Ethiopian mustard (A-19182). Two backcross plants have been recovered to date that could provide additional sources of germplasm for developing black rot resistant cabbages.

Objectives:

[1] To evaluate black rot breeding lines for resistance derived from *B. carinata* and *B. oleracea*.

[2] To develop backcross populations from A-19182 derived interspecific hybrids and evaluate for resistance to black rot.

[3] To self-fertilize all selections from the 2003 trials, and make crosses of the most resistant material to cabbage, broccoli and cauliflower.

Procedures:

[1] Approximately 150 breeding lines will be evaluated with controls in replicated plots during 2003. The lines will represent populations advance to the F_4 generation and beyond, and new crosses made with resistant material. The seed will be planted in 128-cell speedling trays, grown to the 3-week stage and hardened in coldframes for planting. Four replicated plots with 10 plants per plot will be transplanted to the field with 18" spacing between plants and 3' spacing between rows. Plants will be spray inoculated with a backpack mist-

blower at 6-weeks and 8-weeks with a slurry of four NY isolates of black rot grown for 4-days on YDCP. An overhead irrigation sprinkler plot will be set up to maintain high humidity and moisture content in the field that is conducive to pathogen spread. Plants will be evaluated for disease severity at 10 and 12-weeks after planting, relative to susceptible and tolerant resistant control varieties. Field plots will be grown at Geneva with isolation to prevent any risk of spread to neighboring trials or farms. Plants will be evaluated for black rot damage using a scale of 0-5, where 0= completely resistant, and 5= completely susceptible. Comparisons between resistant lines derived from B. carinata will be contrasted to those with B. oleracea derived resistance. Selections from the most resistant lines will be removed from the field, placed in 10" pots and transferred to vernalization chambers for 3-months to induce flowering. An additional trial will contrast the resistance response to needle and backpack inoculation approaches on susceptible and resistant plants in field and greenhouse trials.

[2] Twenty-six interspecific hybrids between black rot resistant *B. carinata* accession A-19182 and *B. oleracea* (broccoli and cabbage) have been generated by rescuing immature siliques prior to abortion and culturing them for 10-14 days. These siliques were then opened and embryos were rescued on sterile Murash and Skoog media. These interspecific hybrids will be used for additional crosses with *B. oleracea* to develop backcross populations for development of black rot resistant germplasm.

[3] Standard breeding methodologies will be employed to advance germplasm and make crosses to commercial material for further testing. Plants will be removed from vernalization in December, cut back and encouraged to flower at 60°C. The flowers are largely self-incompatible, this will be overcome by self pollinating unopened flowers with mature pollen from mature flowers of the same plant. Crosses will also be made by using flowers from one plant to pollinate unfertilized flowers on another. Seed will be collected and cleaned for further field and greenhouse testing in 2004.

Results and discussion:

[1] A total of 180 breeding lines and 12 control varieties/accessions were seeded to screen for black rot resistance in field plots during 2003. Breeding lines were transplanted to the field in 4 replicated plots in June 2003, and were inoculated twice at 21 and 42 days after transplanting using a backpack mist blower. Field plots were irrigated prior to inoculation late afternoon to create high turgor pressure in plants facilitating inoculation. Plants were irrigated daily for seven days after each inoculation using an overhead sprinkler system. Control varieties were used (cabbage, cauliflower and broccoli) representing susceptible and tolerant varietal controls. A total of 91 selections were made for self-pollination and crossing.

Commercial varieties were also evaluated for black rot resistance to identify the most promising cultivars currently on the market for reducing black

rot damage. These varieties were evaluated using four inoculation procedures (juvenile wound, juvenile spray, mature wound and mature spray). The genotype that ranked highest overall was the Wisconsin line Badger #16 developed by Paul Williams at the University of Wisconsin; however, it did not perform significantly better than the lines 'Cornell 102' and PI 436606, and was outperformed in field spray trials by Silver Dynasty (Seminis Vegetable Seeds). All breeding lines had lower disease severity ratings than the commercial varieties except for CXB933256, and the most resistant commercial variety was Silver Dynasty in these trials (Table 1 and Table 2). Tenacity and Matsumo had significantly lower disease symptoms than all 10 'susceptible' varieties except for Bobcat. Ten susceptible varieties of cabbage (Atria, Azan, Bobcat, Fresco, Genesee, King Cole, Morris, Rinda, Superdane, Transam), 3 black rot tolerant varieties (Silver Dynasty, Matsumo, Tenacity) and 6 breeding lines/accessions (Badger # 16, Cornell 101, Cornell 102, NY 4002, CXB93256, PI 436606) were evaluated for black rot resistance in greenhouse and field experiments during 2003. Plants were evaluated in four trials with four replications in each trial.

Table 1: Mean disease severity ratings of juvenile and mature cabbage genotypes inoculated using the wound and spray procedures.

Genotype	No. Tested	Juv Wound	Juv. Spray	Mat. Wound	Mat. 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
CXB93256	136	3.74 b-f	3.28 de	1.40 ij	2.42 e	2.63 f
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 separation according to Duncan's multiple range test ($p \leq 0.05$).

Differences were observed between the inoculation procedures (Table 3) when compared using the Spearman rank correlation. Genotypes including Silver Dynasty performed less well, when challenged with the wound inoculation procedure, ranking 15th in the juvenile wound test, but highest in the field mature spray test. These results suggest that the juvenile wound approach, may not correlate accurately with field resistance in cabbage and that the juvenile

spray inoculation may be a more accurate juvenile test of *B. oleracea* (cabbage) resistance to black rot. All genotypes in these inoculation screens showed symptoms of black rot, the resistance for which is controlled by multiple genes in *B. oleracea* and the genotypes evaluated in this study. While commercial varieties are being developed with increased resistance to black rot, the genetic control of the resistance is incomplete, quantitative and difficult to work with. Alternative approaches to introgressing black rot resistance into cabbage varieties are being concentrated on the use of black rot resistance identified in related mustard species (Indian mustard and Ethiopian mustard). Interspecific crosses made between cabbage and these resistant sources can be used to incorporate new sources of resistance to black rot that are superior and easier to work with than current *B. oleracea* germplasm.

Table 2: Rankings of cabbage genotypes in four inoculation trials.

Genotype	Juv. Wound	Juv. Spray	Mat. Wound	Mat. Spray	Mean Rank
Badger	2	2	2	2	1
PI436606	1	1	1	8	2
Cornell 102	3	3	4	9	3
Cornell 101	8	4	5	5	4
NY4002	9	4	8	3	5
Silver Dynasty	15	7	6	1	6
Tenacity	10	8	9	4	7
Bobcat	6	6	10	11	8
Matsumo	18	9	7	6	9
CXB93256	13	11	3	10	11
Fresco	17	10	12	13	12
Azan	5	14	15	16	14
Transam	11	18	16	14	15
Atria	6	17	14	17	15
Genesee	16	16	18	15	17
Rinda	19	15	17	18	18
Superdane	14	20	21	21	19
Morris	21	21	19	19	20
King Cole	20	19	20	20	21

Table 3: Comparison of inoculation techniques 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

[2] *B. oleracea* does not have complete resistance to the major races of black rot (race 1 and race 4), and the incorporation of incomplete resistance into varieties is complicated due to multiple gene control of resistance. The mustard species (*B. carinata*, *B. nigra* and *B. juncea*) have been associated with race non-specific resistance with single gene control. New sources (A 19182 and A 19183) have been investigated to introgress the gene into *B. oleracea* and develop breeding lines that do not exhibit stability problems previously documented in B. carinata PI 199947 deriived sources. Accessions A19182 and A19183 were identified as immune to race 1 and race 4 NY isolates of black rot (determined through differential cultivar screening). A modified embryo rescue procedure was used to culture hybrid siliques between A19182/A19183 and *B. oleracea*, from which embryo rescued hybrids were obtained (30 in total, all from A19182). A19182 failed to cross with *B. carinata*, suggesting incorrect classification. Morphological/molecular work suggests that this accession is in fact *B. juncea*. Resistant backcross plants have since been recovered that show complete resistance to races 1 and 4 (Table 4). Additional crosses of the interspecific hybrids have been recovered with *B. napus*, to utilize an additional bridging approach.

Table 4: Backcrosses and bridge crosses made with *B. oleracea*, A 19182 and *B. napus*.

Cross combinations	Backcross designations	No of ovaries cultured	No of plants obtained
$F_{1\text{-}1}$ xMajor	$BC_{1\text{-}1}$ $BC_{1\text{-}2}$	747	2
$F_{1\text{-}2}$ x Captain	$BC_{1\text{-}3}$ $BC_{1\text{-}4}$	587	2
$F_{1\text{-}3}$ x A 19182	$BC_{1\text{-}5R}$	23	1
(Atlantis x A 19182) x PI 458949	-	17	1
(A19182 x Titleist) x PI 458949	-	13	2

[3] All selections of black rot resistant material were removed from the field and transplanted to 10" pots. Cabbage selections were moved to cold storage for 3 months to overcome the biennial life cycle of cabbage through vernalization. Plants were placed in greenhouses, and are being self-pollinated and crossed to advance breeding lines and create new populations segregating black rot resistance that can be used for future varietal production.