Selection for Yellow Clover Aphid and Pea Aphid Resistance in Red Clover

H. J. Gorz

G. R. Manglitz

Francis A. Haskins
University of Nebraska - Lincoln, fhaskins@neb.rr.com

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ABSTRACT

Most red clover (*Trifolium pratense* L.) cultivars are susceptible to attack by the yellow clover aphid (*Therioaphis trifolii* Monell) and the pea aphid (*Acyrthosiphon pisum* Harris). Starting with 27 yellow clover aphid-resistant plants selected from a wide diversity of germplasm, phenotypic recurrent selection was used to improve resistance to the two aphids. Through five cycles of testing and selection for yellow clover aphid resistance and three such cycles for pea aphid resistance, a synthetic, 'N-2', was developed that had a high level of resistance to both aphids.

***Additional index words:* *Therioaphis trifolii* Monell, *Acyrthosiphon pisum* Harris, *Trifolium pratense* L., Insect resistance, phenotypic recurrent selection.**

PEA aphids (*Acyrthosiphon pisum* Harris) and yellow clover aphids (*Therioaphis trifolii* Monell) can severely damage red clover (*Trifolium pratense* L.). The pea aphid is considered to be the more serious pest. Heavy concentrations of pea aphids on the leaves and stems of terminal shoots may seriously injure red clover plants by feeding, but the aphids may be even more destructive as vectors of virus diseases (3). Manglitz and Kreitlow (6) reported that alfalfa mosaic virus was transmitted from infected to virus-free plants of Ladino white clover (*Trifolium repens* L.) by both of the above aphids as well as the clover aphid (*Nearctaphis bakeri* Cowen), but only the pea aphid and clover aphid transmitted bean yellow mosaic virus. Thus, the role of aphids in the spread of viruses infecting clovers was demonstrated.

The yellow clover aphid (YCA) is quite similar in appearance to the spotted alfalfa aphid (*Therioaphis maculata* Buckton), and its damage to red clover resembles the damage to alfalfa (*Medicago sativa* L.) caused by the spotted alfalfa aphid (8, 9). Hosts of the YCA are restricted primarily to the genus *Trifolium* (8, 9). No reports of research on resistance to the YCA were found in the literature other than a brief mention (5) of the work on which this paper is based.

Resistance to the pea aphid (PA) in red clover was observed by Cooper (1) who found that certain strains of red clover survived and yielded better than other strains under PA infestation. Jewett (4) reported that no red clover cultivar was resistant in his tests, but he indicated that many individual plants appeared to be resistant. Wilcoxson and Peterson (10) found that 'Dollard' red clover was much more resistant to the PA than the cultivar 'Wegener', and Dollard also had a much lower incidence of mosaic and pea stunt viruses. When mechanically inoculated, both cultivars were equally susceptible to the viruses. The authors concluded that breeding for aphid resistance might be an effective way to control viruses. El-Kandelgy and Wilcoxson (2) demonstrated that the PA also transmits red clover vein-mosaic virus. They also found some aphid-resistant plants in the cultivar 'Lakeland'.

This study was undertaken to determine the ocurrence of resistance to the PA and YCA in red clover cultivars and strains and to evaluate the extent to which resistance could be increased by phenotypic recurrent selection. Information obtained was utilized in the development of a red clover synthetic possessing a high level of combined resistance to both aphids.

MATERIALS AND METHODS

Thirty-five red clover germplasm sources (Table 1) representing all available named cultivars, improved strains from breeding programs, and a few plant introductions were assembled for initial evaluations of resistance. The three entries listed as Early, Medium, and Late Flowering Beltsville Synthetics were broad-based sources derived by bulking seed from a large introduction nursery. Several other entries also were synthetics or bulks with broad genetic bases, and the named cultivars represented a wide range of origin and adaptation. Thus, a wide range of germplasm was represented in this initial evaluation.

In the initial screening, 34 entries were evaluated for resistance to the YCA and 32 for resistance to the PA (Table 1). All tests were conducted by mass-infesting young seedlings grown in a greenhouse maintained at approximately 21 C with an 18-hour photoperiod. Planting was done in wood flats with 12 rows/flat and 35 to 50 seeds/row. Single rows of the entries were replicated three or four times in a randomized, complete block design. This procedure was varied in the initial evaluation for PA resistance in which only two replications were used, and in the initial evaluation for YCA resistance in which six replications were used with 100 seeds/row. Six replications also were used in the final evaluation for both aphids. Following the initial evaluation for YCA resistance, the cultivars 'Tensas' and 'Alaskland' were included in each flat as susceptible checks. Seed was planted in trenches 0.64 cm deep in a 3:1:1 mixture of soil, sand, and peat moss, and covered with a 1:1 mixture of fungicide-treated silica sand (0.11 g Orthocide[3]/liter of sand)

[1] Contribution from the USDA-SEA, AR, and the Nebraska Agric. Exp. Stn., Lincoln. Published as Paper No. 5521, Journal Series, Nebraska Agric. Exp. Stn. The work reported was conducted under Nebraska Agric. Exp. Stn. Projects 12-27, 12-88, and 17-27. Received 7 Aug. 1978.

[2] Supervisory research geneticist and research entomologist, USDA-SEA, AR, and foundation professor of agronomy, Univ. of Nebraska, Lincoln, NE 68583, respectively.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or the Univ. of Nebraska and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Initial evaluation of seedlings of red clover cultivars and strains under heavy aphid infestations of yellow clover aphids (one test with six replications, winter, 1968–69) and pea aphids (one test with two replications, winter, 1970–71).

	Yellow clover aphid			Pea aphid	
	Surviving plants	Resistant plants†	No. of selected plants	Surviving plants	Resistant plants†
	%			%	
Chesapeake, F.C. 39,731	49.8 a*	2.0	9	81.4 a*	34.7
15 Clone Syn, Beltsville	22.7 b	0.3	1	26.8 bc	0.0
Illinois No. 2	22.0 b	1.7	3	26.1 bc	1.4
Lakeland, F.C. 38,914	20.8 bc	0.3	1	3.8 c	0.0
Kenland, F.C. 38,956	15.6 bcd	0.4	1	11.0 c	2.0
Illinois No. 1	15.0 bcde	0.3	1	28.1 bc	0.5
P.I. 233828 (Italy)	14.9 bcde	0.0	0	9.5 c	1.0
Md. Sel. 67-A2	14.6 bcde	1.4	1	40.0 bc	0.0
Wis. Syn H	13.5 bcde	0.0	0	8.3 c	0.0
Wis. Syn F	13.3 bcde	1.4	1	–	–
P.I. Bulk, Beltsville	12.5 bcde	0.0	0	–	–
Wis. Syn C305	11.5 bcde	0.0	0	20.8 bc	0.0
Wis. Syn D2	11.0 bcde	0.7	2	6.7 c	0.0
Wis. Syn C306	9.0 bcde	0.5	1	5.6 c	0.0
Pennscott, F.C. 39,393	8.8 bcde	0.0	0	21.3 bc	9.2
Early Flw. Syn, Beltsville	8.8 bcde	1.0	4	9.5 c	0.0
Med. Flw. Syn, Beltsville	7.5 cde	0.8	1	8.8 c	0.0
Nolin's Red, F.C. 38,903	7.2 cde	0.0	0	0.0 c	0.0
Wis. Syn C2	6.5 de	0.0	0	13.1 c	0.0
Wis. Syn C305	6.0 de	0.0	0	55.0 ab	0.0
Late Flw Syn, Beltsville	5.9 de	0.2	0	9.6 c	0.8
Altaswede, F.C. 38,375	5.5 de	0.0	0	–	–
Mammoth, F.C. 38,949	5.4 de	0.7	0	5.6 c	0.0
P.I. 204508 (Turkey)	5.4 de	0.0	0	4.1 c	1.2
Minn. Common, F.C. 39,444	5.4 de	0.4	1	3.4 c	0.0
LaSalle, F.C. 39,494	4.5 de	0.0	0	19.8 bc	0.0
Ky. Syn A-2, F.C.39,819	3.8 de	0.0	0	29.3 bc	0.9
N1-17-1(64), F.C. 39,495	3.7 de	0.0	0	19.4 bc	0.0
Midland, F.C. 37,796	3.7 de	0.0	0	6.7 c	0.0
Ky. Syn A-3, F.C. 39,818	3.3 de	0.0	0	24.3 bc	0.4
Dollard, F.C. 39,394	2.8 de	0.0	0	9.0 c	0.0
Tensas, F.C. 38,919	1.6 de	0.0	0	0.0 c	0.0
Orbit, F.C. 38,909	1.3 de	0.0	0	2.0 c	0.0
Alaskland, F.C. 38,084	0.7 e	0.0	0	0.0 c	0.0
Michigan Syn	–	–		13.2 c	0.0

* Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
† Data not analyzed statistically because of very small numbers of resistant plants.

and finely pulverized peat moss. Flats were covered for about 4 days after planting with clear plastic sheets. When seedling emergence was complete, a count was made of the number of seedlings in each row.

Except as otherwise indicated, aphid cultures were established each season from collections made in red clover fields located in eastern Nebraska. Collected aphids were first cultured in growth chambers to eliminate parasites and other aphid species. Mass cultures were then established in the greenhouse on susceptible red clover plants. Seedlings at the unifoliolate leaf stage were infested by shaking large numbers of aphids uniformly over the plants in each flat. Additional aphids were added as necessary to maintain a high population. In most tests, aphid infestation was continued for approximately 2 months, although the time varied somewhat depending upon the vigor of the aphid culture and the level of resistance in the germplasm being screened.

Screening tests were terminated when differences between resistant and susceptible plants were considered to be at a maximum. Each plant was assigned a damage rating based on a scale from 1 to 4 as follows: 1—highly resistant, no obvious aphid injury; 2—moderately resistant, somewhat stunted with leaves smaller and lighter green than in Class 1; 3—susceptible, extreme stunting with very small, light green or yellowish leaves, but still alive; 4—highly susceptible, dead. Mean damage ratings were calculated from the number of seedlings observed in each rating class. Calculations of percent resistant were based on the relative number of plants receiving a score of 1 or 2, and percent survival was based on the relative number of plants in Classes 1, 2, and 3. Only plants in Class 1 were retained for propagation or for additional screening with other aphids. Plants to be rescreened were cut back, fumigated, and reinfested with the appropriate aphids as new leaves emerged. Resistant plants selected for propagation were individually transplanted to 10.2-cm clay pots and intercrossed at random by honey bees in a greenhouse cage, or were transplanted to an isolated plot on the University of Nebraska campus. Open-pollinated seed was harvested from individual plants.

A flow chart summary of the various steps involved in the selection and testing procedures is shown in Fig. 1.

Table 2. Progress in selecting for yellow clover aphid and pea aphid resistance in red clover.

Cycle	Accessions screened	Total plants	Resistant plants	Surviving plants	Damage rating, mean
			%		
Yellow clover aphid					
0—Initial evaluation	34	10.885	0.3	10.4	3.89†
1—1st progeny test	27	2,551	19.5	21.3	–
2—2nd progeny test	44	3,261	57.2	77.6	2.43
3—3rd progeny test‡	66	744	–	–	–
4—4th progeny test	124	1,644	88.6	94.4	1.95
5—5th progeny test	1	300	95.6	98.7	1.25
Pea aphid					
0—1st screening	44	764	27.5	53.0	3.09
1—1st progeny test	66	2,339	75.6	85.4	2.18
2—2nd progeny test	124	8,083	60.1	82.8	2.37
3—3rd progeny test	1	354	93.7	100.0	1.41

† Damage ratings were not made during the initial evaluation with YCA but an estimated damage rating was calculated.
‡ Resistant plants selected but readings not made.

RESULTS AND DISCUSSION

The level of resistance to both the YCA and PA was generally low in the 35 sources of red clover germplasm used in the initial screening tests (Table 1). In most cases, the percentage of plants surviving the initial YCA infestation was lower than the percentage surviving the initial PA test. No more than 2% of the plants of any entry were classified as resistant to the YCA, but two entries exceeded this level of resistance to the PA by a considerable amount. The true difference in reaction to the two aphids was actually greater than shown in Table 1 because the YCA test was terminated by fumigation when most of the initial stand of plants had been killed by the aphids. Fumigation was necessary to preserve a small proportion of surviving plants, since all plants had been killed in a preliminary YCA test that was not terminated by fumigation. The cultivar 'Chesapeake' had a higher proportion of surviving and resistant plants in the tests with both aphids than any other entry in the test. Four other sources of red clover germplasm yielded some resistant plants in tests with both aphids, while other sources had resistance to only one of the two aphids. Damage ratings for the initial screening with YCA were not recorded. Although not shown in Table 1, such ratings were recorded for the PA test. For the 32 entries in this test, the correlation of mean rating score with percentage of surviving plants was −0.96 (significant at $P = 0.01$).

In the initial screening for YCA resistance, 27 plants were selected for progeny testing. As shown in Table

Year	Yellow clover aphid (YCA)	Pea aphid (PA)
68-69	Initial evaluation (see Table 1): 27 YCA-resistant plants selected and open-pollinated (OP) seed produced.	
69-70	First progeny test: 44 YCA-resistant plants selected and OP seed produced.	
70-71	Second progeny test: 764 YCA-resistant plants selected, cut back and fumigated	Initial evaluation (see Table 1): No germplasm from this test was used in development of the resistant synthetic, N-1. Initial screening of YCA-resistant plants: Regrowth from the 764 YCA-resistant plants infested, with PA; 66 YCA- and PA-resistant plants selected and OP seed produced.
71-72	Third progeny test: Regrowth from 744 PA-resistant plants infested with YCA; 138 PA- and YCA-resistant plants selected and OP seed produced.	First progeny test: 744 PA-resistant plants selected, cut back and fumigated.
72-73	Fourth progeny test: Regrowth from 1641 PA-resistant plants infested with YCA; 322 plants highly resistant to PA and YCA selected and 50 OP seeds from each plant bulked to form a resistant composite.	Second progeny test: 1641 PA-resistant plants selected, cut back and fumigated.
73-74	Fifth progeny test of seedlings (see Table 3): 5 samples of seed from the resistant composite were compared with parental strains: No plants selected for seed production. Fifth progeny test: Regrowth from 309 PA-resistant plants infested with YCA; 198 plants highly resistant to YCA and PA selected and OP seed produced in 1974 and 1975 was bulked and designated as red clover synthetic 'N-2'.	Third progeny test (see Table 3): 5 samples of seed from the resistant composite were compared with parental strains: 309 PA-resistant plants were selected from the resistant composite, cut back and fumigated.

Fig. 1. Flow chart of steps in the development of a red clover synthetic highly resistant to both the yellow clover aphid and pea aphid.

1, 9 of these plants were from the Chesapeake entry, 4 were from the 'Beltsville Early Flowering Synthetic', 3 were from 'Illinois No. 2', and the remaining 11 plants were from 10 other entries.

A summary of the progress achieved in selection for combined resistance to the YCA and PA in red clover is shown in Table 2. The percentage of plants resistant to the YCA ranged from 0.3% in the initial population to 95.6% in the fifth progeny test. Percent survival and damage rating also demonstrated substantial progress in the incorporation of YCA resistance in each succeeding cycle of selection. Good progress also was achieved in improving the level of resistance to the PA, but progress was less uniform and did not cover as broad a range. It should be pointed out that the initial evaluation results for PA resistance (Table 1) cannot be compared directly with the first PA screening results shown in Table 2 because the conditions of the two tests differed markedly. For example, the PA tests of Table 1 involved 32 red clover entries, but in Table 2 the first screening test for PA resistance involved 764 YCA-resistant plants from the second progeny test for YCA resistance (Fig. 1). The YCA-resistant plants were cut back, fumigated, and the regrowth was infested with pea aphids. Thus, the plant material in the first PA screening test represented a greatly restricted group of red clover germplasm that already contained a high level of resistance to the YCA.

Although overall progress in incorporating PA resistance was excellent, no progress was made in the second cycle of selection (Table 2). The difference in progress between cycles may be related to differences in pea aphid biotypes used in the various screening tests. Some evidence supporting this hypothesis was obtained in a sequel to the initial evaluation for PA resistance. In the initial test, a mixed culture of PA collected from alfalfa, red clover, and sweetclover (*Melilotus* spp.) was propagated on broadbeans (*Vicia faba* L.) before infestation of the red clover seedlings. Large differences in resistance were observed in this test. In the sequel (unpublished data), a PA culture collected from red clover plants in eastern Nebraska and reared on susceptible red clover plants was used to compare the resistance of open-pollinated progeny of resistant plants from the initial evaluation with the

Table 3. Comparison of a YCA- and PA-resistant composite with the unselected parental cultivars and strains from which the composite was derived.

Cultivar or strain	Yellow clover aphid* Damage rating, mean	Yellow clover aphid* Surviving plants	Yellow clover aphid* Resistant plants	Pea aphid* Damage rating, mean	Pea aphid* Surviving plants	Pea aphid* Resistant plants
		%	%		%	%
Res. composite (no. 1)†	1.22 c*	98.6 a	95.6 a	1.52 c*	100.0 a	91.2 ab
Res. composite (no. 2)	1.27 c	100.0 a	97.6 a	1.37 c	100.0 a	94.4 a
Res. composite (no. 3)	1.16 c	98.6 a	97.1 a	1.34 c	100.0 a	94.4 a
Res. composite (no. 4)	1.29 c	96.3 a	94.5 a	1.45 c	100.0 a	93.9 a
Res. composite (no. 5)	1.36 c	100.0 a	93.4 a	1.39 c	100.0 a	94.4 a
Chesapeake	3.03 b	70.9 b	23.9 b	1.43 c	100.0 a	91.0 ab
Illinois no. 1	3.87 a	10.0 d	0.0 c	1.96 b	100.0 a	85.0 ab
Illinois no. 2	3.86 a	14.3 d	0.0 c	2.18 ab	100.0 a	76.2 abc
Early Flw Syn, Beltsville	3.98 a	3.9 d	1.9 c	2.24 ab	100.0 a	60.0 cd
Med. Flw Syn, Beltsville	3.93 a	7.0 d	0.0 c	2.13 ab	100.0 a	71.5 abcd
Alaskland (susc. check)	3.61 a	38.8 c	0.0 c	2.51 a	88.9 a	50.3 d
Tensas (susc. check)	3.92 a	8.6 d	0.0 c	2.34 ab	97.2 a	68.5 bcd

* Means, within a column, followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
† The numbers represent samples of seed drawn from a single composite made by bulking open-pollinated seed from 322 plants resistant to both the YCA and PA.

resistance of unselected cultivars and strains. No increase in PA resistance was observed in the progeny plants. Thus, use of the mixed culture of aphids propagated on broadbeans was apparently ineffective in screening out and identifying genotypes resistant to PA collected from and reared on red clover. Additional evidence on differences in PA biotypes was obtained by Neiman (7), but no evidence of similar biotype differences was apparent in our work with the YCA.

Following the fourth progeny test for YCA resistance and the second for PA resistance, a "resistant composite" was formed that consisted of 50 open-pollinated seeds from each of 322 plants (Fig. 1). Each of the 27 plants (representing 13 entries) initially selected for YCA resistance (Table 1) contributed germplasm to these 322 plants. However, only six maternal sources were represented in the pedigrees of the 322 plants. These sources and the percentages of the 322 plants each contributed were as follows: Chesapeake, 63%; Beltsville Early Flowering Synthetic, 15%; Maryland Selection 67-A2, 10%; Illinois No. 2, 8%; Beltsville Medium Flowering Synthetic, 3%; and Illinois No. 1, 1%. Levels of YCA and PA resistance of plants grown from five samples of seed drawn from the resistant composite were compared with resistance levels exhibited by five of the six maternal sources (Table 3). Seed of the sixth line (Maryland Selection 67-A2) was not available for this comparison. The level of resistance to the YCA was substantially greater in the resistant composite than in any of the original germplasm sources included in this test. Differences in PA resistance were less striking, probably because the PA culture used was weaker than usual. Nevertheless, as shown in the damage rating and resistant plant columns of Table 3, significant differences in PA resistance were observed.

As shown in Fig. 1, the YCA and PA-resistant synthetic, 'N-2', resulted from subjecting the resistant composite to one added cycle of testing and selection. The synthetic is based on 198 plants selected from this final cycle for high resistance to both aphids.

In this report, rapid progress was demonstrated in the development of an aphid-resistant red clover cultivar by the use of phenotypic recurrent selection. Insect-resistant cultivars provide the ideal solution for control of insects because such control is effective and economical, it avoids insecticide hazards, and the protection often lasts for many years. In a forage crop such as red clover, the insect-resistant cultivars are especially appropriate and promising because of the comparatively low acreage value of the crop, the relatively high cost of chemical applications, and the possibility of forage contaminated with insecticide residues. In addition, aphid resistance may reduce infection by viruses, as demonstrated in the previously cited study by Wilcoxson and Peterson (10).

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