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# Insect Transmission of Red Clover Vein-Mosaic Virus and Resistance of Clovers to Aphids<sup>1</sup>

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The pea aphid, *Acyrtosiphon pisum* (Harris), transmitted red clover vein-mosaic virus (RCVMV) after an acquisition feeding of one half minute and an inoculation feeding of two minutes. The clover aphid, *Anuraphis bakeri* (Cowen) failed to transmit RCVMV. Dollard, Lakeland and Wegener red clover varieties contained plants that were resistant or susceptible to the pea aphid. Dollard and Lakeland were more resistant to the pea aphid than Wegener. Resistance was due to non-attractiveness and antibiosis to the pea aphid.

Red clover vein-mosaic virus (RCVMV), described by Osborn (1937), is identical with the "Wisconsin pea stunt" virus described by Hagedorn and Walker (1949), according to Hagedorn and Hanson, (1951). It occurs in midwestern United States (Goth and Wilcoxson, 1962; Graves and Hagedorn, 1956) in peas and clovers, in Finland and Russia in red clover (Hagedorn et al, 1959; Jamalainan, 1957), and in the Netherlands, Switzerland, England, Sweden, Western Germany and Poland, in peas and red clover (Hagedorn et al, 1959).

The most conspicuous symptom of RCVMV infection in red clover is chlorosis along veins of leaflets. Leaflet curling and stunting have been reported on diseased Alsike clover (El-Kandelgy, 1964) and crimson clover (Hagedorn et al, 1959; Osborn, 1937). Diseased peas also become chlorotic along the veins of leaflets but, more particularly, the diseased plants are stunted, with the apical foliar and flower tissues malformed and congested into a terminal rosette (Hagedorn et al, 1959). All the symptoms mentioned are characteristic of the disease and make field diagnosis of the virus in clover and peas fairly certain.

The development of RCVMV resistant varieties appears to be the most feasible way of controlling the virus. The pea aphid, *Acyrtosiphon pisum* (Harris) = *Macrosiphum pisi* (Harris), carries RCVMV from clover to pea and vice versa each year, and so development of varieties that are either resistant to the virus or the vector would aid greatly in control of the virus. Wilcoxson and Peterson (1960) reported that Dollard red clover is more resistant to the pea aphid than Wegener. Their observations also indicated that Dollard was less likely

to become infected with the virus complex present in Minnesota than was Wegener.

Hagedorn et al (1959) reported that the pea aphid can act as a vector after it has acquired RCVMV for 1, 3 and 6 days, and inoculated healthy pea plants for 5, 10, 15 and 30 min. The pea aphid was also reported to acquire and transmit RCVMV after two hours on a diseased host but the virus was lost after the aphids fed a day on healthy clover (Osborn, 1937). This study was made to (a) determine the minimum length of time needed for the acquisition and inoculation feedings for the pea aphid and the clover aphid *Anuraphis bakeri* (Cowen) to transmit RCVMV from diseased clover to healthy Dollard and Wegener red clover plants and, (b) study the aphid resistance of Dollard, Lakeland and Wegener red clover.

## Methods and Results

### Transmission of RCVMV with *A. bakeri* and *A. pisum*

In order to determine the minimum length of time needed for acquisition and inoculation feedings for the pea aphid and clover aphid to transmit RCVMV from diseased clover to healthy Dollard and Wegener red clover, the following procedure was initiated.

A Wegener red clover plant with typical symptoms of red clover vein-mosaic was used as a source of the virus.

Populations of both aphids were started from single adult aphids, the pea aphid on virus-free broad beans, *Vicia faba* L., and the clover aphid on virus-free Wegener red clover.

Dollard and Wegener red clover plants were used as test plants. They were one month old and growing vigorously.

Acquisition feedings were ½, 1, 5, 20, 35 and 60 minutes for both aphid species. Inoculation feedings of one and two minutes followed each acquisition feed. The feedings were timed with a stop watch.

In each acquisition feeding, ten adults of the pea aphid were individually transferred with sterilized moist toothpicks to a trifoliolate leaf of the virus source plant. After the aphids had fed for a particular period of time on the source plant, half (five aphids) were transferred individually to the trifoliolate leaves of five test plants of

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either red clover variety (one aphid per plant) to feed for one minute. The other five aphids were transferred to trifoliolate leaves of another set of five test plants (one aphid per plant), where they were allowed to feed for two minutes. After the aphids had fed on healthy plants they were removed. The same procedure was applied with the clover aphid. Controls were handled in the same manner, except that the aphids were fed on a healthy Wegener red clover plant.

All plants were kept under glass lamp chimneys in a greenhouse at about 24°C. They were observed for one and one half months after inoculation. The success of transmission was judged from symptom development and by indexing on *Gomphrena Globosa* L. Symptoms were first observed five to six weeks after inoculation.

<sup>1</sup>TABLE 1. Insect transmission of RCVMV by adult pea aphid on Dollard and Wegener red clover.

Acquisition feeding in minutes	Inoculation feeding in minutes	Dollard		Wegener	
		No. plants infected <sup>a</sup>	No. plants tested	No. plants infected	No. plants tested
½	1	0/5		0/5	
	2	2/5		1/5	
1	1	0/5		0/5	
	2	2/5		1/5	
5	1	1/5		1/5	
	2	1/5		2/5	
20	1	3/5		2/5	
	2	2/5		0/5	
35	1	4/5		1/5	
	2	2/5		0/5	
60	1	1/5		0/5	
	2	0/5		0/5	

Control=0 transmission

<sup>a</sup>The fractions also indicate the number of pea aphids tested and the number that transmitted RCVMV.

Data in Table 1 indicate that the pea aphid can act as a vector. It is difficult to say which combination of acquisition and inoculation feeding was most effective for obtaining infection because infection resulted from each combination except the extremes. It will require more experiments to determine the optimum feeding times. In this test more Dollard than Wegener plants were infected. This aphid can transmit the virus after a one half minute acquisition feed and a two minute inoculation feed and, further, it can also transmit after a 60 minute acquisition and a one minute inoculation feed. The RCVMV was not transmitted by the clover aphid.

#### Resistance of red clover to *A. bakeri* and *A. pisum*

It was decided to confirm the findings of Wilcoxson and Peterson (1960), with respect to resistance of red clover to *A. pisum*, and to extend the study to include *A. bakeri* as well. Although this aphid was not shown to be a vector it is an important pest of clover and has not been studied.

#### Attractiveness of clovers to the aphids

Dollard, Lakeland and Wegener red clover one and one half months of age were used. In each of five experiments made during the winter of 1963 under artificial lights in a greenhouse at about 24°C, 12 plants (four of

each variety/pot) were transplanted at random into six inch pots. Two pots in each experiment were placed close together in an insect proof cage and a Petri dish cover containing pea aphids was placed so that it rested on the lips of the two adjacent pots. After 24 hours the number of aphids per plant was counted. The same procedure was repeated with respect to the clover aphid. Data are shown in Table 2.

TABLE 2. Attraction <sup>a</sup> of pea aphid to Dollard, Lakeland and Wegener red clover plants.

Trial No.	No. of pea aphids added	Dollard		Lakeland		Wegener	
		No.	%	No.	%	No.	%
1	120	37	31	17	14	60	50
2	100	21	21	14	14	62	62
3	90	26	29	8	9	48	53
4	90	22	24	11	12	44	49
5	60	3	5	23	38	31	52
Average	88	22	26	17	19	53	57

<sup>a</sup>Number of aphids on 8 plants after 24 hours. Between 3 and 13 aphids were lost in each trial after it began. Differences among varieties are significant (P=.95).

An average of 57 per cent of the pea aphids selected Wegener, 19 per cent selected Lakeland and 26 per cent selected Dollard. These differences are statistically significant. About 70 per cent of the Wegener plants had 20 aphids/plant and were considered susceptible to the insect. About 80 per cent of the Dollard and Lakeland plants had 0-3 aphids/plant and were considered resistant to the insect. Resistant and susceptible plants from each variety were saved for use in the other experiments reported below.

The attractiveness study was made twice using the clover aphid but it failed both times because the insects died before they found the plants.

#### Reproduction of aphids on Dollard, Lakeland and Wegener

*Studies with A. Pisum:* This study was done with individual pea aphids on susceptible and resistant Dollard, Lakeland and Wegener plants selected in the attractiveness studies. The plants were two months old when the experiment was performed. Ten pea aphid susceptible plants and ten resistant plants of each red clover variety were transplanted individually into six inch pots containing sterilized soil. When the plants were well established one adult pea aphid was transferred onto each plant. Each test plant was kept under a glass lamp chimney in a greenhouse at about 20°C. After five hours, each plant was examined to assure the presence of the aphid. In a few cases, when the aphid was not found, another was transferred. Test plants were observed daily and the number of progeny was recorded every 24 hours. Final data were taken after ten days. The experiment was repeated. Results are in Table 3.

The population of the pea aphid was greater on Wegener and least on Dollard. Populations were also significantly greater on the susceptible than the resistant plants of each variety. The differences between susceptible and resistant plants, however, were greater with Wegener and Dollard than with Lakeland.

TABLE 3. Populations<sup>a</sup> of pea aphids on susceptible and resistant plants of Dollard, Lakeland and Wegener red clovers on which single aphids were placed for 10 days.

Dollard		Lakeland		Wegener	
S	R	S	R	S	R
No.	No.	No.	No.	No.	No.
274	139	263	280	537	337

<sup>a</sup>Progeny from 20 aphids each on a separate plant 10 days. S and R = susceptible and resistant plants (15-20 aphids/plant and 0-3/plant, respectively) selected in the attractiveness study (Table 2). Differences due to variety, resistance and the interaction were statistically significant (P = .75, .90, and .75, respectively).

Reproductivity of pea aphid on healthy and RCVMV infected Dollard, Lakeland and Wegener plants was also compared. Ten aphids were individually placed on ten plants of each class of plant in each variety and populations were counted at 24 hour intervals. Results are in Table 4.

The data appear to support the idea that healthy plants supported better reproduction than did diseased

TABLE 4. Progeny<sup>a</sup> of the pea aphid on healthy and RCVMV infected Dollard, Lakeland, and Wegener red clover 8 and 24 days after confinement.

Trial No.	Days of confinement	Dollard		Lakeland		Wegener	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	days	No.	No.	No.	No.	No.	No.
1	8	18	0	28	6	84	8
2	24	185	50	0	5	165	263
	Average	101	25	14	5	124	135

<sup>a</sup>Total progeny of 10 aphids individually placed on 10 plants in each trial. None of the differences was statistically significant.

plants, but the differences were statistically nonsignificant.

*Studies with A. bakeri:* The same procedure was used as in the study of reproductivity of the pea aphid except that resistant and susceptible test plants were not used due to the failure of the attractiveness study. After ten days populations of the clover aphid on Dollard were twice those on Wegener or Lakeland (Table 5).

TABLE 5. Populations<sup>a</sup> of *A. bakeri* on 3 varieties of red clover individual plants of which were infested originally with single aphids.

Dollard	Lakeland	Wegener
No.	No.	No.
1043	443	539

<sup>a</sup>Progeny of 20 aphids on 20 plants after 10 days. The population on Dollard was significantly greater than on the other 2 varieties.

### Reproductive life span of individual aphids on Dollard, Lakeland, and Wegener

*Studies with A. pisum:* Ten susceptible and ten resistant plants of Dollard, Lakeland and Wegener varieties, three months old, were individually transplanted into six inch pots containing sterilized soil. One adult of the pea aphid was transferred onto each plant and each test plant was kept under a glass lamp chimney in a greenhouse at 20°C. Test plants were observed daily and the number of progeny was recorded every 24 hours. Table 6 indicates the life span of female aphids and the number of nymphs produced on individual plants.

The life span of adult aphids was longer on susceptible Wegener than on resistant plants of this variety. It was three times longer on susceptible Wegener than on Dollard or Lakeland. Reproductivity was also higher on Wegener than on Dollard and Lakeland.

*Studies with A. bakeri:* This study was done to determine the reproductive life span of the clover aphid on Dollard, Lakeland and Wegener. The plants, three months old, were not selected for resistance. They (ten from each variety) were individually kept under a glass lamp chimney after one adult aphid was transferred onto each plant. They were kept in a greenhouse at about 20°C. Test plants were observed daily and the aphid progeny counted. Table 7 represents the reproductive life span of individual aphids on single plants of the three red clover varieties.

The life span was two times longer (19 days) on Dollard plants than on Lakeland or Wegener. Reproductiv-

TABLE 6. Reproductive life span of individual females of the pea aphid on susceptible and resistant Dollard, Lakeland, and Wegener red clover varieties.

Plant No.	Dollard				Lakeland				Wegener			
	Life span in days	No. of nymphs produced		Life span in days	No. of nymphs produced		Life span in days	No. of nymphs produced				
	S <sup>a</sup>	R	S	R	S	R	S	R	S	R	S	R
1.	5	2	20	7	5	5	28	31	5	16	28	35
2.	3	4	8	0	3	1	3	5	15*	6	51	22
3.	5	4	24	2	5	2	1	3	20*	4	21	13
4.	3	5	12	27	5	1	17	1	25*	15*	51	24
5.	6	3	5	8	5	2	13	10	7	17*	20	27
6.	5	5	16	20	1	8	1	26	7	7	40	3
7.	6	2	27	8	5	8	32	13	7	4	13	10
8.	8	2	16	1	1	5	2	18	7	1	26	1
9.	3	5	14	1	5	8	12	27	4	3	10	2
10.	1	1	6	0	8	1	27	0	19*	3	27	14
Avg.	4	3	14	7	4	4	13	13	11	7	28	15

<sup>a</sup>S and R indicate resistance to pea aphid.

\* Insect still alive but test was terminated.

TABLE 7. Reproductive life span of individual females of the clover aphid on 10 individual plants of Dollard, Lakeland and Wegener red clover.

<i>Dollard</i>		<i>Lakeland</i>		<i>Wegener</i>	
Life span days	No. of nymphs produced	Life span days	No. of nymphs produced	Life span days	No. of nymphs produced
20	105	6*	0	13	25
13	105	13	30	3	0
13	22	13	50	13	37
13	40	13	60	7	6
13	30	13	70	13	110
13	40	13	55	13	47
13	60	7*	0	6	2
25	130	3	0	13	45
30	110	13	45	7	0
27	100	6*	0	13	130
<b>AVERAGE</b>					
19	74	10	31	10	39

\*Insect still alive but test was terminated.

ity was also higher (two times) on Dollard than on either Lakeland or Wegener.

#### **Behavior of aphids on well established plants of Dollard, Lakeland and Wegener**

Observations were made with individuals of the two aphid species on plants of Dollard, Lakeland and Wegener. A magnifying lens was used to observe each aphid on a particular red clover plant.

On Dollard the pea aphids were restless. They moved their antennae in all directions for the first 15 to 20 minutes of confinement. A few of the aphids left the plants after a few seconds. When they were placed on the plants again, they were again restless and did not insert their stylet into the plant tissues until 20-45 minutes had passed. Before they started feeding, their mouth parts were kept at right angle to their bodies. On Lakeland and Wegener, individual adults of the pea aphid appeared calm and started feeding after a few seconds.

The clover aphid preferred to feed on the base of the petiole of all red clover plants. Nymphs, however, were observed on the lower and upper surfaces of trifoliolate leaves of each red clover variety. It was difficult to determine the exact time they started feeding. Striking differences in behavior on the varieties was not observed.

#### **Discussion**

Insect transmission of RCVMV indicated again that the pea aphid can act as a vector. It is difficult to conclude which combination of acquisition and inoculation feeding was most effective for obtaining infection because transmission of RCVMV resulted from the two extremes. For the first time, a minimum acquisition feeding of one half minute and an inoculation feeding of two minutes have been recorded for the pea aphid to acquire and infect healthy red clover plants with RCVMV. The clover aphid failed to transmit the RCVMV in this study.

The findings of Wilcoxson and Peterson (1960) were

confirmed in that Dollard was shown to be more resistant than Wegener to pea aphid. In the present work it was also shown that Lakeland red clover, too, has resistance to pea aphid and this fact may account for the field resistance of Lakeland to virus diseases. It was also found that each variety of red clover tested contained plants that were resistant or susceptible to pea aphid and that the resistance was correlated with nonattractiveness and antibiosis to aphid.

The results with clover aphid were different from those obtained with pea aphid. On Wegener and Lakeland this aphid was short lived and did not reproduce well while on Dollard the aphid was long lived and reproduced abundantly. The insect was not proven a vector of RCVMV and this may have been because Wegener was used a source of the virus. The work clearly points to the possibility that red clover varieties may differ in resistance to different aphid species and this may be an important factor in the development of varieties having field resistance to virus diseases.

A study was also made to determine the effect of red clover plants infected with RCVMV on the reproductivity of individuals of the pea aphid. The results support the idea that healthy plants supported better insect reproduction than did diseased plants.

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