

AN ABSTRACT OF THE THESIS OF

Amy J. Dreves for the degree of Master of Science in Entomology presented on November 21, 1990.

Title: Seasonal Abundance, Distribution, and Migration of the Clover Aphid, *Nearctaphis bakeri* (Cowen) in Red Clover

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Abstract approved: \_\_\_\_\_  
Jack DeAngelis

Four sampling techniques were evaluated to estimate clover aphid, *Nearctaphis bakeri* (Cowen), abundance in red clover fields in the Willamette Valley. The Berlese funnel method detected one to four times more aphids than other sampling techniques. Visual assessment often underestimated the high aphid densities during the flowering and seeding of clover. Sweep net and Schuh shaker techniques had limitations and underrated the numbers of aphids present in the clover fields. Strong correlations ( $r^2 = 0.74 - 0.87$ ) were found between the Berlese funnel technique and visual assessment technique in a 2nd year established field.

Numerical differences in the spatial distribution of *N. bakeri* on clover stems were shown during various stages of plant development over the season. During development of buds and axils, aphid numbers on the lower half of the plant averaged 1.5 times greater than those on the upper half. As the season progressed into the flowering and seeding stage, aphid numbers on the upper stem halves were approximately five times greater than those on the lower half.

Winged clover aphids were too few to show definite peaks of flight using water traps, averaging 2.19 aphids per yellow bucket during the June-August period. No significant differences in aphid attractiveness to the different colors were found among water pan traps. Yellow buckets captured approximately five times more alate aphids than did yellow, red, or green pan traps. Traps placed on the south side of the field contained higher numbers of aphids than in other locations in the red clover fields.

**Seasonal Abundance, Distribution, and Migration  
of the Clover Aphid, *Nearctaphis bakeri* (Cowen)  
in Red Clover**

by

Amy J. Dreves

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**SEASONAL ABUNDANCE, DISTRIBUTION, AND MIGRATION  
OF THE CLOVER APHID, *NEARCTAPHIS BAKERI* (COWEN)  
IN RED CLOVER**

**INTRODUCTION AND LITERATURE REVIEW**

Red clover, *Trifolium pratense* Linn, native to most of Europe and portions of Asia was introduced into North America over 200 years ago. Clover is grown for hay, pasture, cover and rotational crops, silage and seed. Several varieties of clover are grown in Oregon such as white, ladino, sweet, arrowleaf, alsike, crimson and most commonly, red clover. Oregon produced 1/7 of the total production of grass and legume seed in the United States in 1989. In 1989, clover seed was harvested on 1550 acres in Lane, Linn and Benton County, and on 20,660 acres in Washington and Yamhill Counties (Miles 1988). Oregon clover seed is shipped to many parts of the United States and to other countries (Miles 1988). A vigorous stand of red clover persists for 2 or 3 years depending on the variety, soil, and climate. Red clover does best on well drained, fertile loam soils such as found in the north Willamette Valley in Oregon. In Oregon, a hay crop is removed in mid-May. The plants flower in early July, reach full bloom in mid-July and are harvested for seed towards the end of August (Melby 1988).

Red clover has a variety of insect pests and diseases which can pose problems to the grower (Newton 1960, Kamm 1987, Baird et al. 1986). Many polyphagous species of aphids have been recorded in North America on this crop including *Nearctaphis crataegifoliae* (Fitch), the long-beaked aphid; *Myzus persicae* (Sulzer), the green peach aphid; *Acrythosiphon pisum* Harris, the pea aphid; *Aphis coronillae* Ferrari, the black aphid; *Myzus ornatus* Laing, the violet aphid; *Aphis fabae* Scopoli, the black bean aphid; *Macrosiphum euporbiae* (Thomas), the potato aphid; *Therioaphis trifolii* Monell, the yellow clover aphid; and *Aphis gossypii*

Glover, the melon aphid (Eastop and Lambers 1976).

The short beaked aphid, most commonly called the clover aphid, *Nearctaphis bakeri* Cowen, is native to North America. It is known to be a serious pest of clover in the Northwest and can be a destructive pest at damaging levels on red clover (Smith 1919, Johansen 1960, Fisher 1988 and Costa 1988). All parts of the plant may be colonized, particularly bases of stems and axils, and the inflorescences. Although Oregon has been one of the leading producers of clover seed, little attention has been given to the clover aphid. Outbreaks have occurred from time to time, but the relative importance of the clover aphid varies from year to year. In 1907, the clover aphid was first found and later reported as a pest of red clover in the Willamette Valley (Smith 1919). Damaging aphid populations often develop in the spring and sometimes in the fall in the Willamette Valley. Clover seed production has been discouraged, because of the clover aphid (Hickerson 1976).

The clover aphid has six or seven morphs during a season and is easily distinguished from other aphids on clover (Palmer 1952, Richards 1969). Diagnostic characters include light greenish-yellow to pink color of the nymphs and apterous adults; the minute dark spots on the dorsum of apterous forms; the large dark green to blackish quadrate patch on the dorsum of alate forms; the short antennae; and the short cornicles with light areas at their bases (Palmer 1952, Richards 1969).

The holocyclic aphid, *N. bakeri*, overwinters as an egg (Baird et al. 1986) (Figure 1). An average of 23 generations may be produced within a season as recorded by Smith (1923). Alate males finish development on summer food plants, and fertilize females. The oviparous female deposits eggs in the fall at the bases of fruit spurs or buds of primary host trees such as ornamental crab apple, cherry, pear and hawthorn. Eggs hatch in the spring, the stem mothers move to sepals and unfolding leaves to feed on developing foliage and terminal growth of the branches, and two to three generations of nymphs are produced. Second or third generation females produce winged forms which migrate to secondary hosts, particularly *Trifolium* spp. Colonization of red clover by the clover aphid appears to be aided by the

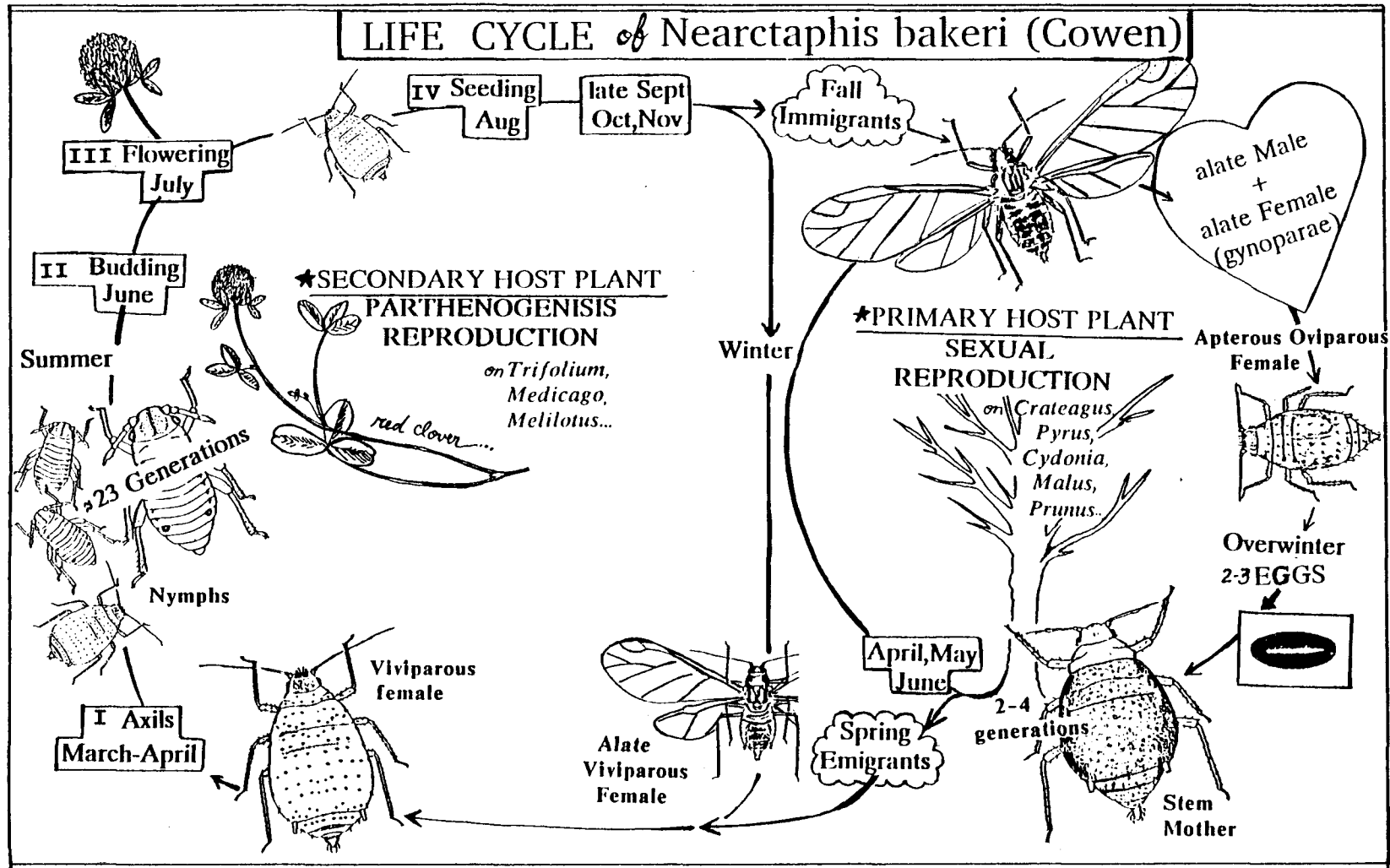


Figure 1. Life cycle of the clover aphid, *Nearctaphis bakeri* (Cowen) on primary and secondary hosts

plant's large clasping stipules and compact blossoms, which provide protection from natural enemies (Smith 1923).

Throughout the warmer parts of a year, secondary host plants include *Melilotus* spp. (i.e. white and sweet clovers), and *Medicago sativa* (alfalfa). The clover aphid apparently never occurs as oviparous forms or as eggs on secondary hosts (Smith 1923). Populations can occur close to the ground and in the crowns of clover. If winter temperatures are not severe, many aphids will overwinter on clover (Gillette 1908).

Blackman and Eastop (1984) reported the primary or winter hosts of the clover aphid as *Crataegus* sp. (i.e. hawthorne), *Cydonia* sp. (i.e. quince), *Malus* spp. (i.e. apple), *Pyrus* spp. (i.e. pear), *Prunus* spp. (i.e. prune, plum, peach, apricot, almond, sweet cherry) and related woody plants. In California, the clover aphid has been reported on sunflower, artichoke, *Cnaphalium* sp. (i.e. cudweed) and *Senecio* sp. (i.e. german ivy) (Taylor 1985). With spring regrowth of the primary hosts, clover aphids infest tips of twigs, leaves and blossom buds. The alate spring migrants disperse and migrate to leguminosae secondary hosts.

The clover aphid feeds on the floral tissues and axils of red clover. Feeding damages plant cells and results in decreased seed yield and market value. The timing of an aphid attack on the clover is important in the effects on seed yield (Smith 1919). The number of flowering branches and vigorous blossoms are reduced due to the weakened vitality of plants under prolonged attack. Seed abnormalities have been reported in 5 to 8 weeks from the date of finding the first colonizing clover aphid alates (Smith 1923). In addition, infested clover blossoms result in blighting and shrinking of seeds, as well as loss of seed when harvesting sticky clover (Smith 1923). The aphids secrete great amounts of honeydew during feeding, reducing the quality of individual seeds. A black mold (*Fumago vagans* Pers.) forming on the honeydew reduces feed value of hay crop. The clover aphid has also been reported to vector alfalfa mosaic and bean yellow mosaic to red clover (Manglitz and Kreitlow 1960).

Literature concerning the clover aphid's importance as a pest on red clover is limited, and there is a particular lack of knowledge for western Oregon. Most research on the clover

aphid has been in the area of chemical control. The earliest record of the clover aphid in North America is that in 1895 by Mr. J.H. Cowen. He reported *Aphis cephalicola* Cowen from heads of white clover (*T. repens*) and the stems of red clover (*T. pratense*), when it was found and first described at the Colorado Experimental Station in 1895 (Gillette and Baker 1908).

The Idaho Agriculture Experiment Station (Smith 1919) published a preliminary report on the clover aphid based largely on observational findings. Bulletins have given some general facts, brief notes, illustrations with discussions on the behavior of the clover aphid, and recommendations for control (Gillette 1908). Detailed investigations of the clover aphid were initiated by the Idaho Agricultural Experiment Station late in the summer of 1916. An extensive review of the life history of the clover aphid in Idaho was written in 1923 by Ralph Smith at the Idaho research station. Included were records of description, natural enemies, damage, alternative hosts, transmittance of viruses and life history.

Several revisions of *Aphis bakeri* Cowen have transpired, moving from the genus *Roepkea* (Richards 1969) to *Nearctaphis* by Hille Ris Lambers in 1970. Robinson (1984) developed a key for 13 species and two subspecies of the genus *Nearctaphis* in North America, which included all species formerly in the genus *Roepkea*. Richards (1969) pointed out that the hosts, life histories, and forms for all species are poorly known and more collecting and host transfer experiments are required. No other publications regarding this insect have been found. Choosing an accurate sampling scheme for collecting the clover aphid is a necessary preliminary study before these other experiments can be carried out.

Developing practical and economical management strategies the clover aphid on red clover will involve its detection, a knowledge of population trends, and prediction of its effects in red clover. Efforts to control this aphid will involve developing IPM strategies, depending in part on suitable sampling methods. Additionally, pest management programs may be designed to maximize the role of natural biological agents and minimize the use of insecticides in maintaining pest populations below economic injury levels. As such, a survey

of predation and parasitic activity on red clover as the season progresses can be important information.

The objectives of this research were to develop an accurate and valid sampling tool to estimate clover aphid population densities and to gain information about the presence of predators on red clover. The present studies were designed to monitor and evaluate the efficiency of various sampling tools for the clover aphid as well as certain other arthropods of the clover community, and to document the seasonal movement and flight in the Willamette Valley with the use of water pan and bucket traps. An understanding of aphid movement patterns is important for developing control programs, for surveying potential pests, and for studying aphid ecology.

## MATERIALS AND METHODS

### Study Areas

Two red clover plots in the Willamette Valley were selected for study. One study area consisted of two red clover varieties, **Kenland**, a standard variety, and **Atlas**, a newer variety. These two clover varieties were seeded on April 18, 1989, at Oregon State University Horticulture farm, 1 mile east of Corvallis on a site designated Field A. Clover seed of the two varieties was inoculated with a culture of *Rhizobia* bacteria (OSU Extension Bulletin-1055, 1981). The field was planted with a Brilliant seed spreader on 60 x 150' plots bordered by filberts, grasses, and vegetables (Figure 2). No insecticides, herbicides, or fertilizers were applied on these plots. A pre-plant herbicide is a practice sometimes used by growers of clover, but was not used on these plots due to the possibility of drift onto other experimental plots nearby. The experimental design chosen for Field A to evaluate different sampling techniques was a randomized block with variety type as the blocking factor. Each block was divided into 4 subplots and replicated twice within each subplot. The blocks were separated by fallow buffer zones plowed periodically. Daily records of minimum and maximum temperatures and rainfall were obtained from the O.S.U. Hyslop Farm weather station to determine their possible effects on efficiency of the sampling techniques. Overhead sprinkle irrigation provided water to the plots. This site was observed again in 1990, and designated as Field C for data analysis purposes.

A third field of commercially grown red clover, designated Field B, also was selected for study. This was a thirty acre red clover field of a Japanese variety called *Hamadori*. The field was located 10 miles South on Hwy 99E off of Smith Loop Road. The field was planted in the fall of 1988 and was in its second seed year at the initiation of this study. Bordering the field were sugar beets, wheat, alfalfa, corn, and beans. Large alder and other deciduous trees were positioned on the southern and northwest sides of the field (Figure 3). Only the east corner of Field B (with north and south borders) was intensively sampled over a 200 x 200'

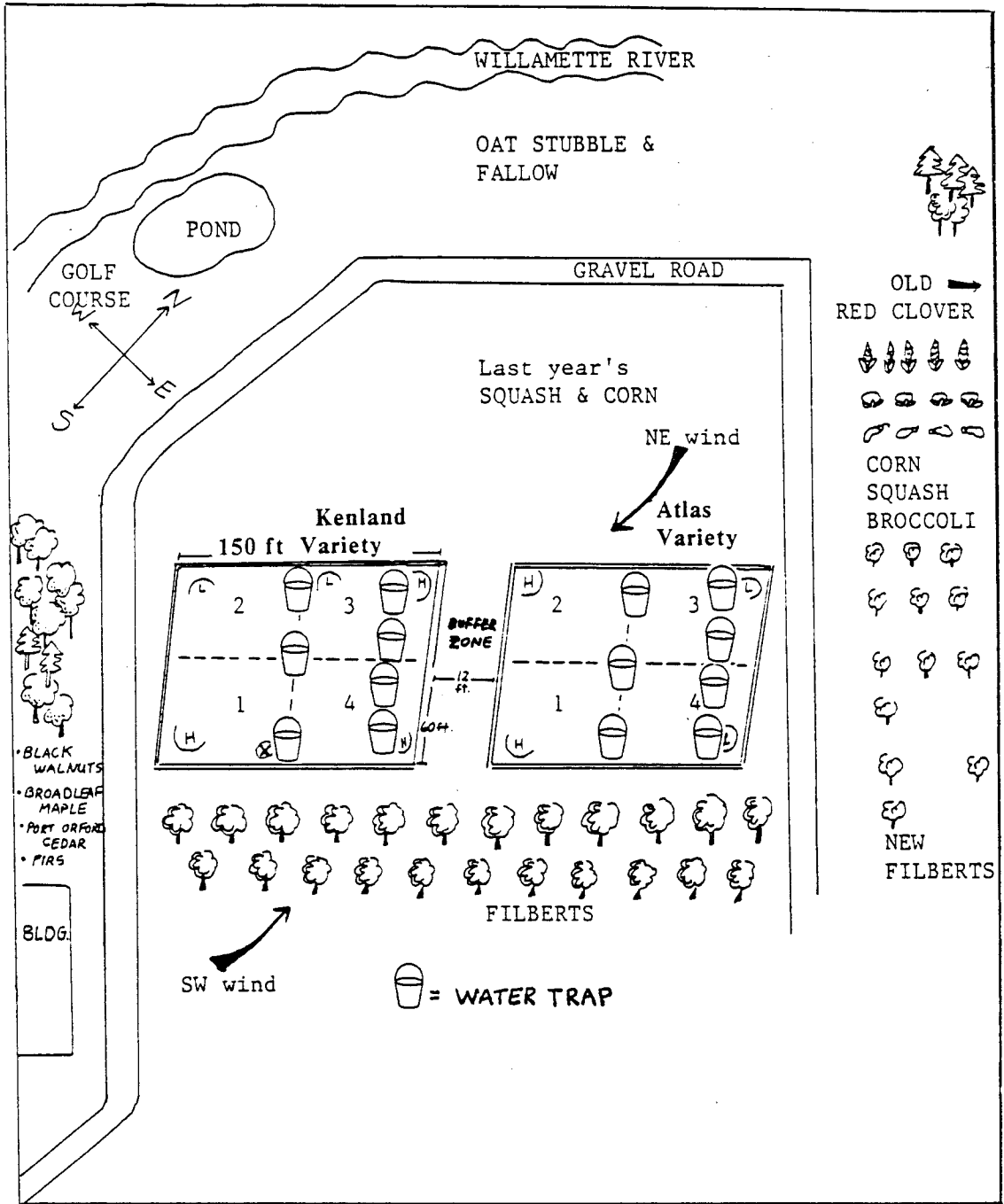


Figure 2. Diagram of Field A site used to sample aphids located 1 mile E. of Corvallis at Vegetable Horticulture Farm



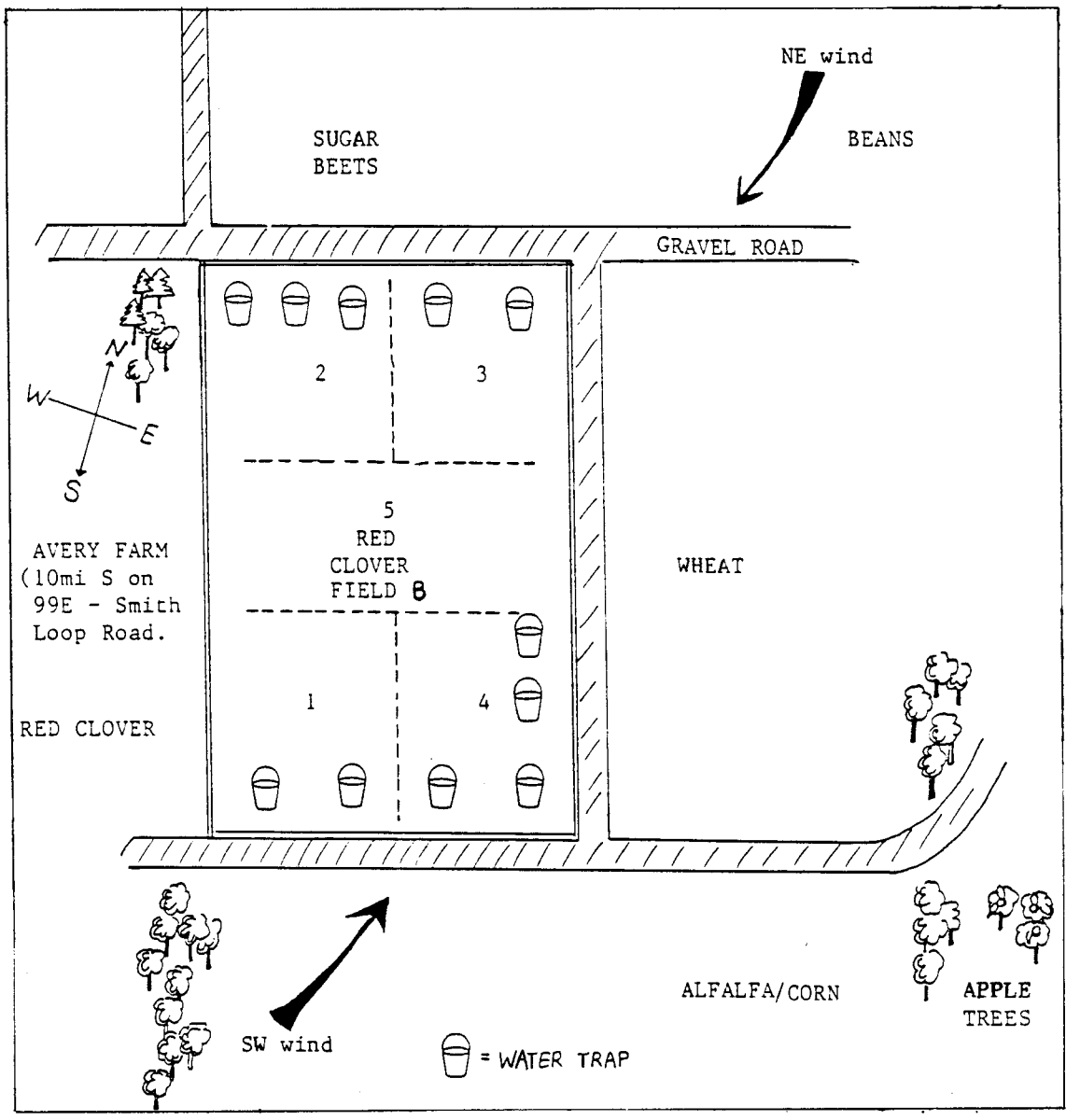


Figure 3. Diagram of Field B site, a 1989 established 2nd year clover stand treated with pesticides, 10 miles South of 99E off of Smith Loop Road

area. A completely randomized block design, divided into 5 subplots was used in Field B. Each treatment was replicated twice within subplots.

### **Plant Development**

To examine the effect of plant development on the sampling methods, plant size and growth stage were recorded through the season. Twenty plants were selected randomly the length of the tallest main stem was used as the measure of height. Growth stage descriptions included: number of axillary growing points that have produced one or more fully opened leaves, budding, flowering, and seeding (Figure 4). Stem density was determined on 1000cm<sup>2</sup> (crown surface) samples.

### **Sampling Techniques**

The development of clover aphid populations throughout the season was measured by various sampling techniques. The purpose was to determine an accurate and valid sampling tool to estimate population densities (Southwood 1978). Visual assessment, sweep net, Berlese funnel, Schuh shaker, and water traps were chosen as the sampling techniques. Comparisons of sampling methods were made simultaneously and recorded from June - August in Fields A and B during the morning hours when permitted.

### **Visual Assessment**

A visual whole-plant search of the clover stem is a common technique used by most farmers and extension agents when scouting for aphid abundance. Twenty stems from each subplot were examined. Plants were randomly selected and clipped at the base close to the crown, with minimal disturbance. The stem was divided in half visually, and clover aphids were counted and recorded according to the upper and lower half. Total aphid numbers of apterous and alate aphids were recorded per 20 stems. The flower heads, main terminal, axils, and stipules were searched thoroughly for the presence of aphids. Each stem was measured for length and categorized by growing stage.

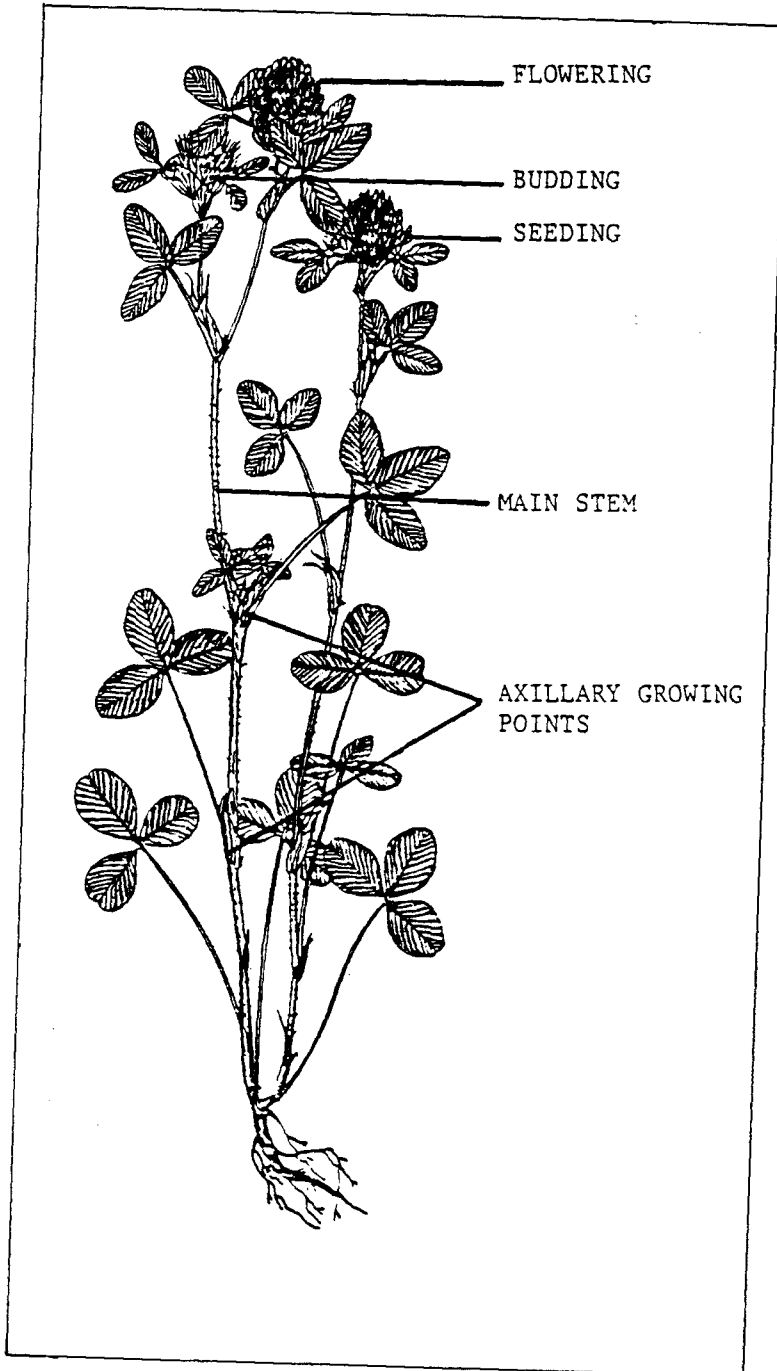


Figure 4. Red clover development with plant stage descriptions

### **Sweep Net**

A sweep net with a diameter of 38 cm and a handle length of 91 cm was used. In areas relatively undisturbed by stem sampling, the sweep net was extended in a horizontal manner, and moved through the upper 30 cm of plant canopy (Figure 5). The mechanics of the sweep net sampling were standardized and consisted of twenty 180° single sweeps per subplot. Only one pass of the net was made over sampled plants. Succeeding strokes were always made in parts of the field not previously disturbed by the net. The sweep net contents were emptied into a 1 gallon plastic freezer bag and stored frozen. Aphids were examined under a microscope to distinguish *N. bakeri* from other species. Numbers of clover aphids and certain natural enemies were recorded. The importance of these natural enemies on clover aphid abundance was not examined in this study and only an initial survey was taken. Red clover fields were surveyed for the presence of natural enemies using the sweep net only during the morning hours, thus, only a small fraction of the daily predator activity was surveyed.

### **Berlese Funnel**

Twenty clover stems were selected randomly, cut at the base and placed in a labeled white plastic bag. Plants were transported back to the laboratory and processed within the Berlese funnel (Figure 6). Collecting jars were filled with 70% alcohol. When the plant material had dried under the 40 watt bulb, 3–5 days depending on the age of the plant, the jars were removed and the contents poured through a Büchner funnel onto a gridded filter paper. The gridded papers were carefully examined under a stereomicroscope at 40x. Numbers of clover aphid and stage of growth for each stem were recorded.

### **Schuh Shaker**

The Schuh shaker (Gray and Schuh 1941) was used to sample clover aphids on flower heads. Twenty heads were collected after 50% bloom and placed in a 1 gallon ziplock bag in

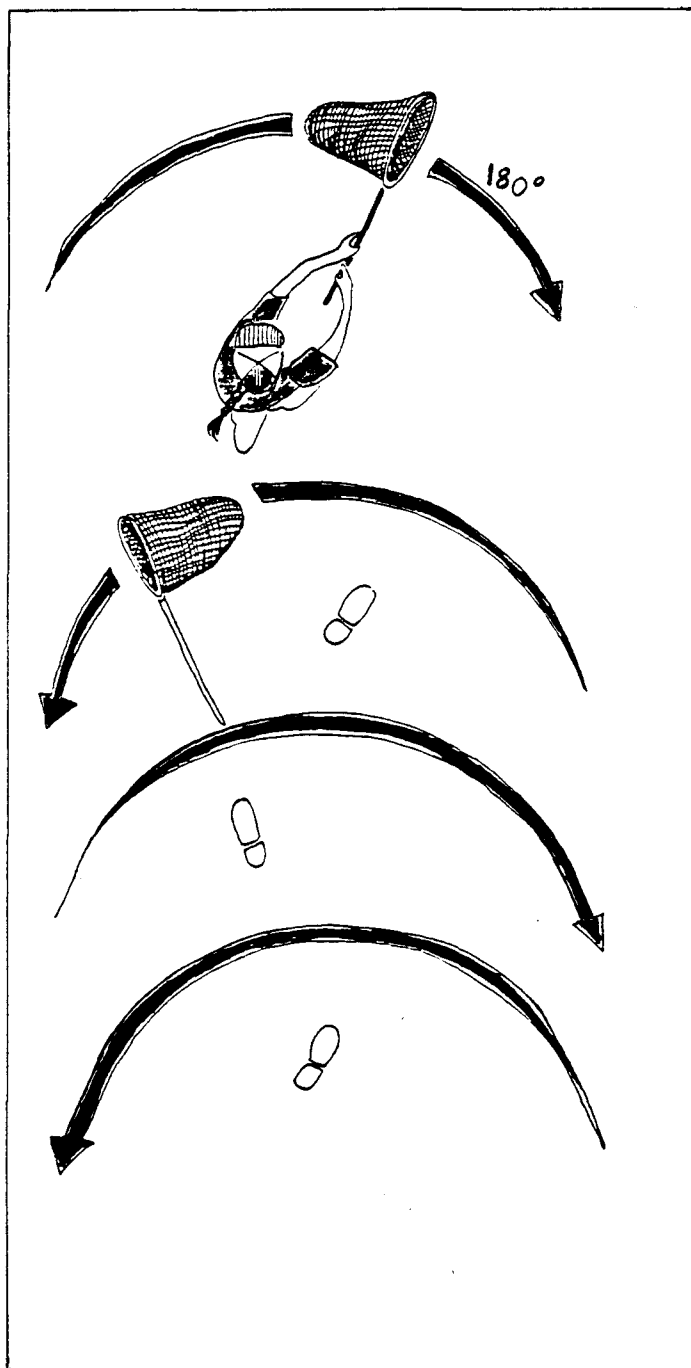


Figure 5. Sweep net sampling technique with 180° arc swing used in red clover

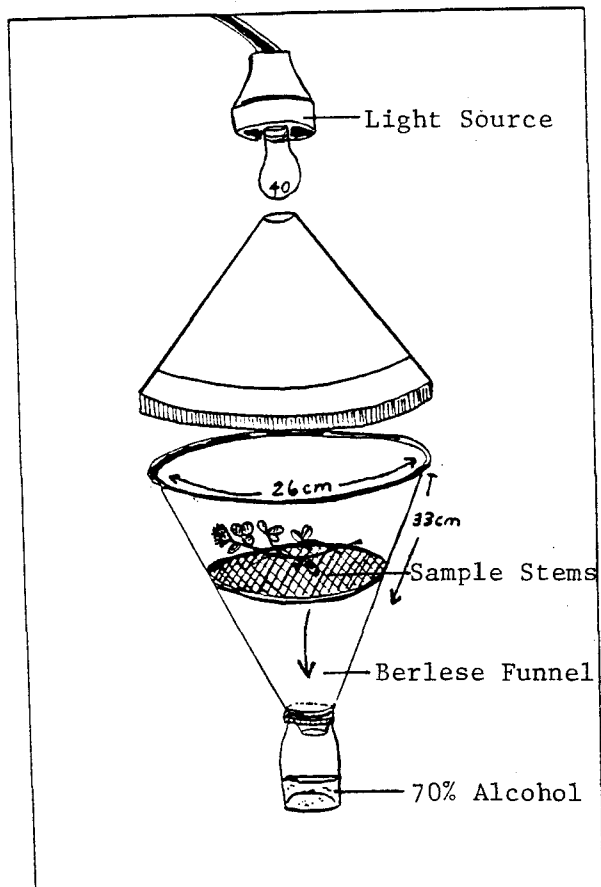


Figure 6. Berlese funnels used to assess aphid infestations from clover stem samples

such a way that disturbance and escape of insects were minimized. A chemical extraction procedure described by Gray & Schuh (1941) used methyl ethyl ketone to force aphids from the plant into a collection container. Clover heads were fumigated for 5 minutes then the canister was shaken 50 vigorous vertical times. Samples were removed and examined for the presence of remaining aphids. A smaller version of the Schuh shaker was designed for field use (Figure 7) to process flower heads.

### **Water Traps**

Water traps of two sizes were used: a standard round water pan trap with an opening of 18 cm and depth of 5cm purchased from Trece, Inc. and a yellow water bucket trap with a diameter of 20.5 cm and a depth of 17 cm. The water pan and bucket trap holder were designed to adjust to plant canopy height over the season (Figures 8 and 9). The water pan trap holders were painted tan to resemble a bare dirt background to highlight the colors of the pan traps and yellow bucket.

The pan traps were individually painted with one of the following three colors: 1. Gloss Yellow-Brite Touch Spray Paint (Borden, Inc.-Columbus, OH); 2. Chinese Red (Red Devil paints & Chemicals-Mt. Vernon, NY); 3. Lawn Green (Red Devil Paints & Chemicals-Mt Vernon, NY). The color of the bucket was a bright yellow. Traps were mounted on the north, south, east, west and center of the fields. Each trap type was rotated systematically into a new position on the field to get an equal chance of receiving winged aphids. Placement was chosen considering wind direction, and wind breaks (Taylor 1965). None of the traps were within 50 m of each other, and no trap was within 50 m of the field edge. Eight to ten traps were placed in each of the fields (Figure 2 and 3). The traps were filled with water weekly 2/3 to 3/4 full. A few drops of detergent or antifreeze were added to reduce the surface tension. A few cap fulls of bleach (5% NaOCl) were added to inhibit fungi.

The water traps were serviced by filtering the contents through a sieve apparatus shown in Figure 10. Trap stands were elevated each week as the clover grew taller. The traps

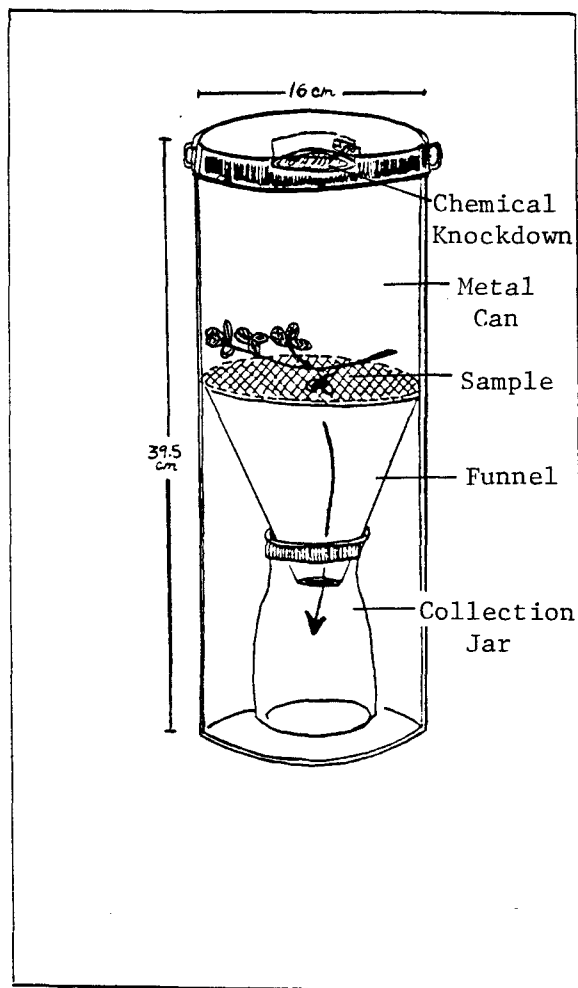


Figure 7. Chemical extraction can-'Schuh Shaker' (Gray, K.W. and J. Schuh 1941) for sampling aphids



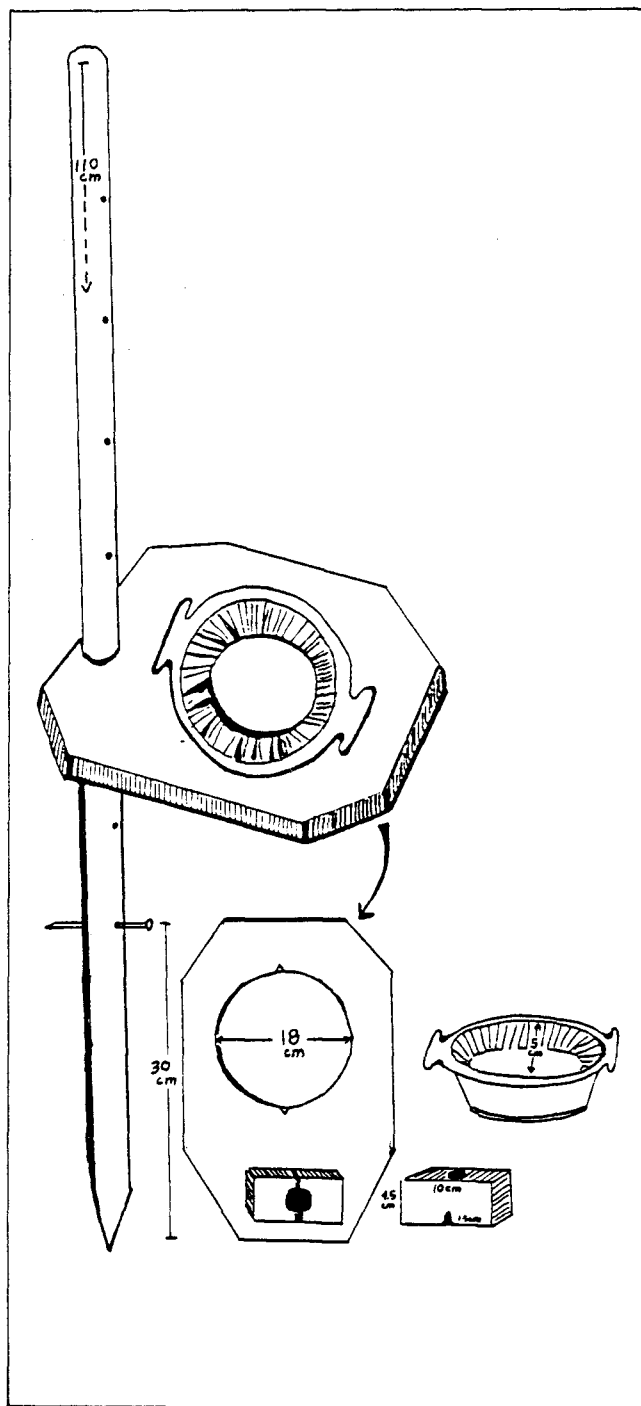


Figure 8. Water pan traps designed to sample aerial migration of the aphid

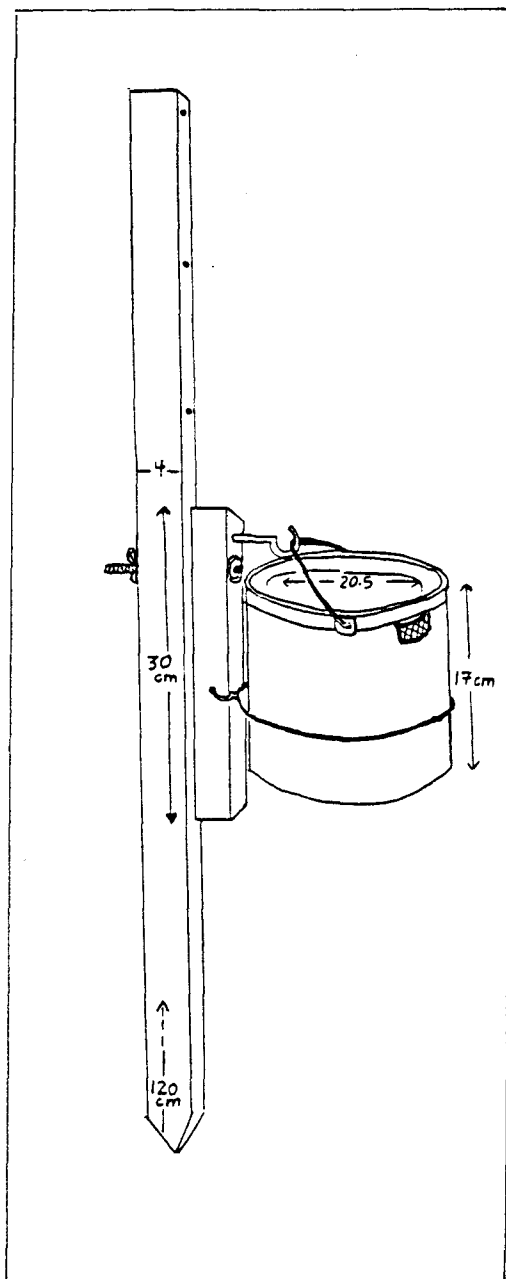


Figure 9. Design of water bucket trap for sampling winged aphids

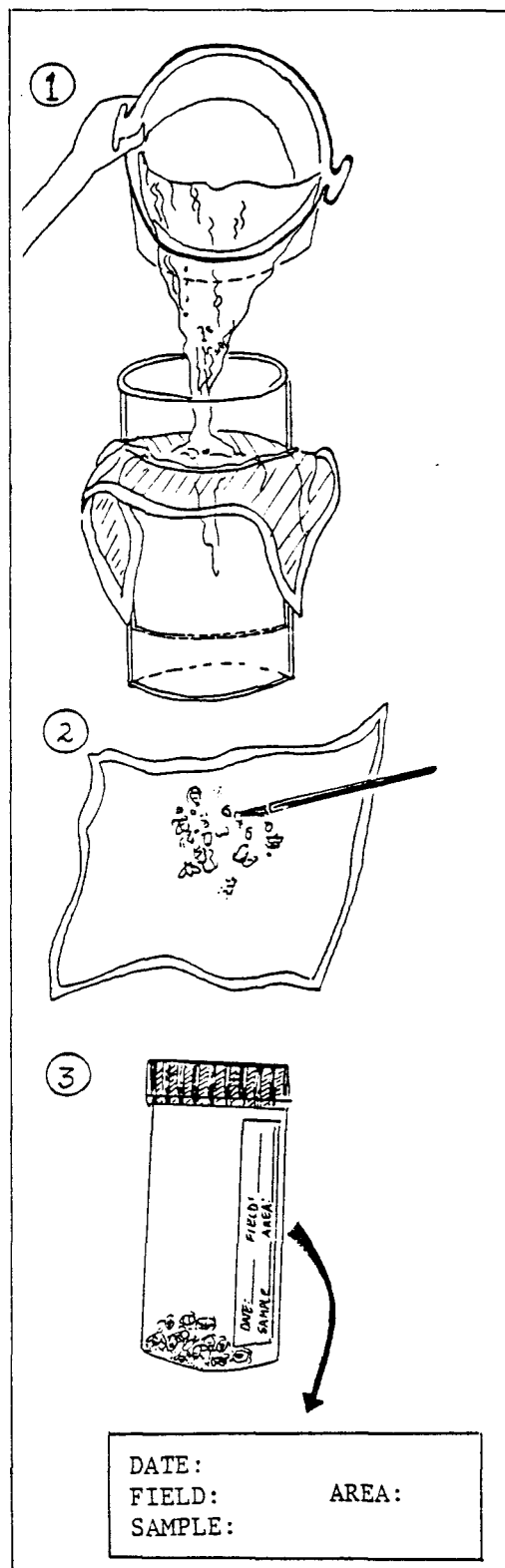


Figure 10. The water trap contents are: 1. Filtered over fine material 2. A fine paint brush or forceps are used to collect aphids 3. Vials with specimens include a label for identification

were covered with plastic bags during irrigation and an overflow system was incorporated into the bucket traps to remove excess water from irrigation and rain. Water traps were monitored in 1989 in Fields A and B and in Field C in 1990.

### **Data Analysis**

Aphid numbers derived from sample technique comparisons were transformed using square root ( $x + 1$ ) both to stabilize sample variances and because many zeros occurred in the data (Sokal and Rohlf 1969). The transformations satisfied the assumptions necessary for proper application of the two-way ANOVA with unequal replication in the sampling technique study (Gomez and Gomez 1984). The Pearson Correlation Coefficient analysis was computed on the square root transformation, normalizing residuals, in order to examine the strength of the relationships that exist between the techniques (Cody and Smith 1987). Sampling dates were combined according to 'stage' of growth, to increase the data points available for the calculation. Correlation coefficients ( $r$ ) were interpreted by looking at the square of the coefficient ( $r^2$ ). High and low distribution counts on stems were compared and tested over the season using one-way ANOVA.

## RESULTS

### Sampling Technique Comparison

Results from field experiments showed differences among the sampling techniques. Only three of the original four sampling techniques were analyzed to assess clover aphid populations. The Schuh shaker technique was found to greatly underestimate the clover aphid abundance compared to the other techniques, so was disregarded for data analysis. Aphids remained tightly affixed within the heads and axils of clover and 80-90 per cent of the aphids would not release into the canister in the presence of methyl ethyl ketone.

Field B, with an over-all mean for the season of 140.9 aphids per 20 stems, was significantly different in aphid abundance from Field A ( $F=25.67$ ,  $df=1$ ,  $p=0.0001$ ), with a mean of 49.5 aphids per 20 stems for the season, therefore, field data were analyzed separately (Field A = 1989 1st year untreated clover; Field B = 1989 2nd year red clover treated with pesticides). Analysis of data collected during 1989 indicated no significant variety differences in numbers of aphids on the two different varieties of clover in Field A ( $F= 0.91$ ,  $df= 1$ ,  $p=0.34$ ). Blocking proved to be ineffective in filtering out extraneous variation of variety differences in the randomized block design, therefore, both variety subplots were combined, increasing the replications.

Sampling dates were combined into 'Stages' of plant growth based on clover plant development for statistical analysis of technique comparisons. The stages included: Stage I = axils and budding (June); Stage II = flowering (July); Stage III = seeding (August); Stage IV = post-harvest (September-October).

Significant Pearson correlations were found between three techniques sampling clover aphid abundance (Table 1). Berlese funnel and visual sampling methods displayed significant positive correlations for all three stages of plant growth in the second year field (Field B). Correlation ( $r^2$ ) ranged between 0.74 and 0.87 for all stages of growth. No relationship or

Table 1. Correlation coefficients<sup>a</sup> for seasonal relationships of three aphid sampling techniques (Berlese funnel, sweep net, and visual assessment) in red clover fields (Field A=1st year untreated clover; Field B= 2nd year clover treated with pesticides) during 1989

STAGE	BERLESE:SWEEP		BERLESE:VISUAL		VISUAL:SWEEP	
Field	A	B	A	B	A	B
<i>I</i>	0.19 <sup>b</sup> (0.37) <sup>c</sup>	0.38 (0.10)	0.35 (0.10)	0.74 (0.0002)	0.13 (0.54)	0.60 (0.006)
<i>II</i>	0.10 (0.58)	0.15 (0.60)	0.05 (0.79)	0.80 (0.0002)	0.52 (0.002)	0.07 (0.81)
<i>III</i>	0.10 (0.63)	0.49 (0.07)	0.24 (0.26)	0.87 (0.0001)	0.03 (0.90)	0.34 (0.21)
<i>IV</i>	0.32 (0.13)	HARVESTED	0.05 (0.81)	HARVESTED	0.30 (0.15)	HARVESTED

<sup>a</sup> Pearson Correlations transformed to square root for analysis (SAS Institute 1988).

<sup>b</sup> Correlation coefficient values ( $r$ ).

<sup>c</sup> Probability associated with coefficient ( $p$ ); significant at  $p < .05$ .

correlation was seen between Berlese funnel and sweep net methods in Field A or B (Table 1). In Field A, visual assessment and sweep net had one significant correlation in Stage II. Another significant correlation was observed between the visual and sweep net methods in Stage I, Field B (Table 1).

In 1989, significant differences among the techniques occurred over the season in Field A ( $F=240.95$ ,  $df=2$ ,  $p=0.0001$ ) and Field B ( $F=56.73$ ,  $df=2$ ,  $p=0.0001$ ). The sweep net consistently and significantly underestimated aphid abundance compared to the other two sampling techniques (Table 2 and 3), thus the sweep net technique was eliminated from the statistical analysis when comparing differences in techniques.

During Stage I, no significant differences were found between Berlese funnel and visual assessment in Field A ( $F=1.19$ ,  $df=1$ ,  $p=0.28$ ) or Field B ( $F=.04$ ,  $df=1$ ,  $p=0.84$ ) (Table 2 and 3). Field A was newly planted in late April, and cut to the ground in early June (to increase clover density), so remained in the axil stage. The plant stage in Field B, a 2nd year field, was represented by bud development.

During Stage II, significant differences were found between techniques in both Field A and B (Table 2 and 3). In Field B, the aphid count reached its highest level during the whole season on July 4th sampled by the Berlese funnel technique. At this point, Berlese funnel resulted in much higher counts of aphids ( $F=9.59$ ,  $df=1$ ,  $p=0.02$ ). An insecticide was applied, dropping the mean aphid count 100x lower (Table 3). At this time no significant differences in numbers of aphids occurred between Berlese funnel and visual assessment techniques ( $F=3.22$ ,  $df=1$ ,  $p=0.11$ ). However, by July 24, clover height and aphid abundance had again increased, and significantly greater numbers of aphids were recorded by the Berlese funnel technique compared to the visual assessment technique ( $F=7.81$ ,  $df=1$ ,  $p=0.02$ ).

In Field A, aphid numbers reached high levels on July 4 (Stage I). On this date, as in Field B, significantly more clover aphids were recorded by the Berlese funnel technique ( $F=5.59$ ,  $df=1$ ,  $p=0.03$ ) (Table 2). However, this clover field was developing slowly, lacked stand uniformity and density, and an abundance of weeds competed with the clover. To

Table 2. Comparison of sampling techniques of *N. bakeri* abundance, recorded in 1st year untreated red clover in Corvallis, Oregon (Field A) during 1989

Date	Mean Aphid Abundance ( $\bar{x} \pm SE$ ) <sup>a</sup>		
	Sweep net	Berlese	Visual
<b>Stage I</b>			
JUNE 12	0.50c $\pm$ 0.33	17.87b $\pm$ 4.13	36.63a $\pm$ 8.55
JUNE 20	1.13b $\pm$ 0.32	22.12a $\pm$ 4.01	13.63a $\pm$ 2.43
JUNE 26	4.88b $\pm$ 1.76	37.88a $\pm$ 5.05	44.13a $\pm$ 3.64
<b>Mean</b>	<b>2.17b <math>\pm</math> 0.71</b>	<b>25.96a <math>\pm</math> 3.03</b>	<b>31.46a <math>\pm</math> 4.09</b>
<b>Stage II</b>			
JULY 4	2.38c $\pm$ 0.32	210.12a $\pm$ 39.29	108.88b $\pm$ 8.61
JULY 11	3.00b $\pm$ 0.38	182.37a $\pm$ 52.37	205.13a $\pm$ 30.74
JULY 18	0.25b $\pm$ 0.20	85.12a $\pm$ 15.47	66.75a $\pm$ 12.06
JULY 24	0.75c $\pm$ 0.25	243.74a $\pm$ 31.70	77.63b $\pm$ 11.64
<b>Mean</b>	<b>1.59c <math>\pm</math> 0.25</b>	<b>180.35a <math>\pm</math> 20.61</b>	<b>114.59b <math>\pm</math> 13.01</b>
<b>Stage III</b>			
AUG 7	0	129.12a $\pm$ 10.07	69.63b $\pm$ 7.12
AUG 21	0.75b $\pm$ 0.53	55.00a $\pm$ 25.52	33.88a $\pm$ 2.13
AUG 28	0.25c $\pm$ 0.16	71.13a $\pm$ 14.90	10.75b $\pm$ 4.11
<b>Mean</b>	<b>0.33c <math>\pm</math> 0.19</b>	<b>85.08a <math>\pm</math> 11.96</b>	<b>38.08b <math>\pm</math> 5.73</b>
<b>Stage IV</b>			
SEPT 5	0	70.25a $\pm$ 14.84	11.38b $\pm$ 1.88
SEPT 11	0.13c $\pm$ .08	24.62a $\pm$ 8.51	17.88a $\pm$ 2.27
SEPT 19	0	56.50a $\pm$ 8.05	13.63b $\pm$ 1.90
<b>Mean</b>	<b>0.04c <math>\pm</math> .04</b>	<b>50.46a <math>\pm</math> 7.22</b>	<b>14.29b <math>\pm</math> 1.25</b>

<sup>a</sup> Means  $\pm$  Standard error. Means within a row followed by a common letter are not significantly different ( $p = 0.05$ ; ANOVA-FPLSD with Square Root Transformation [SAS Institute 1988]). Sweepnet technique means are based on 20, 180° sweeps per sample. Berlese funnel technique means are based on 20 stems per sample. Visual assessment technique means are based on 20 stems per sample.



Table 3. Comparison of sampling techniques for *N. bakeri* abundance, recorded in 2nd year red clover in Corvallis, Oregon (Field B) treated with pesticides during 1989

Date	Mean Aphid Abundance ( $\bar{x} \pm SE$ ) <sup>a</sup>		
	Sweep net	Berlese	Visual
<b>Stage I</b>			
JUNE 12	1.60b $\pm$ 0.60	74.00a $\pm$ 32.07	52.60a $\pm$ 9.85
JUNE 20	36.00b $\pm$ 11.70	158.10a $\pm$ 34.28	213.40a $\pm$ 55.29
JUNE 26	43.00b $\pm$ 14.79	623.10a $\pm$ 94.57	543.00a $\pm$ 71.14
<b>Mean</b>	<b>26.87b <math>\pm</math> 7.57</b>	<b>285.07a <math>\pm</math> 72.30</b>	<b>269.67a <math>\pm</math> 61.31</b>
<b>Stage II</b>			
JULY 4	19.90c $\pm$ 4.19	1061.10a $\pm$ 242.35	434.40b $\pm$ 43.76
JULY 11	0.50b $\pm$ 0.16	10.20a $\pm$ 2.08	4.80a $\pm$ 1.24
JULY 18	0.70b $\pm$ 0.34	16.60a $\pm$ 1.50	10.60a $\pm$ 3.01
JULY 24	0.30c $\pm$ 0.30	86.60a $\pm$ 19.87	29.20b $\pm$ 8.11
<b>Mean</b>	<b>5.35c <math>\pm</math> 2.16</b>	<b>293.63a <math>\pm</math> 116.16</b>	<b>119.75b <math>\pm</math> 42.97</b>
<b>Stage III</b>			
AUG 7	0.60c $\pm$ 0.19	611.50a $\pm$ 166.01	134.00b $\pm$ 32.03
AUG 21	0.50c $\pm$ 0.22	14.30a $\pm$ 2.15	3.00b $\pm$ 0.89
AUG 28	0	16.80a $\pm$ 3.04	25.20a $\pm$ 10.18
<b>Mean</b>	<b>0.37c <math>\pm</math> 0.11</b>	<b>214.20a <math>\pm</math> 90.90</b>	<b>54.07b <math>\pm</math> 18.49</b>

<sup>a</sup> Means  $\pm$  Standard error. Means within a row followed by a common letter are not significantly different ( $P = 0.05$ ; ANOVA-FPLSD with Square Root Transformation [SAS Institute 1988]). Sweepnet technique means are based on 20, 180° sweeps per sample. Berlese funnel technique means are based on 20 stems per sample. Visual assessment technique means are based on 20 stems per sample.

correct this situation, the field was mowed to a height of 5 inches on July 12. At this time and shortly thereafter no significant differences were noted between the two techniques in detecting aphid numbers ( $F= 1.09$ ,  $df=1$ ,  $p=0.03$ ). The plants began producing flowers on axillary shoots, but since these bloomed after the peak of activity, the aphid numbers were relatively low compared to the established field. On July 24th, the clover aphid reached its highest population for the season and once again the Berlese technique recorded greater numbers of aphids relative to the visual counts ( $F=31.9$ ,  $df=1$ ,  $p=0.0001$ ).

During August, Field B was in the seed stage (Stage III). The sampling techniques displayed significantly different results in aphid abundance counts per 20 stem samples for the season. Berlese counts were significantly higher than visual counts ( $F=9.92$ ,  $df=1$ ,  $p=0.01$ ). On August 7, four weeks after the first insecticide application, the aphid population had increased and reached a second peak. At this time a second application of insecticide was made. The insecticides reduced the populations of aphids in the seed crop in August. The two techniques showed no significant differences in their measurements of aphids by seed harvest ( $F=0.74$ ,  $df=1$ ,  $p=0.21$ ). The 2nd year field was cut and harvested for seed on August 29th, with Berlese funnel having significantly higher counts than visual assessment over-all for Stage III ( $F=7.28$ ,  $df=1$ ,  $p=0.01$ ) (Table 3).

Because of poor seeding and late and uneven mowing, Field A contained a high percentage of clover plants in the axil stage throughout the season, while few plants had produced buds or flowers by Stage III and IV in development. There were significant differences between techniques collecting aphids during Stage III, with Berlese funnel counts recording the higher numbers ( $F=21.60$ ,  $df=1$ ,  $p=0.0001$ ) (Table 3). After the cutting in August, the Berlese funnel and visual aphid counts were not significantly different ( $F=0.35$ ,  $df=1$ ,  $p=0.56$ ) (Table 3). By late August and early September, aphids decreased, clover lodged in the field, and the Berlese funnel generally gave significantly larger numbers of aphids on 20 stem samples than the visual technique ( $F=30.71$ ,  $df=1$ ,  $p=0.0001$ ) (Table 3).

The Berlese funnel technique was chosen to estimate aphid abundance in 1990. Field

C was sampled throughout the 2nd year of establishment (Figure 11). Clover development was similar in height and growth stages to that observed in 1989, Field B. Aphid numbers peaked in early July during Stage II (flowering) (Figure 11). During July, the field was very dry, so sprinkle irrigation was applied. The aphid population rapidly declined in Stage III, in the absence of insecticide or mowing.

The efficiency of Berlese funnel and visual assessment in terms of sampling variability during 1989 was measured by the coefficient of variation (CV). Berlese funnel had a CV range of 12.5% - 22.3%. Visual assessment varied from 12.4% - 27.7%.

### Flight Activity

In 1989, replications of three colored water pan traps (red, yellow, and green) were located in Field A and B. No significant differences in aphid attractiveness to the different colors were found among the water pan traps ( $F=2.01$ ,  $df=2$ ,  $p=0.14$ ). In 1989, the yellow bucket traps captured significantly more alate *N. bakeri* ( $2.03 \pm 0.32$ ) than did yellow ( $0.42 \pm 0.10$ ), red ( $0.30 \pm 0.09$ ), or green pan traps ( $0.47 \pm 0.14$ ) in both fields ( $F=13.64$ ,  $df=3$ ,  $p=0.0001$ ).

In Field B, the first winged aphid was collected on May 4. Alate individuals were too few to show definite peaks of flight (Figure 12). Aphid catches ranged from 0.11 - 2.50 averaging less than 1 aphid (0.97) per bucket per week over a 25 week period. A late flight of 11 aphids was recorded in November. There was no differences in number of aphid catches in any one trap location in Field B ( $F=1.16$ ,  $df=2$ ,  $p=0.32$ ).

Figure 12 visualizes three main aphid flights in Field A during 1989. Yellow buckets averaged 2.75 clover aphids per yellow bucket per week over 21 weeks, ranging from .75 to 15.75 per bucket per week. Two and a half times more aphids per bucket per week were recorded from Field A than from Field B during 1989 and 5.85 times more aphids per bucket than for Field C in 1990. Traps on the south side of the field (averaging  $3.78 \pm 9.5$  per trap)

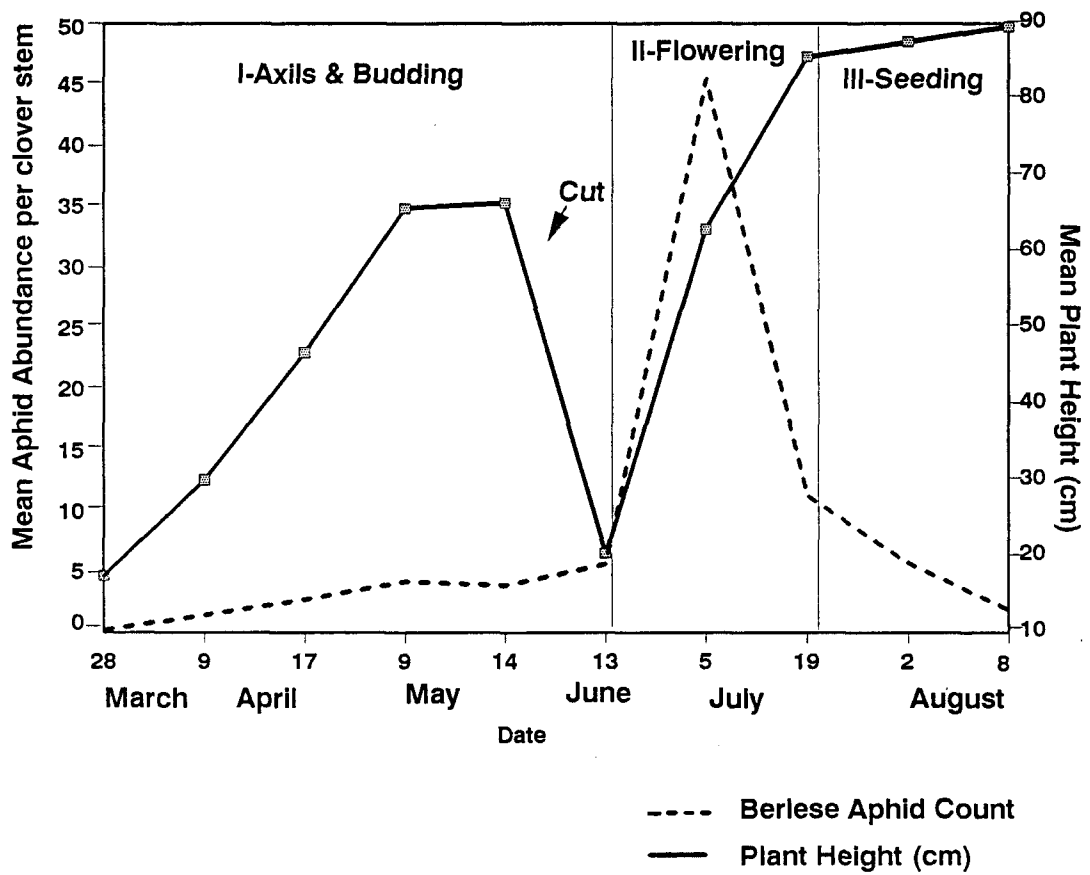


Figure 11. Clover aphid abundance in relation to clover plant development on 1990 2nd year untreated red clover (Field C) in Corvallis, Oregon

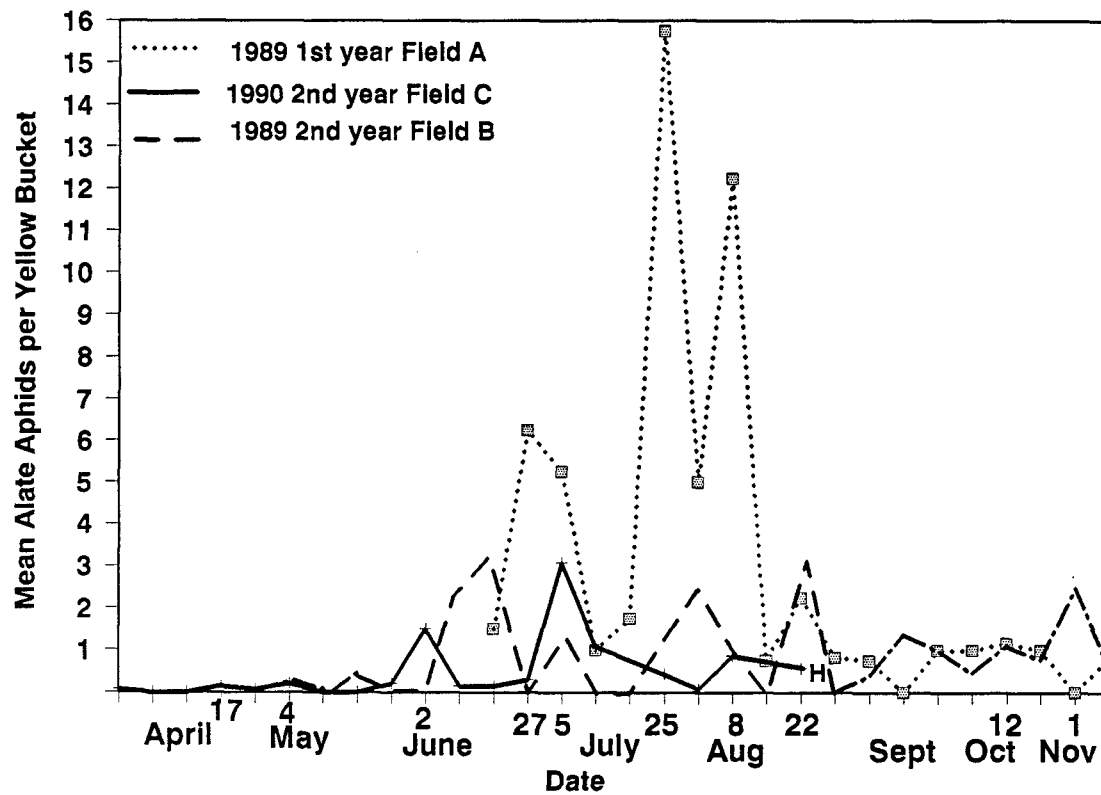


Figure 12. Aphid flight patterns as determined by yellow bucket traps in untreated 1st and 2nd year red clover fields (A and C) and 2nd year clover field (B) in Corvallis, Oregon

caught significantly more aphids than in other locations ( $F=6.05$ ,  $df=6$ ,  $p=0.0001$ ).

Because yellow buckets proved to be more effective in this study than the smaller water pan traps in monitoring the clover aphid, yellow buckets were chosen in 1990 as the only water trap type in Field C. During the 1990 season the first *N. bakeri* (Cowen) was recorded in bucket traps early in the season (March 28). Three peak flights of clover aphid occurred on June 2, July 5, and August 8 (Figure 12). An average of 0.47 winged aphids per bucket, ranging from 0.07 - 3.07 aphids per bucket were collected during 19 weeks in Field C. There was no statistical difference in trap catches in any one position in the field ( $F=0.54$ ;  $df=6$ ;  $p=0.78$ ), however trap placement on the south border of the field displayed higher total aphid numbers (36.3%) for the season.

### **Spatial Distribution**

In 1989 the mean aphid abundance ( $\pm$  SE) per lower stem half was significantly greater than those on the upper half for both Fields A and B during Stage I of clover growth ( $F=160.38$ ,  $df=1$ ,  $p=0.0001$ ;  $F=17.69$ ,  $df=1$ ,  $p=0.0001$ ) (Table 4). Clover plants were primarily in the developing axil stage of growth. Field B clover plants were taller in height and more developed in axils than in Field A. In Field A, Stage II and III (July and August), continued to be represented by axils with only a few budding plants, and showed no differences in aphids distributed on the lower or upper half of the plant ( $F=0$ ,  $df=1$ ,  $p=0.98$ ;  $F=2.24$ ,  $df=1$ ,  $p=0.14$ ) (Table 4; Figure 13). The field was mowed down to 5 inches in July and cut to the ground in August, delaying development of the clover. As a result Stage IV (September) clover plants remained in the axil stage, but a few flowers and buds were present. Aphid numbers on the basal half of the stem were significantly greater than numbers on the distal stem half ( $F=174.05$ ,  $df=1$ ,  $p=0.0001$ ) (Figure 13).

However, during Stage II (July) in Field B, clover plants flowered and grew in height and the mean numbers of clover aphid on the upper stem halves were significantly greater

Table 4. Aphid abundance in the upper and lower halves of red clover plants by visual assessment method in two fields in Corvallis, Oregon (Field A = 1st year untreated stand; Field B = 2nd year stand treated with pesticides) during 1989

MEAN APHID ABUNDANCE ( $\bar{x} \pm SE$ ) PER CLOVER STEM				
STAGE <sup>b</sup>	Field A		Field B	
	LOWER	UPPER	LOWER	UPPER
I	1.45 ± 0.13 <sub>a</sub>	0.13 ± 0.04 <sub>b</sub>	7.45 ± 0.74 <sub>a</sub>	6.04 ± 0.57 <sub>b</sub>
II	2.66 ± 0.17 <sub>a</sub>	3.07 ± 0.24 <sub>a</sub>	0.91 ± 0.20 <sub>b</sub>	5.08 ± 0.58 <sub>a</sub>
III	0.94 ± 0.09 <sub>a</sub>	1.10 ± 0.10 <sub>a</sub>	0.22 ± 0.06 <sub>b</sub>	2.03 ± 0.26 <sub>a</sub>
IV	0.70 ± 0.06 <sub>a</sub>	0.03 ± 0.02 <sub>b</sub>	---- <sup>a</sup>	

Seasonal means were transformed to square root for ANOVA-FPLSD [SAS Institute 1988]; and seasonal means in the same row within a field followed by the same letter are not significantly different ( $p > .05$ )

<sup>a</sup> Field harvested

<sup>b</sup> Stages determined by plant morphology: I=axils & budding; II=flowering; III=seeding; IV=post-harvest

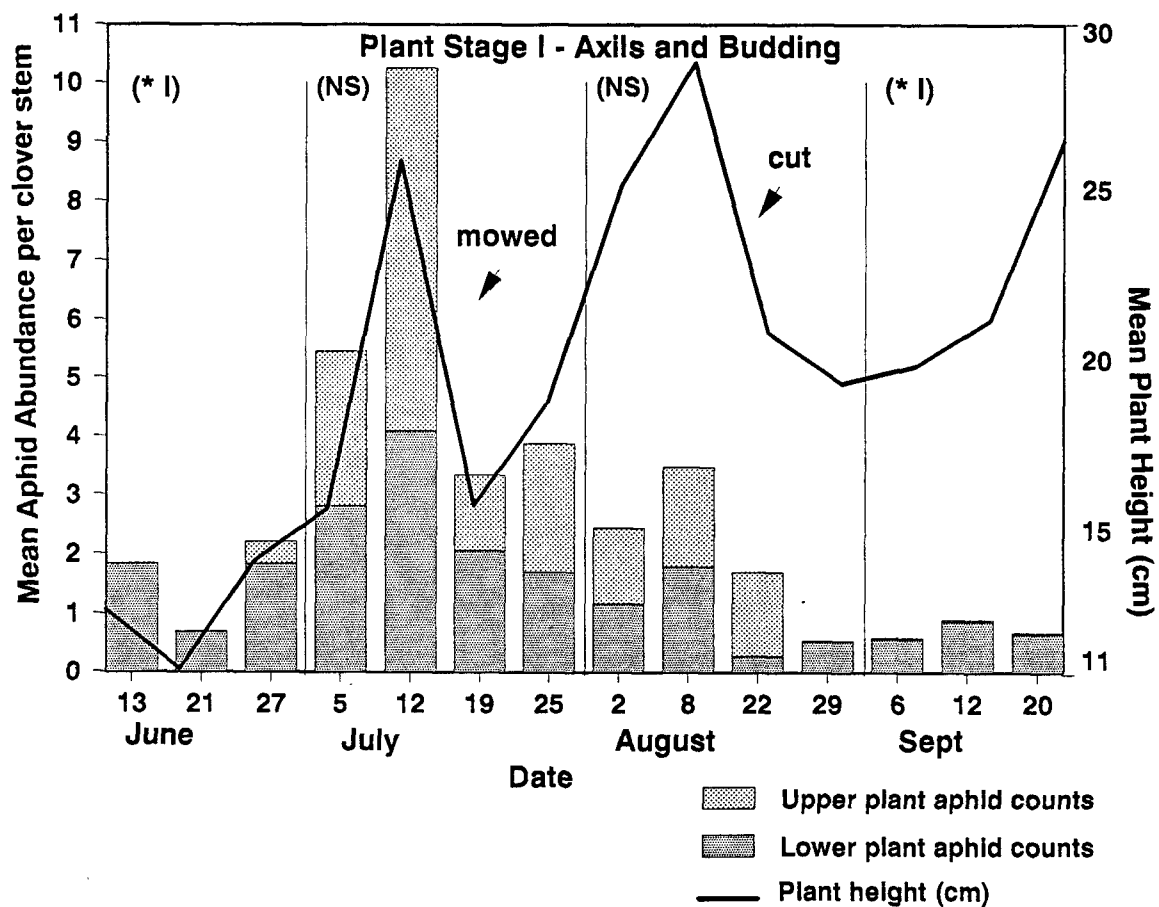


Figure 13. Spatial distribution of clover aphid relative to location on stem (upper or lower half) and plant development on 1989 1st year untreated red clover (Field A) in Corvallis, Oregon. Within plant stage divisions, significant differences in upper (u) and lower (l) distribution of aphids are indicated ( $p > .05$ ; ANOVA. [SAS Institute 1988]: \* I = aphids on lower half of plant significantly higher than on upper half of plant; NS = No significant differences.



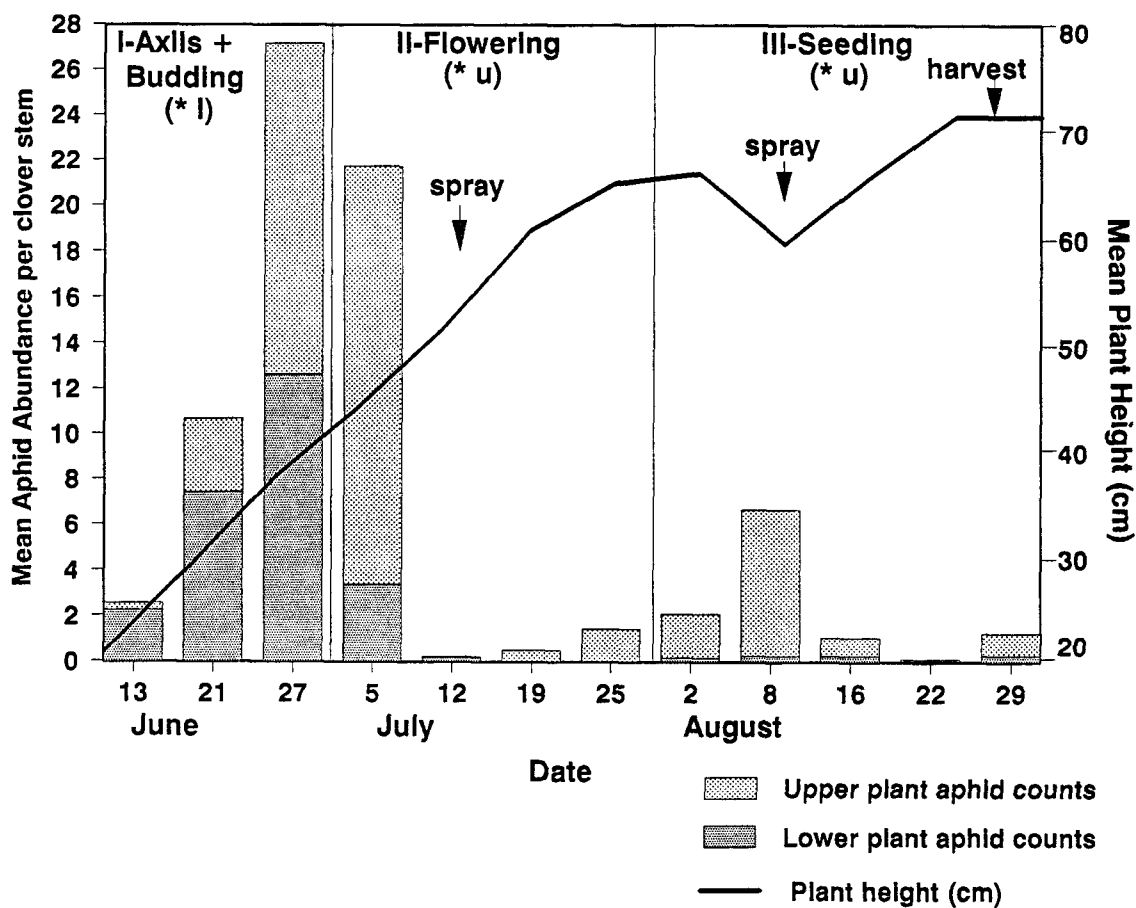


Figure 14. Spatial distribution of clover aphid relative to location on stem (upper or lower half) and plant development (Stage I-III) on 1989 2nd year red clover treated with pesticides (Field B) in Corvallis, Oregon. Within plant stages, significant differences in upper (u) and lower (l) distribution of aphids are indicated ( $p > .05$ ; ANOVA. [SAS Institute 1988]): \* u = aphids on upper half of plant significantly higher than on lower half of plant; \* l = aphids on lower half of plant significantly higher than on upper half of plant; NS = No significant differences.

than those on the lower halves ( $F=139.34$ ,  $df=1$ ,  $p=0.0001$ ) (Table 4; Figure 14). As the 1989 season progressed into the early fall, weather cooled, and clover plants lodged and seeded. Aphid numbers remained significantly greater on the upper half of the plants than on the lower half ( $F=77.78$ ,  $df=1$ ,  $p=0.0001$ ) by harvest.

### **Predator Abundance**

A summary of the most abundant predators in red clover is presented in Table 5, which include Coccinellidae, Anthocoridae, Nabidae, Arachnida, and other taxa.

In Field A, the 1st year clover field, predators were present at low levels in early June. Clover aphid density was low as well (ave.  $25.96 \pm 3.03$  aphids per 20 Berlese stems). Numbers of Coccinellidae and Anthocoridae increased in July, during the flowering season of clover (Figure 15), along with an increase in clover aphid numbers. However, after the July mowing of the clover field, the minute pirate bugs decreased three-fold; while the numbers of ladybugs increased by three-fold (Figure 15). Ladybugs were the predominant predator in July. At this time clover aphid density reached a peak of  $243.74 \pm 31.70$  per 20 Berlese funnel clover stems. Numbers of coccinellids decreased in August with declines actually occurring prior to cutting. Aphid numbers declined in August as well. The field was again cut in late August, after which Coccinellidae did not return to the previous high levels in July. Sweep net counts of adult nabids, Arachnida and anthocorids increased in August and early September, while the coccinellids remained low.

In Field B, predacious Arachnida remained at consistent numbers throughout the season in 1989. Several species of Coccinellidae were most abundant in June and July, as well as high populations of aphids sampled at  $1061.00 \pm 242.35$  aphids per 20 Berlese stems. Aphids and coccinellids decreased to very low numbers after the second chemical application of chlorpyrifos in August (Figure 16). Interestingly, damsel bugs and particularly minute pirate bugs increased to high levels in August (flowering and seeding stage of clover), after

Table 5. Arthropod predators found on red clover foliage by sweep net sampling in Willamette Valley, Oregon. Aphid means  $\pm$  SE per twenty 180° arc sweeps taken in three fields; Field A = 1989 1st year untreated clover; Field B = 1989 2nd year clover treated with pesticides; Field C = 1990 2nd year untreated clover

PREDATORS <sup>b</sup> (All life stages)	Means $\pm$ SE per 20 Sweep Net Catches					
	Field	pre- season	I-June	II-July	III-Aug	post- season
<b>Coccinellidae</b>						
<i>Coccinella trifasciata</i>	A	--- <sup>a</sup>	1.69 $\pm$ 0.42	12.34 $\pm$ 2.04	4.81 $\pm$ 1.46	0.63 $\pm$ 0.19
<i>Hippodamia convergens</i>	B	2.29 $\pm$ 0.50	2.30 $\pm$ 0.47	9.08 $\pm$ 7.30	7.30 $\pm$ 2.37	harvest
<i>Hippodamia sinuata</i>	C	3.36 $\pm$ 0.84	0.38 $\pm$ 0.18	6.50 $\pm$ 1.09	3.75 $\pm$ 0.70	harvest
<b>Anthocoridae</b>						
<i>Orius tristicolor</i>	A	--- <sup>a</sup>	2.38 $\pm$ 0.45	8.59 $\pm$ 1.08	15.68 $\pm$ 0.30	4.52 $\pm$ 0.59
	B	0	0.73 $\pm$ 0.25	4.48 $\pm$ 0.58	42.55 $\pm$ 6.80	harvest
	C	0.44 $\pm$ 0.22	0	16.75 $\pm$ 3.80	43.38 $\pm$ 4.86	harvest
<b>Syrphidae</b>						
<i>Syrphus americanus</i>	A	--- <sup>a</sup>	1.55 $\pm$ 0.30	0.53 $\pm$ 0.14	0.69 $\pm$ 0.53	0.04 $\pm$ 0.04
	B	0	0.53 $\pm$ 0.25	1.88 $\pm$ 0.31	0.43 $\pm$ 0.40	harvest
	C	0.44 $\pm$ 0.15	0	1.06 $\pm$ 0.25	0.75 $\pm$ 0.25	harvest
<b>Nabidae</b>						
<i>Nabis alternatus</i>	A	--- <sup>a</sup>	0.38 $\pm$ 0.11	2.56 $\pm$ 0.82	6.03 $\pm$ 1.27	6.69 $\pm$ 1.27
<i>Nabis americanoferus</i>	B	0.24 $\pm$ 0.10	3.20 $\pm$ 0.52	3.60 $\pm$ 0.52	9.93 $\pm$ 2.00	harvest
	C	0.28 $\pm$ 0.09	0.25 $\pm$ 0.16	5.56 $\pm$ 0.58	7.25 $\pm$ 1.46	harvest
<b>Chrysopidae</b>						
<i>Chrysopa spp.</i>	A	--- <sup>a</sup>	0.19 $\pm$ 0.10	0.44 $\pm$ 0.13	0.06 $\pm$ 0.04	0.10 $\pm$ 0.05
	B	0.12 $\pm$ 0.07	0.20 $\pm$ 0.11	1.10 $\pm$ 0.20	1.05 $\pm$ 0.22	harvest
	C	0.48 $\pm$ 0.14	0.15 $\pm$ 0.09	0.56 $\pm$ 0.18	1.00 $\pm$ 0.27	harvest
<b>Arachnids</b>						
	A	--- <sup>a</sup>	1.67 $\pm$ 0.25	2.81 $\pm$ 0.33	5.00 $\pm$ 0.70	5.63 $\pm$ 0.51
	B	2.88 $\pm$ 0.43	5.97 $\pm$ 0.96	3.10 $\pm$ 0.37	2.93 $\pm$ 0.53	harvest
	C	3.64 $\pm$ 0.43	3.80 $\pm$ 0.75	3.44 $\pm$ 0.35	16.13 $\pm$ 2.11	harvest
<b>Miscellaneous</b>						
Carabidae	A	--- <sup>a</sup>	0.88 $\pm$ 0.26	0.44 $\pm$ 0.20	0.56 $\pm$ 0.12	3.35 $\pm$ 0.91
Staphylinidae	B	0.44 $\pm$ 0.18	0.20 $\pm$ 0.09	0.43 $\pm$ 0.11	0.10 $\pm$ 0.05	harvest
<i>Geocoris spp.</i>	C	1.20 $\pm$ 0.31	0.32 $\pm$ 0.10	0	0.13 $\pm$ 0.13	harvest

<sup>a</sup> No data available

<sup>b</sup> Predator species listed are the most abundant in family named

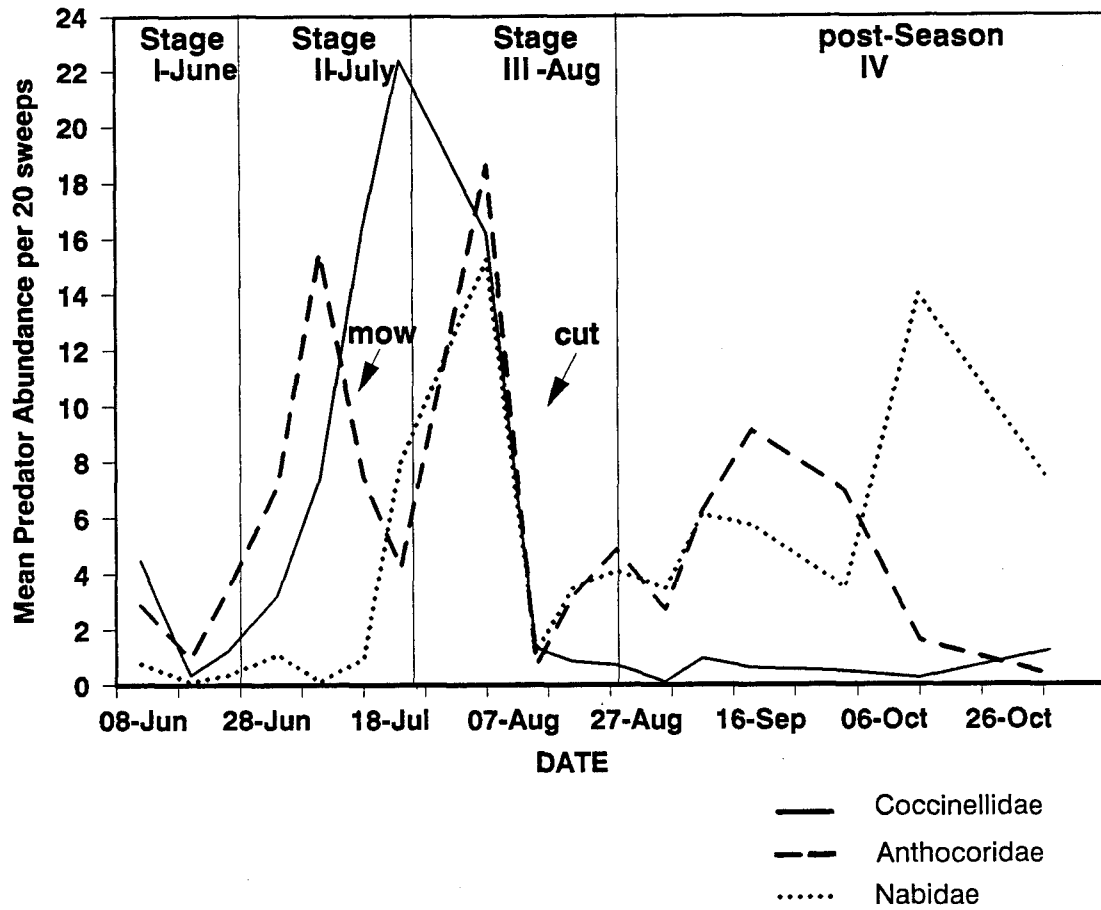


Figure 15. Predator abundance measured by sweep net technique in 1989 1st year untreated red clover (Field A) in Corvallis, Oregon

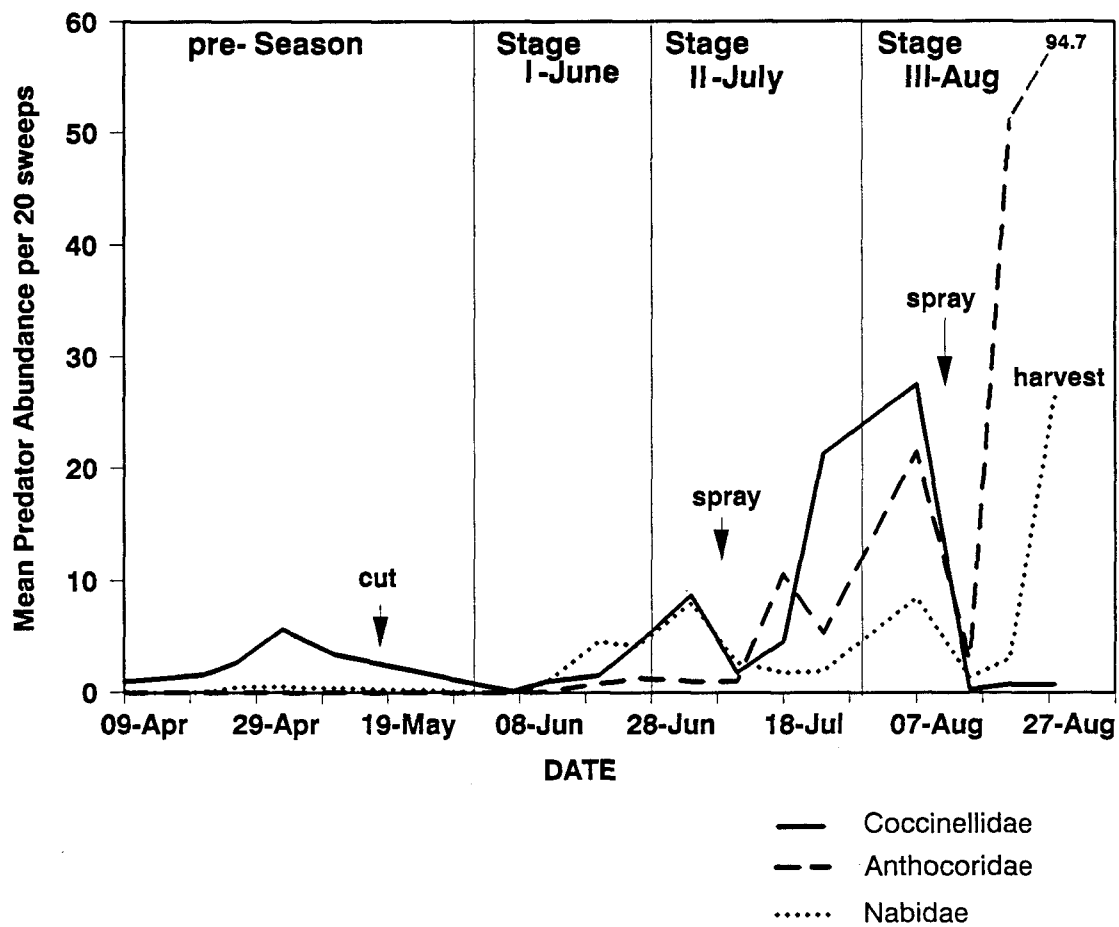


Figure 16. Predator abundance measured by sweep net technique in 1989 2nd year red clover field treated with pesticides (Field B) in Corvallis, Oregon

the August insecticide application.

Prior to Stage I, predators were surveyed in Field C (Field A, 1989) during 1990 using the sweep net technique, while species of Coccinellidae and Arachnida were the most abundant predators (Figure 17). Significant numbers of spiders were collected by sweep net during Stage I after the field was cut and subjected to rainfall. The spider population increased dramatically during Stage III. Populations of coccinellid species increased near the end of Stage I reaching peak numbers at the end of Stage II (flowering clover). In addition, clover aphids were at very high levels ( $909.9 \pm 83.0$ ) aphids per 20 Berlese stems). Ladybug and aphid populations decreased sharply during Stage III. The minute pirate bug population increased near the end of Stage I and experienced a rapid increase through Stages II and III.

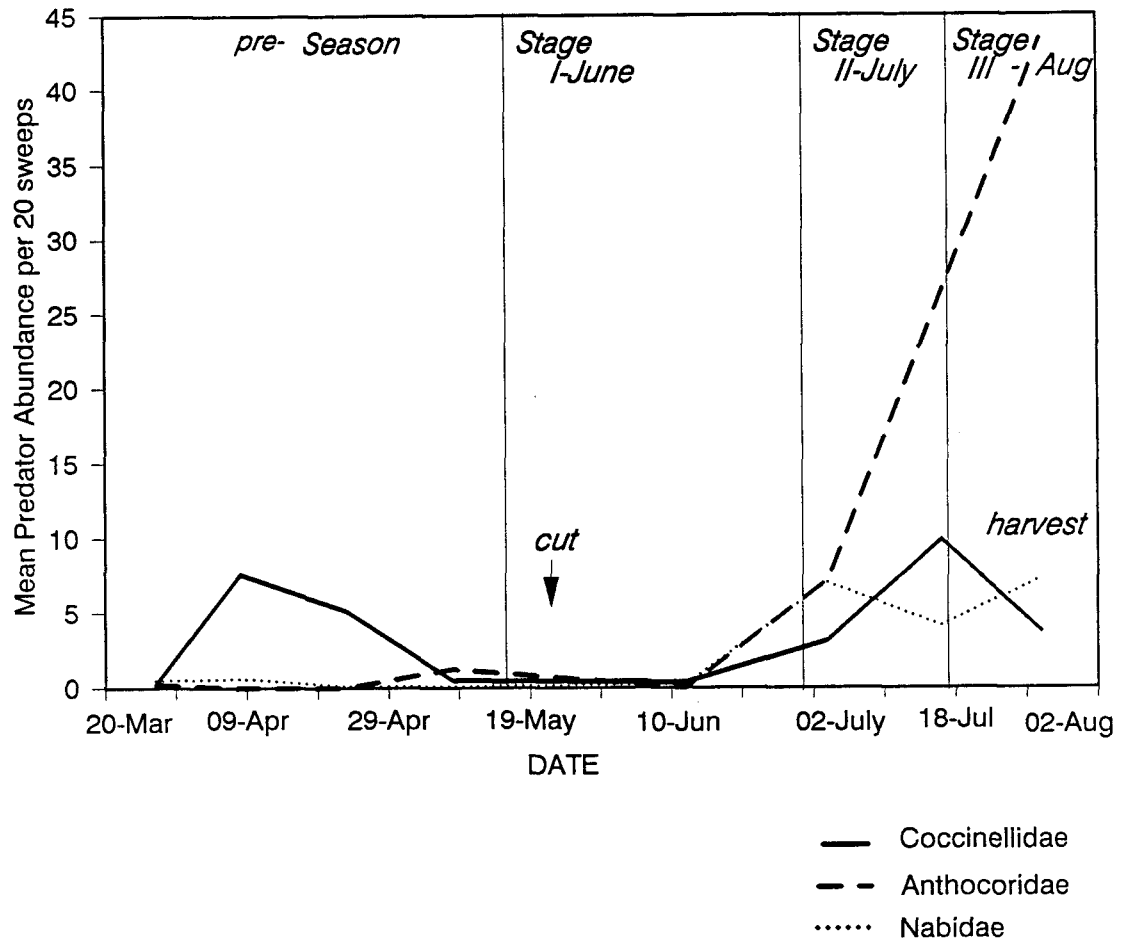


Figure 17. Predator abundance measured by sweep net technique in 1990 established 2nd year untreated red clover (Field C) in Corvallis, Oregon

## DISCUSSION

### Sampling Techniques

Evaluation of the different sampling techniques to monitor field clover aphid populations indicated that the Berlese funnel technique detected significantly greater numbers of aphids throughout the season than the other sampling techniques. However, either the visual assessment or the Berlese funnel technique can be used to provide an approximate measure of clover aphid populations throughout the season.

Sweep net sampling can provide reliable estimates of aphids for determination of control actions. One would assume, that chances of missing a more heavily infested area of aphids in the field (due to the clumped distribution of the aphid) using Berlese funnel or visual assessment, would be much greater than with the use of a sweep net. The sweep net samples more area than the other techniques, however, the sweep net does not provide an accurate measure of clover aphid populations as discussed below. Many variables can influence sweep net counts of insect species studied (Saugstad et al. 1967; Schotzko and O'Keeffe 1986, 1989). The time of day, environmental and physical factors, size and condition of plant, and the individual sweeping could account for variation in the sweep contents (DeLong 1932). Realizing these limitations, the sweep net was used for monitoring the clover aphid and other members of the community. Weeds and uneven stands prevented, in part, the successful use of the sweep net when sampling clover aphids. Short, sparse and newly developing spring plants were most efficiently sampled with higher abundance counts by the sweep net method, although very few clover aphids were actually collected compared to Berlese and visual assessment methods. As the plants grew taller and lodged, fewer of the stems were upright and available for proper sweep net sampling. Plant growth and insect dispersion can greatly affect the efficiency of the sweep net as shown in this study (Byerly et al. 1978). As the plant grew, the canopy began to close then collapse, arthropods have more



places to disperse and sampling became less efficient.

Sweep net sampling did not compensate for the location of insects within the plant canopy being sampled throughout the season. Only the upper part of the plant stem from mid to late season was sampled. Sampling only the portion of the plant canopy used by the insect at the time it is sampled would be preferable. These difficulties hinder efforts to equate samples taken using sweep net for comparative analysis with Berlese funnel and visual assessment. Also the clover aphid is usually located between the leaf axils and the stem or compacted within the head with its own honeydew, making it difficult to remove with the sweep net. In addition, the clover aphid does not have the typical arthropod behavior of dropping upon disturbance, so very few were captured in the net.

Prior to this study, the visual assessment technique was considered to be an accurate technique that might have instant field application (Costa 1988). This study showed that visual assessment often underestimates the aphid population during flowering and early seeding. Clover plants in Field A never matured because of mowing during the growing season. With shorter stems and less aphid density, visual counts were more accurate during July, differing little from the Berlese funnel count. When mature plants were inspected as in Field B, the sepals were peeled back for examination. The aphids settle deep inside of the plant crowns, sepals, and in flower heads. This caused the aphids to fall deeper into the shadows and depth of the axil, not allowing the inspector to observe all aphids. It appeared that as the clover aphid populations increased and as the plant matured in height and growth stage, visual assessment became more difficult and the variation in counts increased with increasing aphid abundance means. Foliage density, lodging in late season, and large numbers of aphids made counting very difficult. Also a decrease in the proportion of aphids counted by visual assessment may be associated with handling of the plant during the inspection process. Some of the aphids fell from the plant stems or were lost deeper in the axils with intensive handling.

It appeared that visual assessment was as reliable a technique in measuring aphids as Berlese funnel at the beginning of the season when the plants were small and when aphids were scarce. This early sampling period might be important in estimating the abundance of aphids that might develop later in the season. Soon after hay crop harvest in the spring and again near seed harvest, when aphid numbers have greatly decreased, visual assessments approximated the numbers measured by the Berlese funnel technique. During bloom the clover aphid numbers increased when measured by either method. As the clover lodged the visual assessment technique underestimated clover aphid numbers compared to those of the Berlese funnel technique. Light intensity, eyesight of the observer, inspector's technique, also affected the estimation of abundance, causing greater variation in records.

Because *N. bakeri* is small, cryptically colored, and often feeds deep in the leaf axils in dense foliage, it was necessary to extract the aphids from the plant for ease of counting. Fenton and Howell (1957) found Berlese funnels to be a good technique when sampling alfalfa stems for the pea aphid. The Berlese funnel technique had a high range of variability, but the counts consistently estimated the highest aphid numbers, relative to the other techniques. A high degree of variation in clover aphid sampling presented problems in interpretation of aphid abundance data. Aphid populations tend to have aggregated or contagious distributions (Edelson and Estes 1983). The variation is presumably caused by the non-random distribution patterns of the aphid in the field, and operators technique. The Berlese technique appears to be more responsive and sensitive to the actual population in the field than the visual assessment technique. However, there are drawbacks. Extracting many individual samples was time consuming and did not give immediate results. Although time requirements for each clover aphid sampling technique were not individually measured, high aphid numbers required increased searching time for a visual assessment and was less accurate as the numbers increased. The Berlese funnel technique required several days of drying time in the laboratory before aphids could be counted. In addition, the Berlese funnel would not necessarily be available to a clover grower.

The significance ( $p$ ) of a correlation coefficient is a function of the degree of the correlation and the sample size. Correlations were calculated between techniques over several julian dates according to plant 'Stage' to increase the data points, and improve the strength of the relationships. Stages were based on clover plant height and plant development over the season.

The Berlese funnel and visual assessment technique were highly correlated in their assessments of aphids in all three stages of plant development in Field B, 2nd year red clover. In Field A, no correlations were found. Field B consisted of 30 acres of a relatively even stand of clover with very few weeds to interfere with application of the technique, unlike the small 1/4 acre newly planted clover field (Field A). Field A displayed inconsistent stages of plant growth due to uneven field cutting, weed competition, poor distribution in planting of seed. Field B also had more aphids present than the newly planted field. Possibly the aphids were distributed a little more evenly throughout the field after a year of establishment. The new field was only beginning to acquire populations of aphids. Variations in computed correlations may be accounted for by differences in distribution patterns, variation in plant stage differences, and operators techniques. In general, as Berlese funnel counts increased, so did visual counts. After clover aphid populations exceeded approximately 500 aphids per 20 stems in Stage I, and/or when the plant matured into Stage II, visual assessment counts, independently from Berlese funnel counts, decreased and leveled off.

Both Berlese funnel and visual assessment technique are adequate clover aphid sampling techniques. Berlese funnel and visual assessment gave similar results at the beginning of the clover season when clover aphid populations were low and plant size was manageable, but as the season progressed and the clover aphid population increased, Berlese funnel technique was more sensitive to changes in the aphid population. Visual assessment could be used to classify the early season populations and knowing the certain levels of precision that it offers. During flowering season, a Berlese funnel count could be taken to assess a more absolute aphid population count. In summary, both methods of sampling can

be used to estimate clover aphid abundance, since aphids not readily collected by one technique in the early season may be collected by another, later in the season.

### Flight Activity

In many aphid species, flight plays a significant role in the dispersion of aphids between and within fields. Water traps can indicate which seasons and under what conditions flight, if any, takes place. The aerial movement of aphids is important in anticipating necessary control measures. Investigating the movement of aphids in the atmosphere has usually involved sampling with sticky or water pan traps (Moericke 1955; Taylor 1965; Heathcote et al. 1969). Aphid catches using these methods can be affected by placement, color, light, temperature and wind speeds (Taylor 1963).

The weather station at the Hyslop farm, approximately 2-3 miles from the field sites, recorded prevailing winds from the southwest. Placement of the traps in this study were based on wind direction and the knowledge that many aphid species infestations occur at field edges (Johnson 1949). Traps were placed on the borders of the fields, adjusted appropriately to the plant height and colored traps were displayed against a tan background. Broadbent (1948) found that trapping efficiency was increased when pans were situated above ground level at plant canopy. Water pan traps are best used at vegetation level where the wind speed is low and where their angular outline will cause least turbulence in the air flowing past them (Lewis 1959). Aphids appear to fly upwind behind trees because the lee shelter slows the wind allowing the aphid to land (Lewis 1967). Moericke (1955) showed that a yellow pan against a bare soil background of vegetation is more effective in trapping aphids than a pan against a background of vegetation.

Larger numbers of alate clover aphids were collected on the south side of the fields, but the numbers were not highly significantly different. Windbreaks formed on the south by filbert trees near Field A and the coniferous and oak trees near Field B provided a possible

shelter effect, perhaps influencing the numbers of aphids in buckets. Aphids can take-off in wind (Haine 1955) and almost all winds have lulls that permit take-off in the shelter of vegetation. Lewis and Stephenson (1966) described the increased numbers of flying insects near natural windbreaks of trees and hedges. Wind blows small insects into the sheltered zone near windbreaks because the air there moves more slowly and they are therefore less likely to be blown away (Lewis 1967). Studies, therefore suggest that the numbers of aphids caught in water traps placed in different positions in the clover field, might be associated with shelter and with wind direction. Because these clover fields were rather small in size, one might expect that each side of the field received equal wind and that aphid numbers in traps on one side of the field did not differ significantly.

Problems considered when using water pan traps for surveying aphid flight (van Emden 1972) are dust from access roads, animal disturbance, placement and attractiveness in relation to movement or disturbance (van Emden 1972). Water pan traps require frequent attention or the aphids rot and water evaporates when exposed to high temperatures and wind. Aphids may be lost when the traps over flow during heavy rains or unexpected field irrigation, unless traps are fitted with an over flow filter. Pan traps were less practical than bucket traps. Buckets were easier to service and more dependable in recovering alate aphids due to the increase in volume, filter system and possibly color and size attractiveness. Results with *N. bakeri* confirm the attractiveness of yellow traps to the clover aphid species, although the numbers of alate were minimal relative to other aphid species (*M. persicae*, *A. fabae* Scopoli, etc) captured. Results from other studies confirmed that many aphids that feed on dicotyledons are more attracted to yellow than species that feed on grasses or sedges (Heathcote 1957, Palmer 1952 and Taylor 1965).

Each species of aphid varies in its flying pattern, and attractiveness to color, alighting behavior, height of flight, willingness to alight and ability to land (van Emden 1972). The optimum color of the trap, light intensity, and changes in environment differ with different species of aphid. For example, *M. persicae* is attracted to yellow 80 times as strong as *S.*

*graminum* (van Emden 1972). If the clover aphid is attracted to clover by color, there may have been differences in spectral characteristics of the water pan and bucket traps that reduced the suitability and number of catch of this species of aphid. The response of *R. maidis* to yellow differs with intensity and amount of sunlight in which the aphid is attracted to yellow. It has been shown that the color of the paint from various manufacturers behave differently in the number of aphids attracted (van Emden 1972; Lewis 1959). The "yellow" paint used on pan traps most likely is not the same yellow as the bucket nor the yellow used in other experiments. It is important to specify the yellow pigment used for comparison of results. There could be variable levels of attractiveness of the yellow traps to different aphid species. To pursue this study further one might determine the percentage of reflectance of all trap types and colors by use of a spectrophotometer equipped with a color analyzer reflectance attachment.

During the flight study there was no indication of major flight peaks, particularly none in the 2nd year field, Field B. It may be that alates were not affected by color or placement of water traps, although, none of the other techniques showed much alate activity either. This might suggest that flight is not an important factor in within-field dispersion of the clover aphid and is not the major element in the infestation of new fields. Red clover fields could become initially infested from surrounding roadside clovers or from other secondary host plants in the area. However, winged migration is still likely to be the only means by which new infestations of the clover aphid are established. Perhaps a field, such as Field A, was initially infested by a few migrants from other fields. More alate aphids were recorded in the 1st year newly planted field. It seemed improbable at first, that a great area of clover could become infested by means of relatively few migrants which developed on the winter host trees. But even though the number of winged aphids was comparatively small, it is probably sufficient to bring about a general infestation of clover. Flight activity was recorded early in the season in 1990. Unseasonably, high temperatures in the first nineteen days with a maximum temperature of 84°F in April might explain the early flight activity.

In 1989, the water traps in the newly planted field had the highest number of winged aphids on July 25, and on this date the Berlese funnel sample of 20 stems showed the highest number of aphids. In Field C in 1990, peak catch in the water traps occurred on July 5th, which correlated with the highest count of aphids on stems sampled by the Berlese funnel. This suggests that high density of aphids correspond to aphid flight trends. Field B, the established commercial field, aphids were extremely abundant on clover stems on July 5th, but were treated with an insecticide, thus decreasing the aphid numbers, possibly lessening the need for flight.

According to Smith (1923) the ratio of apterous to alate clover aphids was 1 winged to 4.4 apterous. Only a small number of migrant aphids (males were identified) develop in autumn and go onto fruit trees in the fall, the greater percentage of the aphids remaining on the clover attempt to pass the winter, as was observed in overwintering crowns using Berlese funnel in this study.

I believe the hypothesis that the degree of crowding of newly molted alates and nymphs determines whether aphids fly (Dix and Wangbaonkang 1983). In these clover fields, clover aphids appear to be evenly dispersed amongst the axils and heads. Little overcrowding and flight activity was observed. Winged adults were seldom seen in the fields, although increased numbers were recorded in the newly planted field, A. Other factors such as insecticide application, or early clover hay cutting may keep clover aphid populations low. A personal observation of caged clover aphids demonstrated that alates were produced under crowded conditions. A large number of alates appeared as the aphid density increased in the limited space of a cage. Stress on high population densities, competition among aphids, or decreasing host suitability would induce winged aphid flight (Minks and Harrewijn 1987). Density of alate and apterous aphids may be dependent on the size or condition of the field, and/or particular polymorphism of the clover aphid (Hille Ris Lambers 1966).

Clover aphids have been reported as being host-alternating species, requiring two or more host plant species to complete their life cycle (Smith 1923). There is a possibility based

on small numbers of alates, that a monoecious mode of life could be evolving from a heteroecious one as did the aphid, *M. persicae* (Sulzer). For instance, *M. persicae* (Sulzer), exhibits a heteroecious holocyclic type of life cycle in areas with cold winters, using *Prunus* spp. (Rosaceae) as the primary host and various herbaceous plants as secondary hosts (Minks and Harrewijn 1987). But in more temperate regions the species is anholocyclic, on the basis of the sexually producing generation, on its secondary host (Blackman 1974). Clover aphid population introduced into the Old World are apparently entirely parthenogenic on secondary hosts, like *Trifolium* spp. (Blackman & Eastop 1984). Non-migratory winged aphids do not always remain on their host but may undertake short flights (Kring 1972). Adult and nymph aphids can be redistributed in clover by walking from one leaf to another within the canopy (Kring 1972).

### **Spatial Distribution**

As the clover plant developed, the aphid population increased in the upper half of the plant. The clover aphid was more abundant on the lower half of the plants early in the season. Many of the aphids were observed at the crown level. Cool weather of the early spring could have played a role here in keeping the aphids low to the ground. Clover aphids became more numerous on the upper half of the plant, particularly in the flower heads of Field B as the season progressed. By late season, during seed set, clover aphid numbers decreased and clover aphids were once again more numerous on the lower half of the plant. Early in Stage II, which occurred in the month of July, aphid populations peaked in 1st and 2nd year Fields (A and B), with greatest numbers appearing in the upper half of the plant. Archer and Bynum (1986) described greenbug infestations and their upward movement on sorghum plants. They speculated that this movement might be related to changes in host physiology as plants matured. The same may be true for the clover aphid. When the fields were treated with insecticide, mowed, or in the late developmental stages of growth, aphid numbers decreased



on the upper stem half and higher numbers were observed on the lower half of the plant. Clover aphids were distributed relatively evenly on the plants of the newly seeded field which was cut several times during the season and not allowed to flower.

### Predator Abundance

The Coccinellids, an aphidophagous predator, may play a primary role in suppressing high population of aphids in the early budding and flowering stage of clover. The ladybug numbers declined after insecticide application, and when temperatures dropped in August; clover aphid populations dropped at this time. The ladybugs appear to respond to density of aphid populations (Frazer et al. 1976). This is supported by Leather and Lehti (1982) who observed oat-bird cherry aphid populations increase in wheat, barley and rye, and coccinellid populations increasing as well. Ladybugs decreased when aphid numbers were reduced. Rockwood (1952) observed that beetles occurred in great numbers when the pea aphid was in great abundance and conversely, the population was sparse when the pea aphid was reduced. The ladybugs could have a great impact on the aphid population in the spring, because of their voracious feeding habits, powers of dispersal, and the fact that both larvae and adults feed on aphids (Rockwood 1952). The clover aphid situates itself deep in the stipules and at the bases of blossoms, making accessibility difficult for the large size of both the ladybug larvae and adult (Smith 1923). Interestingly, coccinellid numbers remained high even after the clover was mowed, suggesting that the practice of mowing was not disruptive to the predators.

The aphid population rapidly declined in Stage III of Field C, in the absence of insecticide or mowing. This decline may be attributable to the presence of predation, irrigation practices, and/or decrease in reproductive activity on mature clover. Smith (1923) related a sudden disappearance of the clover aphid to effects of irrigation, drowning, and various natural controls, including a parasitic fungi.

The anthocorid populations, were not greatly reduced by the insecticide treatment in August in Field B, and even surpassed prespray levels rapidly. The omniphagous or general predators, like the nabids, spiders and the anthocorid species, were quite abundant, especially at the flowering and seeding stage of the clover plant. When Field A was mowed in July, the numbers of anthorcorids in samples were reduced, perhaps due to migration to other parts of the plant or outside of the field.

Spider populations were consistently high throughout the season and may have played a role with other generalist predators in suppressing the aphid. Riechert and Bishop (1990) found prey numbers and levels of plant damage to be lower in plots with enhanced spider densities.

In addition, aphid parasites were collected later in the season tucked under the axils of clover stems. *Aphelinus lapsiligni* Howard was reared from clover aphid mummies. Smith (1923) reported that 97 per cent of clover aphid parasitism is due to this species. The only other recorded host for *A. lapsiligni* is *Brachycaudus helichrysi* Kalt., also on clover (Johansen 1957). Hyperparasites are very commonly obtained from *A. lapsiligni* Howard according to the literature (Smith 1923).

Chrysopids, Mirids, and *Georcoris sp.* were found in the early season. Clancy and Pierce (1966) report that common hemipterous predators feed on *Lygus* nymphs and aphids in alfalfa, but are frequently parasitized. The impact of each predator on the clover aphid is unknown, and future studies are needed to determine the possibilities of potential biological controls on the clover aphid (Tamaki et al. 1974).

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