APHID TRANSMISSION OF BLUEBERRY SHOESTRING VIRUS AND SEASONAL POPULATIONS OF ITS VECTOR *ILLINOIS PEPPERI* (MACGILLIVRAY)

By

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ABSTRACT

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AND SEASONAL POPULATIONS OF ITS VECTOR
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The quantity of blueberry shoestring virus (BBSSV) taken up by its known aphid vector Illinoia pepperi (MacGillivray) reached a threshold with an acquisition access period (AAP) of 24 hr. Transmission occurred with a 24 hr AAP and a 1 hr inoculation access period. Field populations I. pepperi were monitored weekly from May through September. Populations alatae and apterae were greatest in June. Apterae were found throughout the growing season; few alatae were observed after mid-July. Individual I. pepperi were tested for BBSSV with radioimmunoassay (RIA). Percentages of viruliferous aphids ranged between 5 and 15% throughout the season. There was wide variability in the quantity of virus detected in individuals with up to 250 ng BBSSV detected per aphid. Field transmission of BBSSV to blueberry trap plants occurred throughout the season; however, incidence of infection was highest in May and June when the I. pepperi populations were greatest.

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INTRODUCTION

Blueberry shoestring disease, caused by blueberry shoestring virus (BBSSV), is an economically important virus disease of highbush blueberry, Vaccinium corymbosum, L. In Michigan, the nation's leading producer of highbush blueberries, blueberry shoestring disease is the most widespread virus-caused disease of highbush blueberries. Infected bushes have decreased vigor and are eventually debilitated by the disease. The only known vector of BBSSV is the blueberry aphid, Illinoia pepperi (MacGillivray). In the past, sole control of the disease consisted of rogueing infected bushes to remove the source of inoculum. Only recently, growers have begun to spray insecticides to control the aphid vectors in addition to removing the diseased bushes. Prior to this work there had been no epidemiological studies of blueberry shoestring disease. The aphid-vector relationship, aphid dispersal, aphid population dynamics, and aphid-mediated transmission all needed to be studied in order to develop better control measures for the disease.

The first research objective was to determine the optimal times for BBSSV acquisition and inoculation by blueberry aphids. The virus-vector relationship plays an important role in determining whether or not insecticidal sprays may be effective in preventing the spread of an aphid-vectored plant virus.

The second research objective was to determine if blueberry aphids overwinter within the blueberry field. At the time this research project was started it was unknown whether blueberry aphids overwintered within the blueberry field or immigrated into the field from an outside source. It was suspected, however, that the aphids overwintered within the field.

The third objective was to monitor the movement of alate blueberry aphids inside and outside an isolated blueberry field with yellow pan traps and to determine the percentage of alatae which were viruliferous. In western Michigan there are often blueberry fields adjacent to each other. Winged (alate) blueberry aphids could probably easily fly to adjacent blueberry fields and spread the disease. Recently developed ultrasensitive serological assays would be used to determine if the vectors carried virus.

The fourth objective was to determine the seasonal blueberry aphid population trends within the field and to determine the percentage of wingless (apterous) blueberry aphids that were viruliferous. These data would provide information for timing control measures.

Lesney et al. (1978), using van der Plank's model, which tests for randomness of spread of a plant disease (van der Plank, 1944), determined that shoestring disease spreads down the row. Within the field, blueberry bushes usually touch and overlap adjacent bushes. This provides a natural avenue for walking aphids to move to adjacent plants and transmit virus. The fifth objective was to determine whether or not BBSSV is as likely to be transmitted to adjacent

trap plants not touching BBSSV-infected source plants as trap plants which touch source plants.

The final objective was to determine when during the season BBSSV-infection occurs within the field and at what levels relative to aphid populations. This study would provide information for timing control measures.

All of these objectives were directed toward having a better understanding of the spread of blueberry shoestring disease, which would eventually lead to the development of better control measures.

LITERATURE REVIEW

Blueberry Shoestring Disease and the Causal Virus

Blueberry shoestring virus (BBSSV) disease was first reported in New Jersey on highbush blueberry, Vaccinium corymbosum L., by Varney (1957). Since that time, shoestring disease has been reported in Michigan (Stretch & Hilborn, 1970), Washington State (P. Bristow & D. Ramsdell, unpublished data), North Carolina (R. Milholland, personal communication, 1983), and Nova Scotia (Lockhart & Hall, 1962). The probable mode of spread of shoestring disease of blueberry to these areas was through infected nursery stock which could be traced back to New Jersey (J. Nelson, personal communication, 1983).

In Michigan, shoestring disease is the most widespread virus-caused disease of highbush blueberry. A 1983 Michigan Department of Agriculture survey of Ottawa County, which produces 39% of the state's blueberry crop, identified 2435 shoestring diseased plants on the basis of symptomatology (H. Marlow, personal communication, 1983).

The most common symptom on shoestring diseased plants is elongated reddish streaking on current and 1-year-old shoots. Severely affected leaves are crescent or strap-shaped. It is this strap-like "shoestring" symptom that is the basis for the descriptive shoestring disease name. Other common symptoms of the disease include red vein banding or red oak leaf patterns and a red to purple cast to

immature berries. In addition, berry production progressively decreases as the infected bushes decline in vigor.

Hartmann, Bath, and Hooper (1973) found virus-like particles (VLPs) in epidermal, palisade, spongy mesophyll, and xylem parenchyma. They did not, however, find VLPs in the phloem vascular tissue. In addition, crystalline arrays of VLPs were found in leaf epidermal cells and root xylem cells, with larger masses of VLPs in the roots.

Transmission studies by Lockhart and Hall (1962) and localization studies by Hartmann, Bath, and Hooper (1973) indicated a virus-like causal agent of shoestring disease. It was not until later that Lesney et al. (1978) showed that shoestring disease is caused by a virus--blueberry shoestring virus (BBSSV).

Blueberry shoestring virus is a spherical single stranded (ss) RNA virus that is 28 nm in diameter (Ramsdell, 1979a). It is not serologically related to viruses with similar physical and chemical properties (Lesney et al., 1978), but it does have physical and chemical properties that are similar to those of members of the southern bean mosaic virus group (Ramsdell, 1979a,b).

The host range of BBSSV is quite limited. The only known host plants are highbush blueberry, V. corymbosum (Varney, 1957) and lowbush blueberry, V. angustifolium (Lockhart & Hall, 1962). The virus can be transmitted between blueberry plants by chip budding (Lockhart & Hall, 1962; Schulte, 1983) and rub-inoculation using purified virus (Lesney et al., 1978). Attempts to transmit purified BBSSV to herbaceous plants have been unsuccessful (Lesney et al., 1978).

Blueberry shoestring virus has been shown to be vectored by the blueberry aphid, Illinoia pepperi (MacGillivray). The virus was transmitted by blueberry aphids having acquisition access periods of 2 min and inoculation access periods of 100 hr (Ramsdell, 1979b).

Biology of the Blueberry Aphid

The blueberry aphid, I. pepperi, is commonly found in areas of blueberry production in Michigan (Giles, 1966; Elsner, 1982). A general life cycle of I. pepperi is shown in Figure 1 (Elsner, 1982). The egg stage overwinters on or underneath the blueberry bush. Apterous (wingless) female aphids emerge from the eggs and produce second-generation viviparous females that reproduce parthenogenically. Many of the second-generation aphids develop into alate (winged) adults that migrate to other blueberry bushes where they produce apterous young. Some colonies produce alate aphids at a constant rate (approximately 2%) throughout the season (M. Whalon, personal communication, 1984). Newly colonized bushes subsequently support several generations of apterous females during the growing season. As the blueberry leaves age physiologically toward the end of the season, the aphid population declines. Very late in the season the few remaining viviparous aphids produce oviparous females. These oviparous females produce the overwintering eggs.

Giles (1966) noted that the blueberry aphids preferred the upper surface of the blueberry leaves as the primary feeding site. Elsner (1982) reported, however, that the blueberry aphids prefer to feed underneath tender leaves, on succulent growing shoots, and on swelling buds of the blueberry plant. The feeding aphids are sessile unless

Figure 1.--Seasonal life cycle of the blueberry aphid, Illinoia pepperi (MacGillivray). (From Elsner, 1982.)

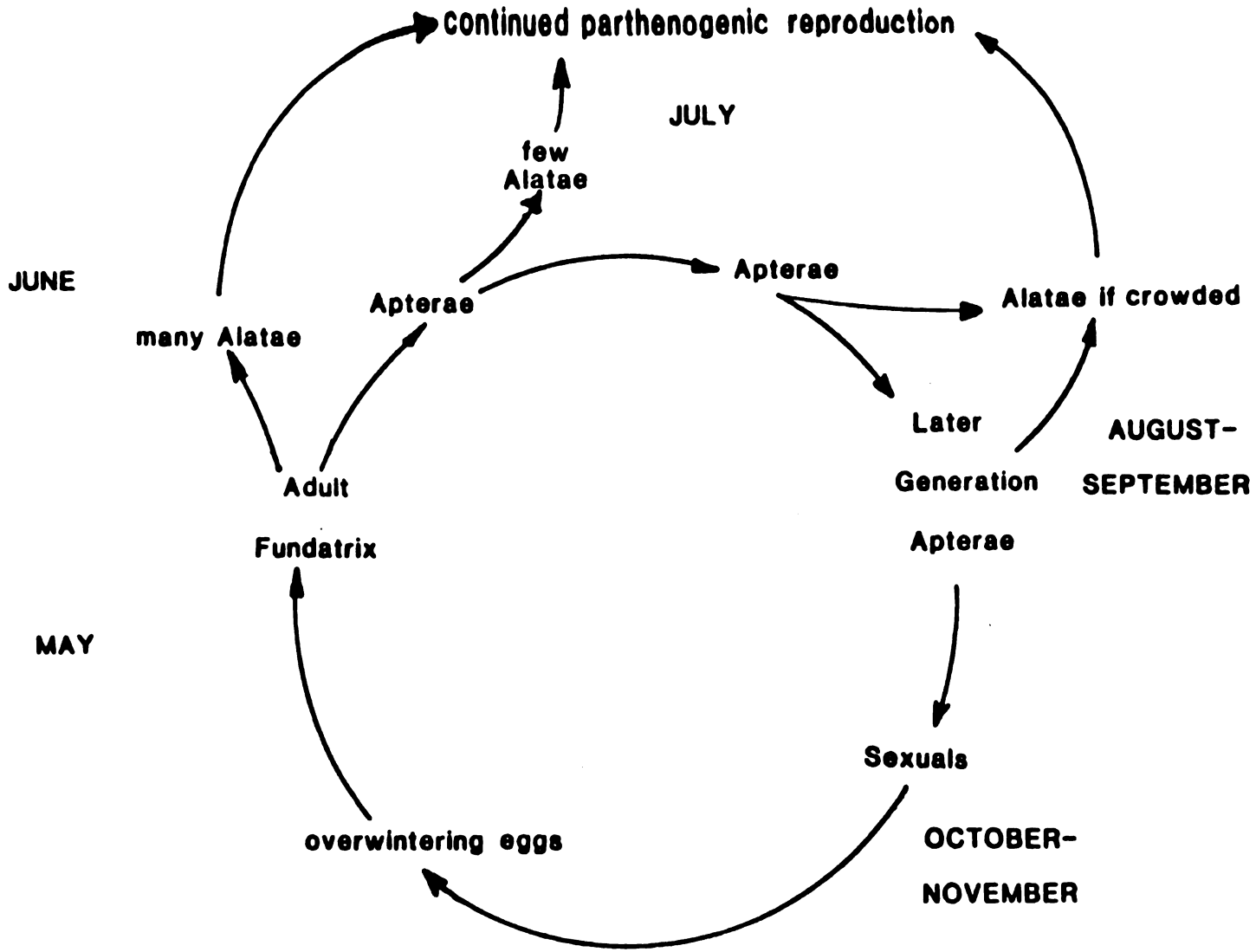
continued parthenogenic reproduction
JULY
few
Alatae
JUNE
Apterae
Apterae
Alatae if crowded
many Alatae
Later
Generation
Apterae
AUGUST-
SEPTEMBER
Adult
Fundatrix
MAY
Sexuals
overwintering eggs
OCTOBER-
NOVEMBER

Figure 1

crowded conditions or poor food quality cause them to move (Elsner, 1982); once disturbed, however, the aphids readily move.

Blueberry aphids have been observed feeding and reproducing on woody plants other than V. corymbosum (Elsner, 1982). These plants included Quercus rubra (red oak), Nyssa sylvatica (black gum), Acer rubrum (red maple), Ilex verticillata (winterberry holly), and Prunus spp. They did not, however, seem to be significant alternate hosts (Elsner, 1982).

Plant Virus Transmission by Aphids

Plant viruses must be able to disperse to new plants in order to reproduce. Common modes of plant virus transmission include transmission through seed, pollen, and infected propagation stock. The most common mode of plant virus transmission in nature, however, is by insect vectors. The insect order Homoptera contains the largest number of plant virus vectors. Included in this order are the aphids (suborder Sternorrhyncha), which vector approximately 200 different viruses (Harris, 1981; D'Arcy & Nault, 1982).

There are three classifications of plant virus transmission by aphids: nonpersistent, semi-persistent, and persistent based upon the length of time the virus is retained by its vector. Nonpersistent virus transmission was characterized by Watson and Roberts (1939) as having very short acquisition and inoculation threshold times on the order of minutes. Both acquisition and inoculation of virus occur during the brief periods that aphids probe or sample the host plants. In addition, virus is retained in its vector for very short intervals. Other characteristics of nonpersistent viruses are that they are not

retained through a molt, there is no latent period after acquisition before virus can be transmitted, and there is increased efficiency of virus transmission with preacquisition fasting of the aphids.

Kennedy, Day, and Eastop (1962) suggested that the term "stylet-borne" be used instead of nonpersistent. They believed that the virus is carried on the vector's stylet. These nonpersistent viruses are said to have a low vector specificity (Sylvester, 1969) because they can be transmitted by many different aphid species.

Examples of viruses that are transmitted in a nonpersistent manner are those that are members of the following virus groups: potyviruses, cucumoviruses, carlaviruses, caulimoviruses, and alfalfa mosaic virus.

Watson and Roberts (1939) also described persistent transmission. Persistent transmission is characterized by very long acquisition and inoculation time thresholds. The term "persistent" relates to the long length of time (days) that these viruses are retained by their vectors. Black (1959) called this type of virus-vector relationship "circulative." These circulative viruses are believed to pass through the vector's gut lining into the hemolymph, where they circulate and bathe the internal organs. Eventually the virus passes into the salivary glands from where the virus is inoculated into the host plant. There is a characteristic latent period between acquisition and inoculation that corresponds to the time it takes the virus to reach the salivary glands of the vector. In addition, since the virus is associated with the hemolymph and internal organs, it is retained through a molt.

Luteoviruses, which include barley yellow dwarf virus, beet western yellows virus, and potato leafroll virus, and two other

nonluteoviruses, lettuce necrotic yellows virus and sowthistle yellow vein virus, are examples of viruses transmitted by aphids in a persistent manner.

Those viruses which are acquired and inoculated after intermediate acquisition and inoculation times (hours to days) were termed semi-persistent by Sylvester (1956). Day and Venables (1961) suggested that these viruses are stylet-borne, but with different physical properties and in different distributions in the host plant tissues. Semi-persistent viruses are retained by the vectors for 1 to 2 days. There is no requisite latent period before the vector is able to transmit the virus.

Closteroviruses such as citrus tristeza virus, beet yellows virus, and beet yellow stunt are semi-persistent viruses.

Currently, the terms nonpersistent, semi-persistent, and persistent are most commonly used in virus-vector relations studies.

Ramsdell (1979b) reported transmission of BBSSV to blueberry seedlings by I. pepperi having acquisition access periods (AAPs) of 2 min and inoculation access periods (IAPs) of more than 100 hr. Transmission did not occur with AAPs of 1 or 24 hr. Viruses that are taken up and transmitted with short AAPs on the order of minutes are nonpersistent viruses. Optimal IAPs for nonpersistent viruses, however, are also short, being on the order of minutes or a few hours rather than several days. The results of the BBSSV transmission test indicate that BBSSV does not clearly fit into any of the virus-vector relationship classifications. Additional experiments need to be

conducted to determine what type of virus-vector relationship BBSSV has with its aphid vector.

Virus and Vector Sources

Crop plants, weeds, and seeds are common sources of plant viruses. Many virus diseases of potato are perpetuated through infected seed pieces which provide virus sources for aphid dissemination. Potato virus diseases that are transmitted by aphids include potato virus Y (PVY), potato virus A (PVA), potato virus M (PVM), potato aucuba mosaic virus (PAMV) (transmitted with helper virus PVA), alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), and potato leafroll virus (PLRV) (Beemster & Rozendaal, 1972). All of these viruses are nonpersistently transmitted except for PLRV, which is persistently transmitted.

Overlapping crops often serve as virus sources. Mangold clamps in England are virus sources of beet yellows virus, beet mild yellowing virus, and beet mosaic virus (Broadbent et al., 1949), all of which infect nearby sugar beet fields. The clamps also serve as protective sites where the virus vector, the green peach aphid [*Myzus persicae* (Sulz)] overwinters.

The beet crop itself is the main source of beet yellows virus in both the United States (Duffus, 1963) and in Europe (Broadbent et al., 1949). Shepherd and Hills (1970) have reported that beet western yellows virus overwinters in the first-season beet plants and suggested that new beet fields be planted up to 20 miles away from overwintering beet fields.

Perennial crops are important as continuous virus sources. Viruses of perennial plants are often spread through infected

propagation stock. Blueberry shoestring disease is a prime example of this. Many of the BBSSV-infected fields in Michigan were planted with infected nursery stock which originated from New Jersey (J. Nelson, personal communication, 1983).

Duffus (1971) has reviewed the role of weeds in the incidence of virus diseases. Quite importantly, weeds may be a reservoir of both viruses and their aphid vectors.

Certain aphid species are dioecious; that is, they have alternate hosts. These aphids overwinter on a primary host (a woody plant) usually in the egg stage. In the spring, winged (alate) aphids migrate to secondary hosts. Myzus persicae is an example of a dioecious aphid. The aphid will usually overwinter as eggs on peach trees or other Prunus species. In warmer climates or during mild winters, the green peach aphid will overwinter as adults in weeds or field crops. Potato storage sheds, greenhouses, and bedding plants also serve as overwintering sites to parthenogenic M. persicae (Whalon, 1979). This is very important epidemiologically because spring migrants develop earlier on secondary hosts (Duffus, 1971). Not only is the migration period longer when the aphid overwinters on the secondary hosts versus primary hosts (Doncaster & Gregory, 1948), but these aphids are more likely to carry viruses than aphids that overwinter on primary hosts (Duffus, 1964; Wallis, 1967; Heathcote et al., 1965).

Wallis (1967) has found that there is a greater incidence of beet western yellows virus (BWYV) in sugar beet plants next to ditches where M. persicae overwinters in the viviparous summer form than next to peach trees where the aphid overwinters in the egg stage. Both early

infection and a longer growing season appear to be the reasons for a higher incidence of BWYV near Walla Walla, WA (Wallis, 1967).

Vector Dispersal

The production of alatae in the spring is associated with physical contact between aphids or "crowding" (Lees, 1966). The physiological condition of the host plant (Johnson, 1966a) as well as the effects of temperature and photoperiod (Johnson, 1966b) also affect aphid wing development.

Once in the air, alatae are carried primarily by surface winds. Any change in the ground or crop surface causes a change in the air turbulence, which affects deposition of the winged aphids (Lewis, 1965). This change in air movement accounts for the edge effects of primary infections that are seen in many crops (Broadbent, 1957; Doncaster & Gregory, 1948). There is no evidence that alatae recognize fields of host plants and then alight at the edge of the field (Swenson, 1968).

Kennedy et al. (1959) found that flying aphids are just as likely to land on nonhost plants as well as host plants. Later Kennedy (1962) summed up these findings quite concisely by saying that "dispersal takes precedence over host finding."

Monitoring Populations

Monitoring aphid population is an important aspect of studying the epidemiology of an aphid-transmitted virus disease. Irwin and Goodman (1981) used horizontal colored tiles (HCT) to monitor aphids in soybean field studies. These lime-green-colored traps were designed especially

for monitoring aphids alighting on soybeans (Irwin, 1980). The incidence of alighting aphids was closely correlated with the incidence of soybean mosaic virus (Irwin & Goodman, 1981). The numbers and species of aphids caught in the HCT traps were very similar to those found on the soybean plants (Irwin & Goodman, 1981).

Live vectors can be collected from vegetation to use for infectivity tests. Aphids within crops may be collected using suction traps (D-VAC) or nets (Howell, 1794). Different types of traps are used for vectors flying into the field. Suction traps (Plumb, 1971), vertical nets (Halbert et al., 1981), or water pan traps (Demski, 1981) have been used to trap incoming vectors. Once collected, the live insects are placed onto test plants. Any aphids that transmit virus are then identified.

The seasonal spread of virus diseases may be studied by assessing virus incidence in the field either by noting symptoms or by indexing the plants. There are two problems associated with this method of studying the seasonal spread of virus diseases: (1) the long length of time required before test or field plants show symptoms and (2) multiple infections in the field. Broadbent and others (1950) averted this problem of multiple infections by exposing potted potato plants to the potato field containing infection foci for limited time periods. Other researchers have also used this trap plant method for estimating seasonal infection pressures in the field (Schwartz, 1965; Madden et al., 1983).

Although transmission tests are the most reliable ways of determining if a vector is infective, it does take a long time before

results are obtained. Ultrasensitive, serological tests have been developed recently which are able to detect virus in vectors within 1 or 2 days.

Gera, Loebenstein, and Raccah (1978) were the first to detect a plant virus (cucumber mosaic virus) in an aphid vector using enzyme-linked immunosorbent assay (ELISA). Since then, other plant viruses have been detected by ELISA in aphid vectors. Potato leafroll virus (PLRV), a persistent virus, was first detected in groups of aphids by Clarke, Converse, and Kojima (1980). Later, Tamada and Harrison (1981) were able to detect PLRV in single aphids and study the seasonal differences of virus content in the vector. Pea enation mosaic virus (PEMV), another persistent virus, can also be detected in individual aphids (Fargette, Jenniskens, & Peters, 1981). ELISA can also detect potato virus Y (PVY), a nonpersistent virus (Carlebach, Raccah, & Loebenstein, 1982), and citrus tristeza virus (CTV), a semi-persistent virus (Cambra et al., 1981), in groups of aphid vectors.

Derrick's (1973) method of serologically specific electron microscopy (SSEM), also known as immunosorbent electron microscopy (ISEM), has been used by Plumb and Lennon (1981) to detect barley yellow dwarf virus (BYDV) in single aphids. Gillett et al. (1982) compared ISEM to ELISA and radioimmunoassay (RIA) for detecting BBSSV in its aphid vector. RIA was the most sensitive for this purpose. ISEM was not suitable due to the low virus concentration in the aphids and the insect particulate matter which obstructed viewing.

The use of sensitive tests such as ELISA and RIA has made transmission and epidemiological studies of blueberry shoestring

disease a practical possibility. Blueberry shoestring virus infection may be latent in the blueberry host plant up to 4 years before symptoms are apparent (Ramsdell et al., 1980). With ELISA, test plants may be assayed for the presence of BBSSV instead of waiting for symptoms to develop years after infection. In addition, the movement of BBSSV-carrying blueberry aphids may now be studied by using RIA, which is capable of detecting BBSSV in individual aphids (Gillett et al., 1982).

METHODS AND MATERIALS

Virus Purification

Blueberry shoestring virus was purified from frozen BBSSV-infected blossoms as described by Ramsdell (1979a). All purification procedures were at 0-4 C. One hundred grams of frozen blossoms were homogenized in a Waring blender with three volumes cold 0.1 M potassium phosphate buffer, pH 7.0, containing 0.01 M 2-mercaptoethanol and 0.005 M thioglycolic acid. Triton X-100 [8% (v/v)] was added to the homogenate and the mixture was stirred overnight.

The homogenate was strained through two layers of cheesecloth. Chloroform and butanol (5% each, v/v) were added to the solution and stirred for 15 min. The emulsion was centrifuged for 15 min at 2000 g in an IEC No. 872 rotor (International Equipment Co., Needham Hts., MA 02194). The aqueous phase was pipetted off and adjusted to 8% (w/v) polyethylene glycol (PEG), mol. wt. 6000, and 0.1 M sodium chloride while stirring. The mixture was stirred overnight and then centrifuged for 30 min at 3500 g in an IEC No. 872 rotor. The PEG pellet was resuspended overnight in 10% of the initial aqueous phase volume with 0.05 M phosphate buffer, pH 7.0, containing 0.001 dithiothreitol (P-DTT).

The suspension was clarified by a low-speed centrifugation for 30 min at 3500 g in an IEC No. 872 rotor, and concentrated by

ultracentrifugation for 2 hr at 28000 rpm in a Beckman No. 30 rotor. The pellet was resuspended overnight in 0.2 ml P-DTT per tube.

The preparation was layered onto 0-30% linear sucrose gradients made in P-DTT the previous night. The sucrose gradients were centrifuged for 90 min at 38000 rpm in a Beckman SW 41 rotor. The single virus band was collected using an ISCO density gradient fractionator and UV-analyzer (Instrumentation Specialties Co., Lincoln, NE 68505). The sucrose fractions containing the virus were diluted threefold with 0.05 M phosphate buffer (PB), pH 7.0, and centrifuged for 3 hr at 38000 rpm in a Beckman No. 40 rotor. The pellet was resuspended in PB overnight. The concentration of the virus preparation was determined using the molar extinction coefficient of BBSSV, $E^{0.1\%}_{260\ nm} = 5.2$.

Antiserum Production

A female New Zealand white rabbit was initially bled from the marginal ear vein to collect preimmune serum. The rabbit was injected intramuscularly with 1.2 mg purified BBSSV emulsified with an equal volume (1.1 ml) of Freund's complete adjuvant (Difco Products Co., Detroit, MI 48232). Two subsequent intramuscular injections at 7-day intervals consisted of a total of 2.3 mg purified BBSSV emulsified with an equal volume (2.1 ml total) of Freund's incomplete adjuvant.

Five days after the final injection, the rabbit was bled from the marginal ear vein at 3- to 6-day intervals for 1 month. The fresh blood was placed in a 37 C water bath for 2 hr and then kept at 4 C overnight to coagulate the red blood cells. The serum fraction was pipetted from the coagulated material and a few crystals of

chlorobutanol (Sigma Chemical Co., St. Louis, MO 63178) were added to the serum as a preservative. The serum was lyophyllized and stored at -20 C.

The anti-BBSSV-serum was titered against purified BBSSV (0.1 mg/ml) in an Ouchterlony gel double diffusion test. The agar consisted of 8% agarose (w/v) (Sigma Type I, Sigma Chemical Co., St. Louis, MO 63178), 0.85% sodium chloride (w/v), and 0.15% sodium azide (w/v).

Enzyme-Linked Immunosorbent Assay

Gamma Globulin Purification

Anti-BBSSV-gamma globulin was purified by the procedure described by Clark and Adams (1976). The gamma globulin was diluted 1:10 (v/v) in distilled water and added dropwise to 10 ml saturated ammonium sulfate solution while stirring. After 30 to 60 min stirring, the mixture was centrifuged for 5 min at 6000 rpm in a Beckman No. 30 rotor. The precipitate was collected and dissolved in 2 ml half-strength PBS (0.01 M sodium-potassium phosphate buffer, pH 7.4, containing 0.8% sodium chloride (w/v), and 0.01% sodium azide (w/v), diluted 1:1, (v/v) in water). The gamma globulin preparation was dialyzed three times against 500 ml half-strength PBS then filtered through a 5 cm high bed of DEAE (Whatman DE-22) cellulose in a 10 ml pipette. Half-strength PBS was used to pre-equilibrate the column and elute the gamma globulin. Two ml fractions were monitored at $A_{280\ nm}$ and the first protein fractions to elute were collected. The gamma globulin preparation was adjusted to 1 mg/ml and stored at -20 C.

Conjugation of Alkaline Phosphates to Gamma Globulin

Gamma globulin was labeled with alkaline phosphatase with the method described by Clark and Adams (1976). Two mg alkaline phosphatase (Type VII-S, Sigma Chemical Co., St. Louis, MO 63178) were centrifuged for 5 min at 6000 rpm in a Beckman No. 40 rotor. The precipitate was dissolved with 1 mg purified gamma globulin preparation and dialyzed three times against 500 ml PBS. Glutaraldehyde (electron microscope grade, Sigma Chemical Co., St. Louis, MO 63178) was added to make a final glutaraldehyde concentration of 0.05% (v/v). The solution was thoroughly mixed and kept at room temperature for 4 hr. The glutaraldehyde was removed by dialysis, three times against 500 ml PBS. Bovine serum albumin (BSA) was added to make a final concentration of 5 mg/ml. The conjugate was stored at 4 C.

Assay Procedure

The double antibody sandwich method of enzyme-linked immunosorbent assay (ELISA) (Clark & Adams, 1976) was used to detect BBSSV in blueberry plant tissue. Flat bottom polystyrene microtiter plates (Dynatech Laboratories, Alexandria, VA 22314) were used for ELISA. The plates were coated with 1 µg/ml anti-BBSSV-gamma globulin in coating buffer (0.05 M sodium carbonate-bicarbonate buffer, pH 9.6) at a rate of 200 µl per well and incubated for 3 hr at 37 C.

Blueberry plant samples were triturated with a Tissumizer homogenizer (Tekmar Co., Cincinnati, OH 45222) in 1:10 (w/v) extraction buffer consisting of 0.01 M sodium potassium phosphate buffer, pH 7.4, containing 0.02% sodium azide (w/v), 0.8% sodium chloride (w/v), 0.5%

Tween 20 (v/v), 2.0% polyvinyl pyrrolidone (mol. wt. 40000, Sigma Chemical Co., St. Louis, MO 63178) (w/v), and 0.2% ovalbumin (grade II, Sigma Chemical Co., St. Louis, MO 63178) (w/v). After the homogenates were filtered through two layers of cheesecloth, aliquots of the samples were added at a rate of 200 µl per well. The plates containing the samples were incubated at 4 C overnight.

Enzyme-conjugate, at a dilution of 1:800 (v/v) in extraction buffer, was added at a rate of 200 µl per well and incubated 4 hr at 37 C.

Between each step the plates were flooded with PBS-Tween at least three times to remove any loosely or nonadsorbed reactants.

One mg/ml enzyme substrate, P-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO 63178), was freshly dissolved in substrate buffer (10% diethanolamine, adjusted to pH 9.8 with HCL) and added to the plates at a rate of 200 µl per well. After 1 hr incubation at room temperature, the $A_{405\ nm}$ was measured spectrophotometrically with a microELISA minireader (Dynatech Laboratories, Alexandria, VA 22314).

The threshold used for positive reaction for each plate was the mean $A_{405\ nm}$ value of healthy samples plus three standard deviations. Samples in each test plate with $A_{405\ nm}$ values greater than the threshold were considered positive.

Radioimmunoassay

Iodination

Purified anti-BBSSV-gamma globulin from the DEAE cellulose column was iodinated using the method described by Greenwood et al. (1963).

To 50 µl gamma globulin, 150 µl PBS, 1 mCi $Na^{125}I$ and 5 µl chloramine-T (5 mg/ml in water) were added. The contents were thoroughly mixed and incubated 15 min on ice. Sodium metabisulfite (5 mg/ml in water), 5 µl, was added to stop the reaction. Sodium iodide (20 mg/ml in PBS), 25 µl, and 0.5% bovine serum albumin (w/v) in PBS (PBS-BSA), 100 µl, were added to act as carriers for the ^{125}I and gamma globulin.

The mixture was loaded onto a Sephadex G-50 column (10 cm x 1 cm) pre-equilibrated with PBS-BSA. One ml fractions were eluted with PBS-BSA and collected. Aliquots of each fraction were counted in a Beckman Biogamma II gamma counter. The protein fractions were pooled and dialyzed three times against PBS.

Assay Procedure

A double antibody sandwich system similar to that described for ELISA was used for solid phase radioimmunoassay (RIA). Flexible disposable polyvinyl "V" bottom microtiter plates (Dynatech Laboratories, Alexandria, VA 22314) were coated with gamma globulin, 5 µl/ml, in coating buffer, at a rate of 100 µl per well, and incubated 3 hr at 37 C.

Blueberry tissue samples were prepared as previously described. Aphid samples were triturated with a stirring rod in a test tube containing 100 µl extraction buffer. The entire contents of each test tube were transferred with a pasteur pipet to a plate well. Test samples were incubated overnight at 4 C.

As with ELISA, the plates were washed at least three times between each step to remove any nonadsorbed reactants. The wash solution for

RIA consisted of 0.5-1.0% bovine serum albumin (w/v) in PBS (PBS-BSA) or in PBS-Tween (PBS-Tween-BSA).

Approximately 55,000 cpm ^{125}I-anti-BBSSV-gamma globulin diluted in PBS-BSA was added to each well at a rate of 100 µl per well. After a 4 hr incubation at room temperature, the nonadsorbed gamma globulin was aspirated out of the wells. The plates were then washed four times with PBS-BSA or PBS-Tween-BSA as previously described.

The sides of the flexible plates were cut off with scissors and the top was cut off with a hot wire to obtain individual wells. Each well was individually placed into a counting vial and counted by the gamma counter.

Samples with counts per minute (cpm) greater than three times the mean of the healthy sample wells plus three standard deviations were considered positive for BBSSV.

Virus-Vector Studies

Aphid Culture

The blueberry aphid culture used in the acquisition and inoculation access time studies was started from an aphid culture maintained by Erwin Elsner, Department of Entomology, Michigan State University. Gravid apterous (wingless) adult aphids were placed in petri plates containing moist filter paper. The ensuing nymphs were used to establish the virus-free blueberry aphid colony.

One-year-old rooted cuttings of highbush blueberry cv. Jersey were used as host plants for the aphid colony. The plants were tested by ELISA prior to use to ensure that they were not infected with BBSSV.

The culture was maintained with an 18 hr day photoperiod with day and night temperatures of 23 C and 18 C, respectively.

Acquisition Access Period Studies

To study the acquisition kinetics of BBSSV by the blueberry aphids, late instar nymphs and apterous adults were allowed access to three different sources of BBSSV:

1. symptomatic BBSSV-infected leaves on a detached shoot in water.
2. purified BBSSV preparation in 20% sucrose contained in a Parafilm® membrane feeding cage (sachet).
3. ^{125}I-labeled purified BBSSV preparation in 20% sucrose contained in a Parafilm® membrane feeding cage.

Three different sources of virus were used because BBSSV is present in blueberry tissue in low titers. Since it was initially unknown whether or not BBSSV could be detected in its vector, the aphids were allowed access to very high concentrations of virus under membrane feeding conditions.

Sachets for feeding aphids were made from plexiglass cylinders (3.8 cm in diameter x 4 cm high). After one end of the cylinder was covered with Parafilm® and 20-30 aphids were placed inside the sachet, the top of the sachet was covered with a piece of very thinly stretched Parafilm®. Approximately 200 µl of purified BBSSV in 20% sucrose, ^{125}I-BBSSV in 20% sucrose, or a control of 20% sucrose alone was pipetted onto the very thinly stretched Parafilm® and then enclosed by a second piece of Parafilm®.

Aphids were allowed access to the BBSSV-infected tissue or the purified BBSSV in 20% sucrose contained in sachets for acquisition

access periods (AAPs) of 10 min, 1, 6, 12, 24, 48, and 72 hr. AAPs of 10 min, 1, 6, 12, 24, and 48 hr were used for aphids allowed access to the ^{125}I-BBSSV in 20% sucrose.

The test aphids that fed on BBSSV-infected tissue of the purified BBSSV in sachets were tested in groups individually with RIA. Those that fed on ^{125}I-BBSSV were counted individually directly by the gamma counter.

Inoculation Access Period Studies

To determine the optimum IAP, aphids were first allowed access to BBSSV-infected tissue for a constant AAP. Late-instar nymphs and adult apterous blueberry aphids were transferred to a symptomatic BBSSV-infected shoot contained in a vase of water for an AAP of 24 hr. An AAP of 24 hr was chosen because acquisition kinetics studies showed that there was no significant additional uptake of BBSSV with AAPs greater than 24 hr. After the requisite AAP, the aphids were transferred to potted healthy 1-year-old rooted blueberry cuttings cv. Jersey in groups of 15. Inoculation access times of 1, 6, 12, 24, 48, 96, and 192 hr were used. At the end of each IAP, the aphids were removed and the test plants were sprayed with Pirimicarb (5,6-Dimethyl-2-dimethylamino-4 pyrimidynl dimethylcarbamate). There were 15 test plant replications per IAP treatment arranged in a randomized complete block experimental design.

After 6 months of incubation in the greenhouse, leaf samples of the test plants were tested by ELISA for BBSSV infection. The test plants were then put into a dark, cold room (4 to 6 C) to satisfy a dormant period. After a dormant period of at least 1000 hr, the test

plants were moved to the greenhouse. Leaf samples taken from new growth after dormancy was broken were tested for BBSSV infection with ELISA, as previously described.

A second IAP was conducted. Aphids were allowed access to symptomatic-BBSSV-infected shoots and leaves for a constant AAP of 24 hr. Groups of 15 aphids were transferred to test plants as before for IAPs of 0, 1, 6, 12, 24, 48, 96, and 129 hr. There were seven replications per treatment set up in a randomized complete block design. After 6 months of incubation in the greenhouse, leaf samples were collected from the test plants and tested for BBSSV-infection with ELISA.

Location of Field Studies

Experiments were conducted in Ottawa County, west-central Michigan, to study the spread of BBSSV by the blueberry aphid.

In 1982 the field plots were set up at the Frank VenRoy blueberry farm, Eastmanville, MI. The cv. Jersey bushes were approximately 20 years old and planted on a 10 x 3 foot spacing. The field was clean cultivated. In 1981 the field was mapped for BBSSV infection by Adele Childress (unpublished data). Any bushes without blueberry shoestring disease symptoms were tested for BBSSV with ELISA.

Source of Overwintering Blueberry Aphids

A caged bush experiment was conducted to determine whether or not blueberry aphids overwinter within the blueberry field. Fourteen BBSSV-infected bushes (hereafter referred to as source plants) at the VenRoy farm were selected and pruned to a uniform size and number of main

shoots. Seven of the source plants were each enclosed in a 16 mesh screen cage before bud break, while the other seven were not caged. The source plants were monitored weekly for the presence of blueberry aphids.

Alate Blueberry Aphid Activity

Alate Aphid Movement Outside the Field

A study was conducted to monitor the movement of alate blueberry aphids outside of an isolated blueberry field using yellow pan traps. The traps were goldenrod-colored plastic dish pans (30 cm x 38 cm x 16 cm) filled with water within 3 cm of the rim. The traps were placed on 2 m high platforms at 100, 200, and 300 m intervals from the east, west, and south edges of the VenRoy blueberry field. Each week blueberry aphids were collected from the traps and the traps were cleaned and refilled with water. The aphids were placed into test tubes containing 100 µl extraction buffer. The tubes were corked and kept at 0 to 4 C until processed for RIA.

Two blueberry plants in one-gallon plastic pots were placed at the base of each trap stand outside the field to attract aphids. These trap plants were checked also for blueberry aphids each week.

Alate Aphid Activity Within the Field

Alate activity within the blueberry field was monitored also with yellow pan traps. The traps were placed on 30 cm boxes and on 2 m high platforms (the height of the canopy) in the corners and center of a block of the VenRoy blueberry field. *I. pepperi* were collected

weekly from the yellow pan traps, as previously described, and were individually tested for BBSSV with RIA.

Alate Aphids on Screen Cages

Alate blueberry aphids were collected on 11, 18, 25, and 26 June from the walls of the screen cages enclosing the caged source plants in 1982. Aphids were collected into test tubes as earlier described and then individually tested for BBSSV with RIA.

Seasonal Trap Plant Infection

Blueberry trap plants were exposed to BBSSV-infected source plants in the field for 4-week intervals to determine when BBSSV infection occurs during the growing season. The five time intervals that the trap plants were exposed to the source plants in 1982 were (1) 7 May to 4 June, (2) 4 June to 2 July, (3) 2 July to 30 July, (4) 30 July to 27 August, and (5) 27 August to 23 September.

Two-year-old highbush blueberry cv. Jersey plants in one-gallon plastic pots served as trap plants. The plants were obtained from the John Nelson Blueberry Nursery, South Haven, MI. Prior to placement in the field, the plants were tested for BBSSV infection with ELISA and sprayed with DDVP (2,2-Dichlorovinyl 0,0-dimethyl phosphate), a low residual, contact/fumigant insecticide. After each 4-week exposure period the trap plants were sprayed with DDVP and kept in isolation outside at Michigan State University. After a winter dormant period, leaves were sampled and tested for BBSSV infection with ELISA.

Seasonal Blueberry Population Dynamics and Bush-to Bush Movement of Blueberry Aphids

Aphids may move from plant to plant by walking across overlapping branches of adjacent bushes. A study was conducted to determine if aphids are likely to move to adjacent trap plants whether or not they are touching aphid source plants.

The same trap plants used to determine the seasonal BBSSV infection were used in this study. Ten trap plants were placed around each of the 14 source bushes previously described. Five of the 10 trap plants were placed around the source bush to that the trap plants and source plant touched and had overlapping shoots. The other five trap plants were placed around the perimeter of the source plant 0.5 m to 1.0 m away so that the trap plants did not touch the source plants.

Alate and apterous (late instar nymphs and adult) blueberry aphid populations on the trap plants and source plants were directly counted at weekly intervals from 7 May to 23 September 1982. Samples of alate and apterous aphid populations were also collected weekly and then tested for BBSSV with RIA to determine if the aphids were viruliferous. The apterous aphid samples collected from 15 May to 4 June were tested in groups of five. Thereafter the apterous aphids were individually tested for BBSSV. All of the alate blueberry aphids were individually tested.

Degree day (base 38 F) accumulation from 1 January to 31 March 1982 for aphid population studies was estimated from National Oceanic and Atmospheric Administration (NOAA) data for Grand Haven, MI. This figure was added to the degree day accumulation (base 38 F) obtained

from the agricultural weather observation station at Allendale, MI, for 1 April to 23 September 1982.

The aphid populations on the five touching or five nontouching trap plants for each source plant replicate were summed (aggregated) using SPSS (Statistical Package for the Social Sciences, Vogelback Computing Center, Northwestern University, Evanston, IL 60201) run on the Control Data Corporation Cyber 750 computer at Michigan State University. The summed trap plant aphid populations and the source plant aphid populations were then analyzed using BMDP2V, analysis of variance with repeated measures (University of California, Los Angeles, CA), converted for use on the CDC 6000 and Cyber series computers by the Vogelback Computing Center, Northwestern University, Evanston, IL 60201.

RESULTS

Virus Purification

Yields of the purified BBSSV ranged from 75 to 150 mg of purified virus per 100 g of frozen infected blueberry blossoms. The purified virus concentrations were determined spectrophotometrically using an extinction coefficient of $E_{260\ nm}^{0.1\%} = 5.2$ (Ramsdell, 1979a).

Serology

Antiserum prepared against purified preparations of BBSSV reacted with purified preparations of BBSSV to a dilution of 1:1024 (v/v) in 0.85% sodium chloride in gel double diffusion tests. There was no reaction of the antiserum with purified healthy blossoms.

ELISA

ELISA could detect purified BBSSV diluted twofold in extraction buffer at a concentration of approximately 0.5 ng/ml (Figure 2). This was equivalent to approximately 0.1 ng per well. Purified BBSSV diluted in ELISA extraction buffer with extracts of single blueberry aphids (one aphid per 0.2 ml extraction buffer) was detected at a concentration of 3.0 ng/ml or 0.6 ng BBSSV per single aphid extract (Gillett et al., 1982).

Figure 2.--ELISA absorbance values (405 nm) of a twofold dilution series of a purified preparation of blueberry shoestring virus (BBSSV) in ELISA extraction buffer. The dilution of anti-BBSSV-gamma globulin in coating buffer was 1 mg/ml, while the dilution of enzyme-conjugated gamma globulin was 1:800 (v/v). Each point represents the mean of six replicates. The dashed line represents the threshold of detection determined by the mean value plus three standard deviations of healthy blueberry leaf samples.

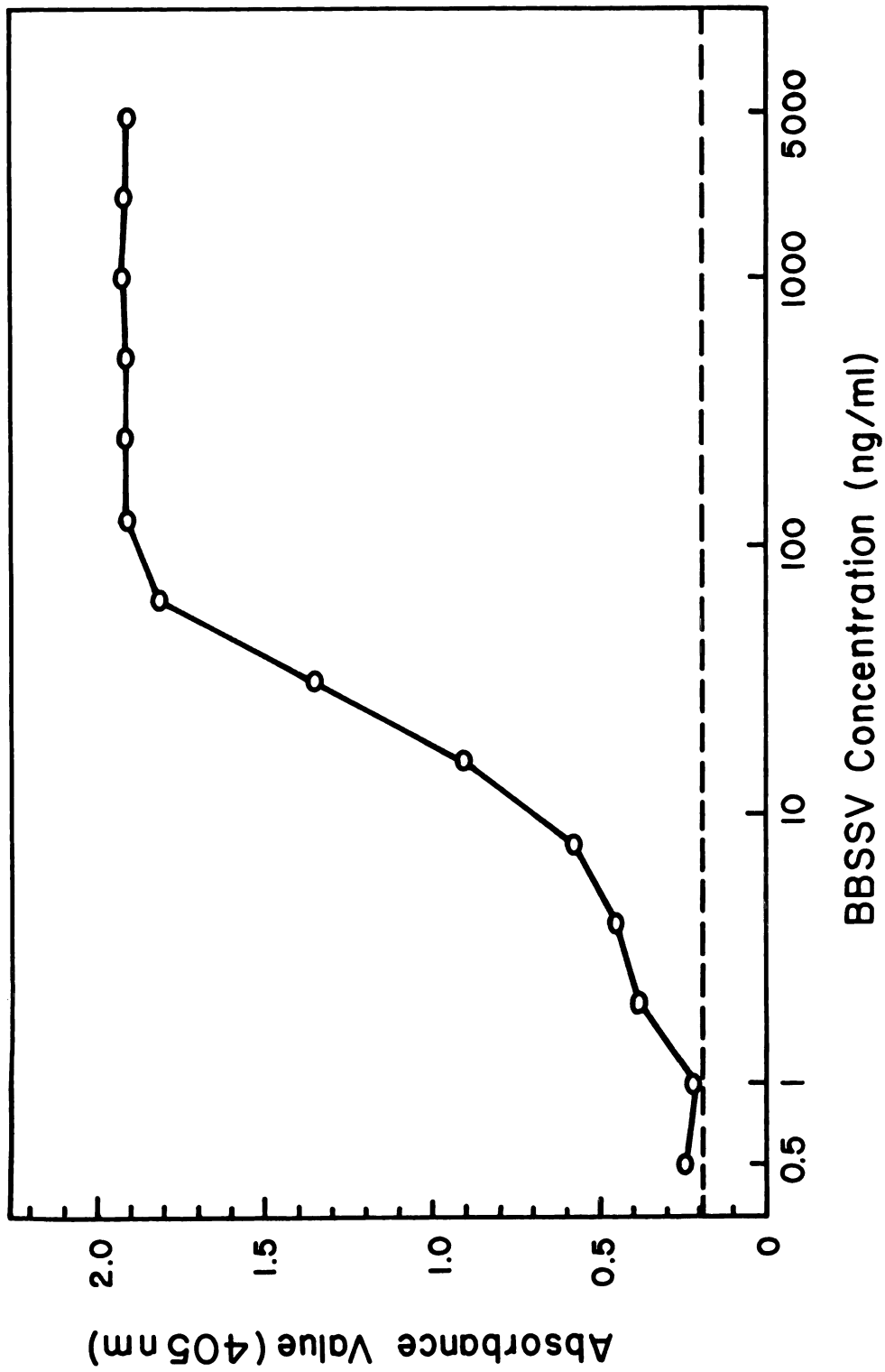
Absorbance Value (405 nm)
2.0
1.5
1.0
0.5
0
0.5
1
10
100
1000
5000
BBSSV Concentration (ng/ml)

Figure 2

RIA

Using RIA, purified preparations of BBSSV diluted twofold in ELISA extraction buffer could be detected at levels down to 0.5 ng/ml (Figure 3). In addition, the assay could detect purified BBSSV diluted twofold in extraction buffer with homogenized single blueberry aphid extracts (one aphid per 0.2 ml buffer) at 0.75 ng/ml (Gillett et al., 1982). This corresponds to a detection level of 0.15 ng per aphid.

BBSSV Acquisition by Blueberry Aphids

Aphids allowed access to BBSSV-infected leaf tissue acquired increasing amounts of virus with increasing AAPs (Figure 4). The first significant ($P < 0.05$) amount of measurable virus uptake occurred at an AAP of 12 hr. There were no significant differences in amounts of BBSSV acquired with AAPs of 24 hr or more. There was, however, large variability in the amount of BBSSV acquired at the 48 hr AAP. The large variability in the quantity of virus taken up with the 48 hr AAP may have been due to several aphids acquiring very small quantities of virus. This is very likely since the virus is unequally distributed within the plant tissue.

Purified BBSSV contained in sachets was acquired by aphids at the greatest rate during the first 24 hr (Figure 5). After 24 hr virus uptake continued to increase, but at a much slower rate. There appeared to be a threshold of virus uptake at the 24 AAP.

The rate of ^{125}I-BBSSV acquisition from sachets was steady with increasing AAP except for a decrease in virus uptake at 12 hr (Figure 6). This slight decrease at 12 hr may have been due to the greater number of aphids that did not acquire any ^{125}I-BBSSV at that AAP.

Figure 3.--RIA counts per minute (cpm) of a twofold dilution series of a purified preparation of blueberry shoestring virus (BBSSV) in ELISA extraction buffer. The dilution of ^{125}I-labeled gamma globulin was approximately 55,000 cpm in PBS-0.5% BSA. Each point represents the mean of six replicates. The dashed line represents the threshold of detection determined by the mean value plus three standard deviations of extraction buffer.

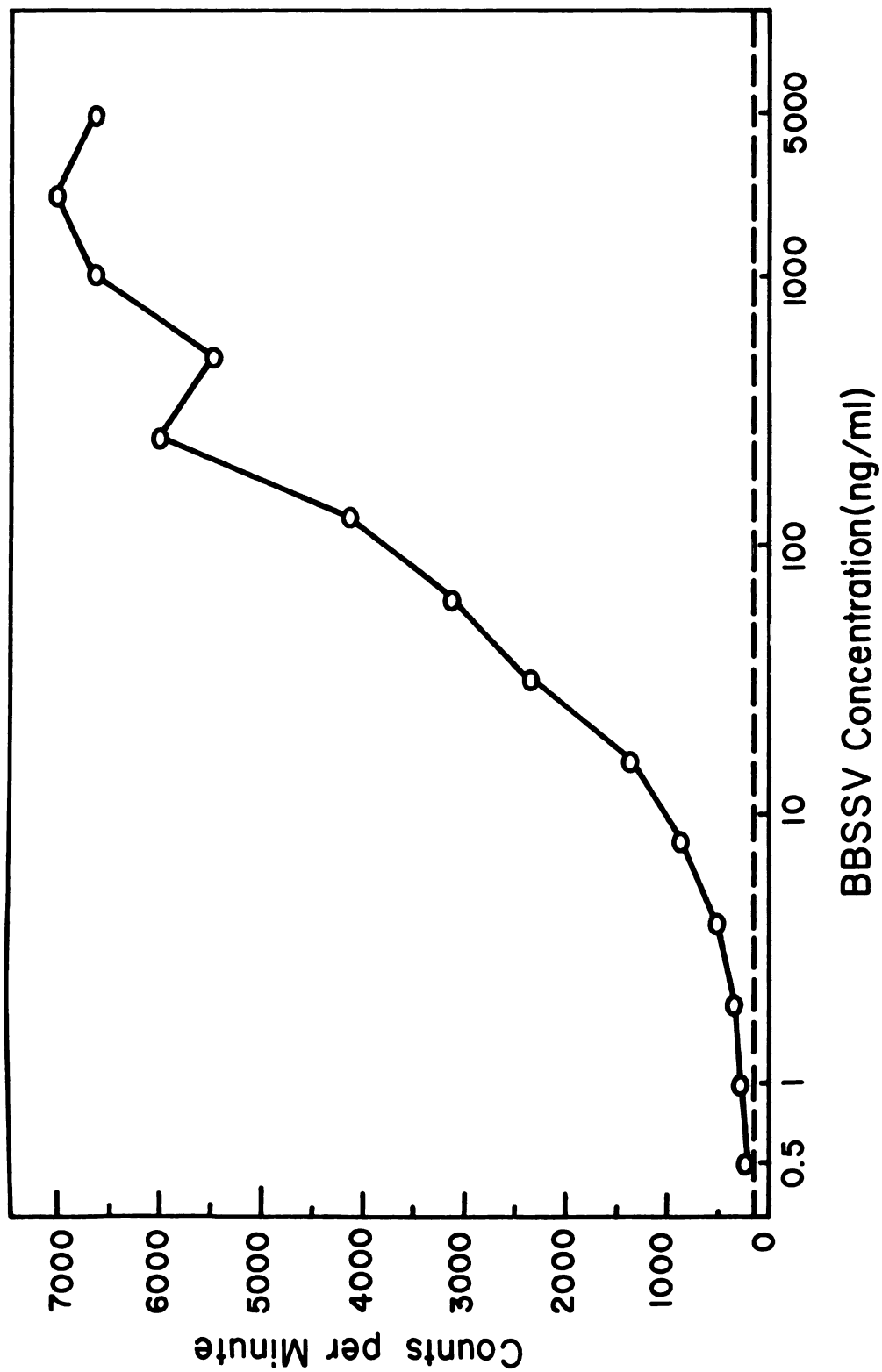
Counts per Minute
7000
6000
5000
4000
3000
2000
1000
0
0.5
1
10
100
1000
5000
BBSSV Concentration(ng/ml)

Figure 3

Figure 4.--Acquisition kinetics of blueberry shoestring virus (BBSSV) uptake by blueberry aphids which fed on BBSSV-infected plant tissue for 10 min, 1, 6, 12, 24, 48, and 74 hr. Aphids were individually tested for presence of BBSSV with RIA. Thirty aphids were used per acquisition access period. Bars represent 95% confidence intervals.

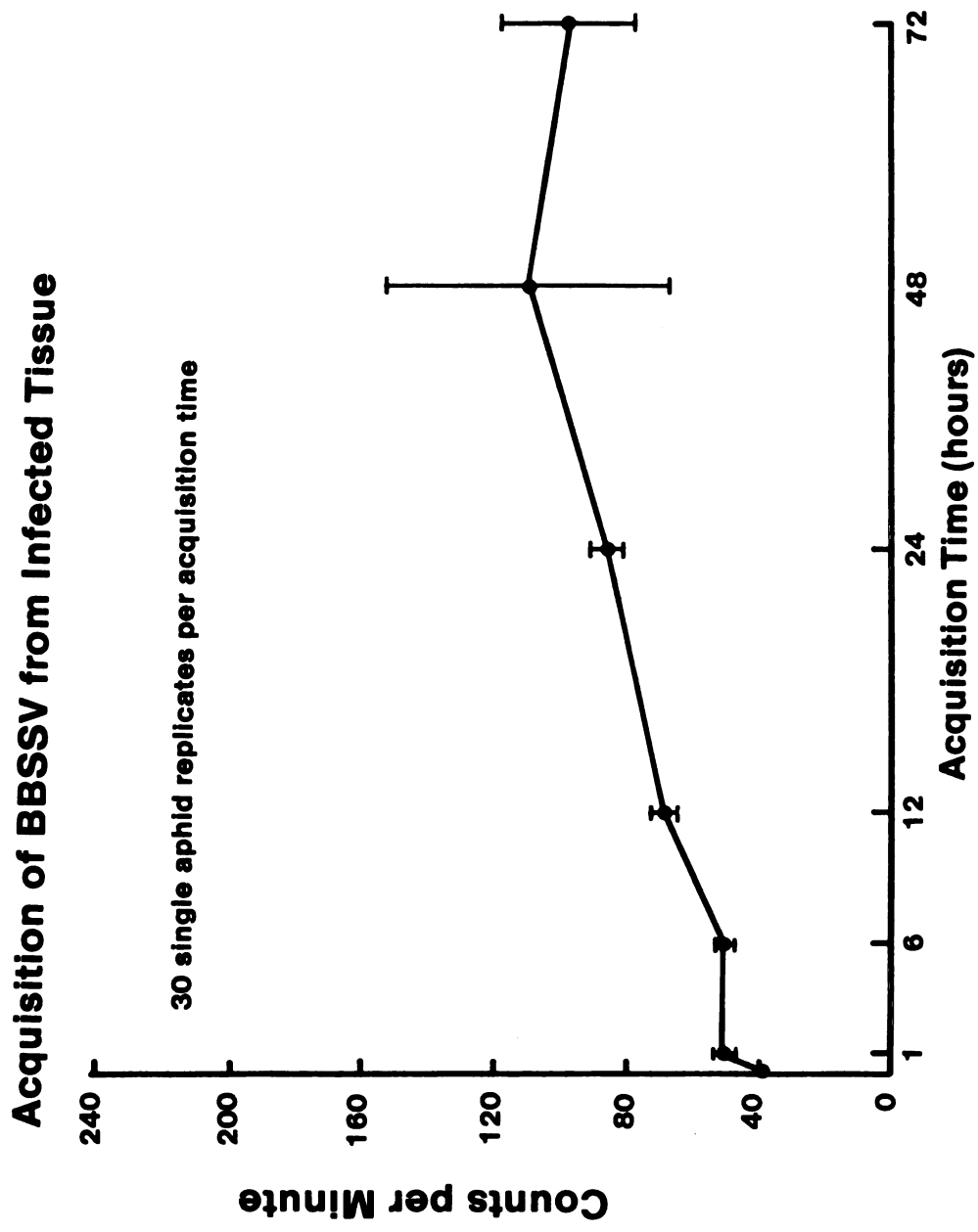
Acquisition of BBSSV from Infected Tissue
30 single aphid replicates per acquisition time
Counts per Minute
240
200
160
120
80
40
0
1
6
12
24
48
72
Acquisition Time (hours)

Figure 4

Figure 5.--Acquisition kinetics of blueberry shoestring virus (BBSSV) uptake by blueberry aphids which fed on purified preparations of BBSSV in sachets. Aphids were individually tested for the presence of BBSSV with RIA. Thirty aphids were used per acquisition access period. Bars represent 95% confidence intervals.

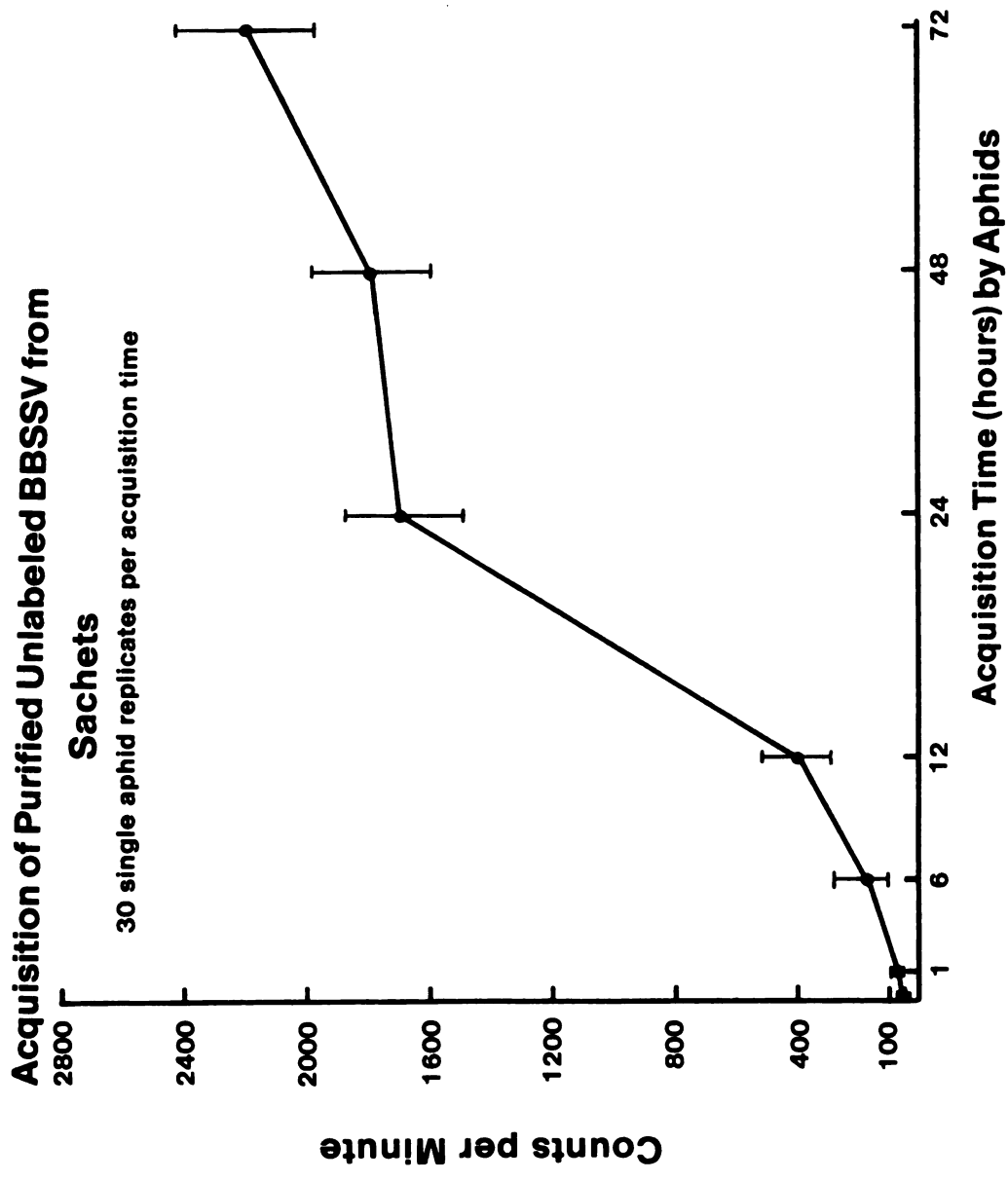
Acquisition of Purified Unlabeled BBSSV from Sachets
30 single aphid replicates per acquisition time
Counts per Minute
2800
2400
2000
1600
1200
800
400
100
1
6
12
24
48
72
Acquisition Time (hours) by Aphids

Figure 5

Figure 6.--Acquisition kinetics of blueberry shoestring virus (BBSSV) uptake by blueberry aphids which fed on ^{125}I-labeled BBSSV in sachets. Aphids were individually counted directly by the gamma counter. There were 10 aphid replicates per acquisition access period. Bars represent 95% confidence intervals.

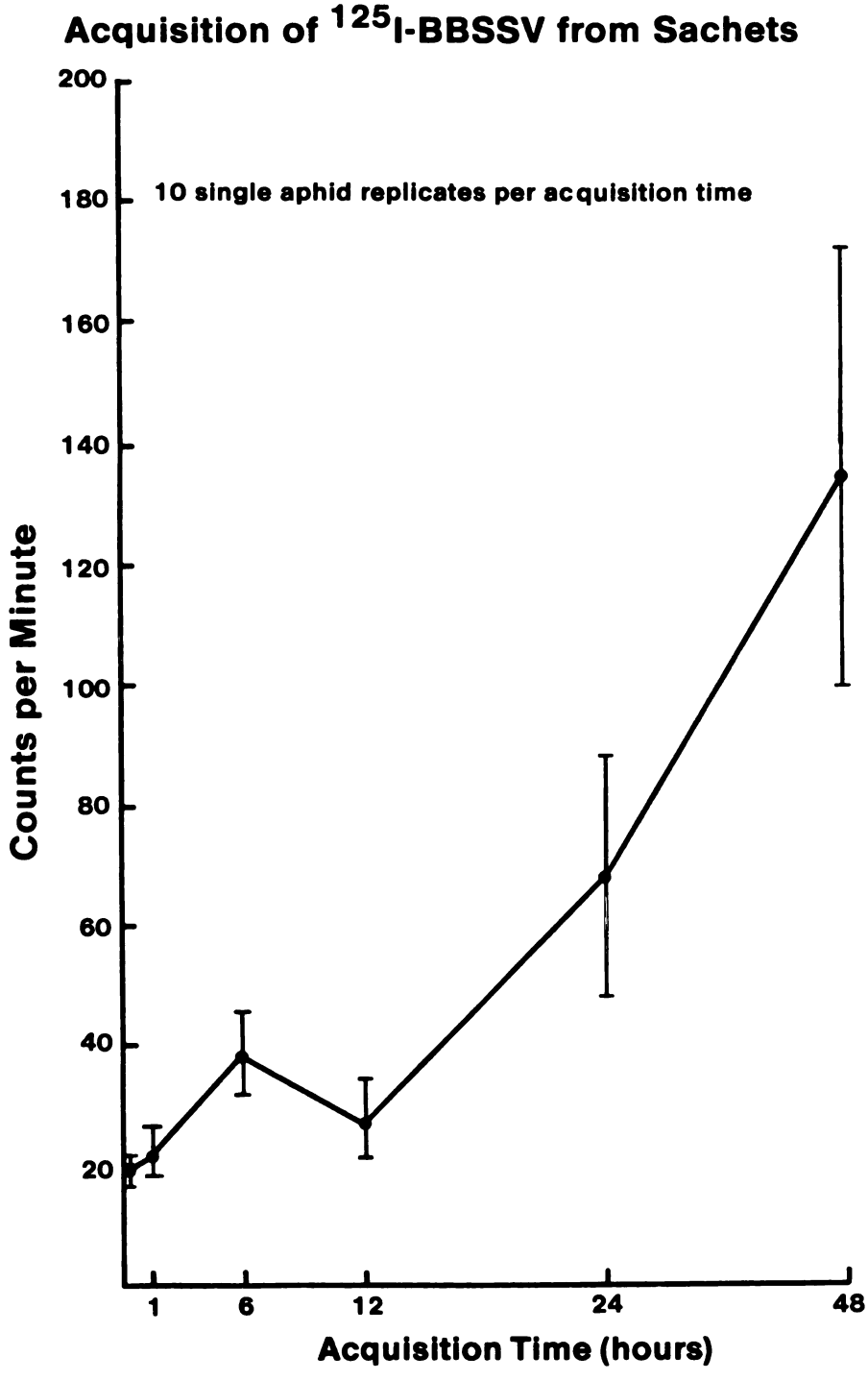
Acquisition of 125I-BBSSV from Sachets
10 single aphid replicates per acquisition time
Counts per Minute
200
180
160
140
120
100
80
60
40
20
1
6
12
24
48
Acquisition Time (hours)

Figure 6

The effect of AAP on the proportion of viruliferous aphids is shown in Figure 7. In general, there was an increase in the proportion of viruliferous aphids with increasing AAP up to a threshold at 24 hr. This occurred when aphids were allowed to feed on either BBSSV-infected plants or purified BBSSV. The proportion of viruliferous aphids which fed on BBSSV-infected leaves decreased slightly with AAPs greater than 24 hr. This slight decline, which did not occur with those aphids that fed on the purified BBSSV, may have been due to the unequal distribution of virus in the plant tissue, as previously discussed.

The percentage distribution of RIA counts per minute (cpm) of individual late-instar and adult apterous blueberry aphids that fed for a 24 hr AAP on BBSSV-infected tissue is shown in Figure 8. The threshold for presence of BBSSV was 85 cpm--the mean plus three standard deviations of individual aphids that fed on healthy tissue. Forty-three percent of the individual aphids tested for BBSSV contained detectable quantities of the virus. A maximum of 1 ng (250 cpm) of BBSSV per individual aphid was detected.

Figure 9 shows the percentage distribution of cpm of the 30 individual late instar nymph and adult apterous blueberry aphids allowed a 24 hr AAP on purified BBSSV in 20% sucrose contained in sachets. All of the individuals were viruliferous as determined by the threshold value of the mean plus three standard deviations of control individuals which fed on 20% sucrose in sachets. The aphids had cpms ranging from 308 to 3265, which corresponded to a range of 1.5 to 60 ng of virus.

Figure 7.--The relationship between acquisition access period and number of viruliferous blueberry aphids which fed on purified blueberry shoestring virus (BBSSV) in sachets or BBSSV-infected tissue. Aphids were individually tested for the presence of BBSSV with RIA. Each point represents the number of viruliferous aphids out of 30 aphids tested.

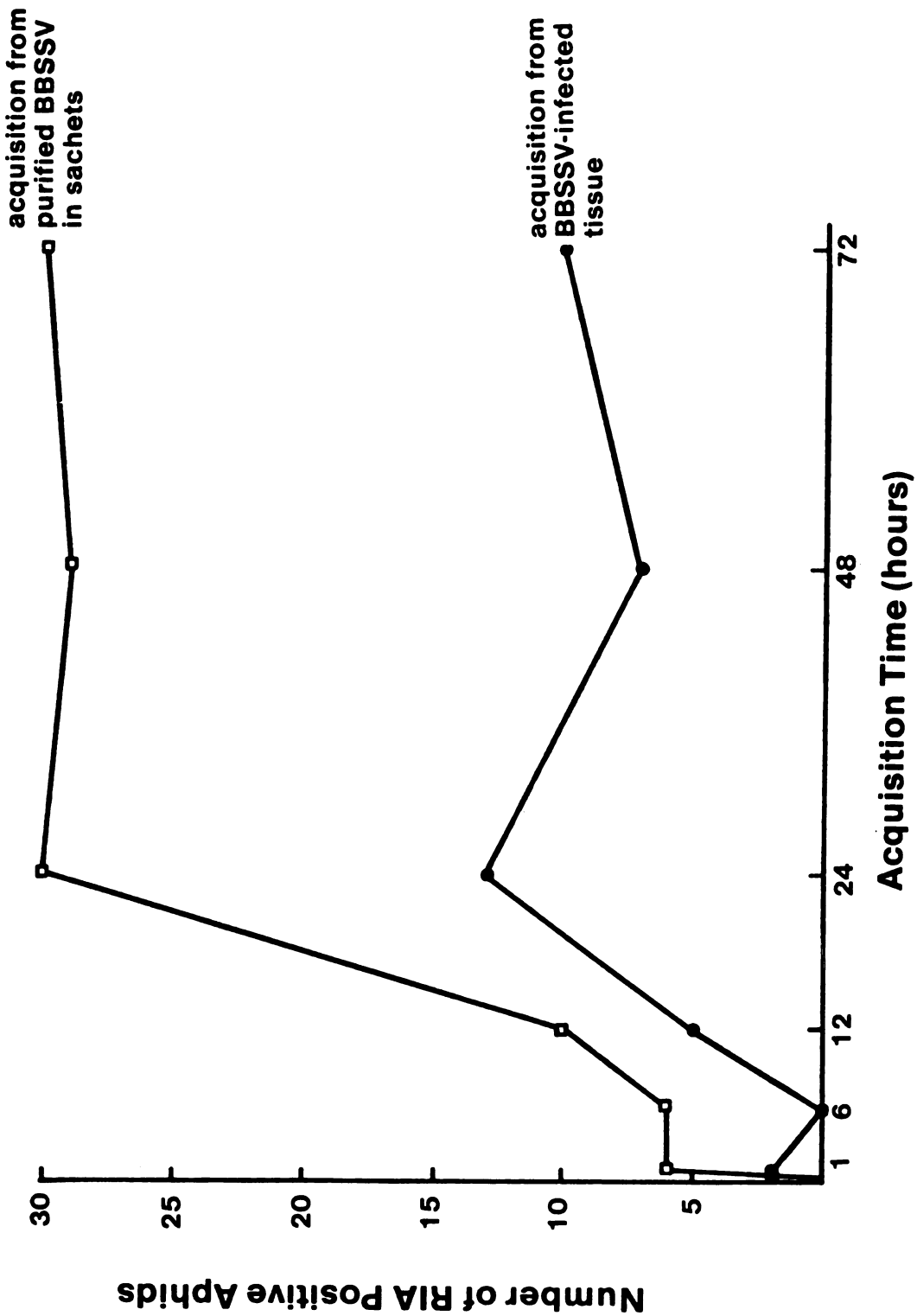
acquisition from purified BBSSV in sachets
acquisition from BBSSV-infected tissue
Number of RIA Positive Aphids
30
25
20
15
10
5
1
6
12
24
48
72
Acquisition Time (hours)

Figure 7

Figure 8.--The percentage distribution of RIA counts per minute (cpm) of 30 individual late-instar and adult apterous blueberry aphids that fed for 24 hr on BBSSV-infected tissue. The shaded bars represent the percentage of viruliferous aphids while the nonshaded bar represents the percentage of nonviruliferous aphids.

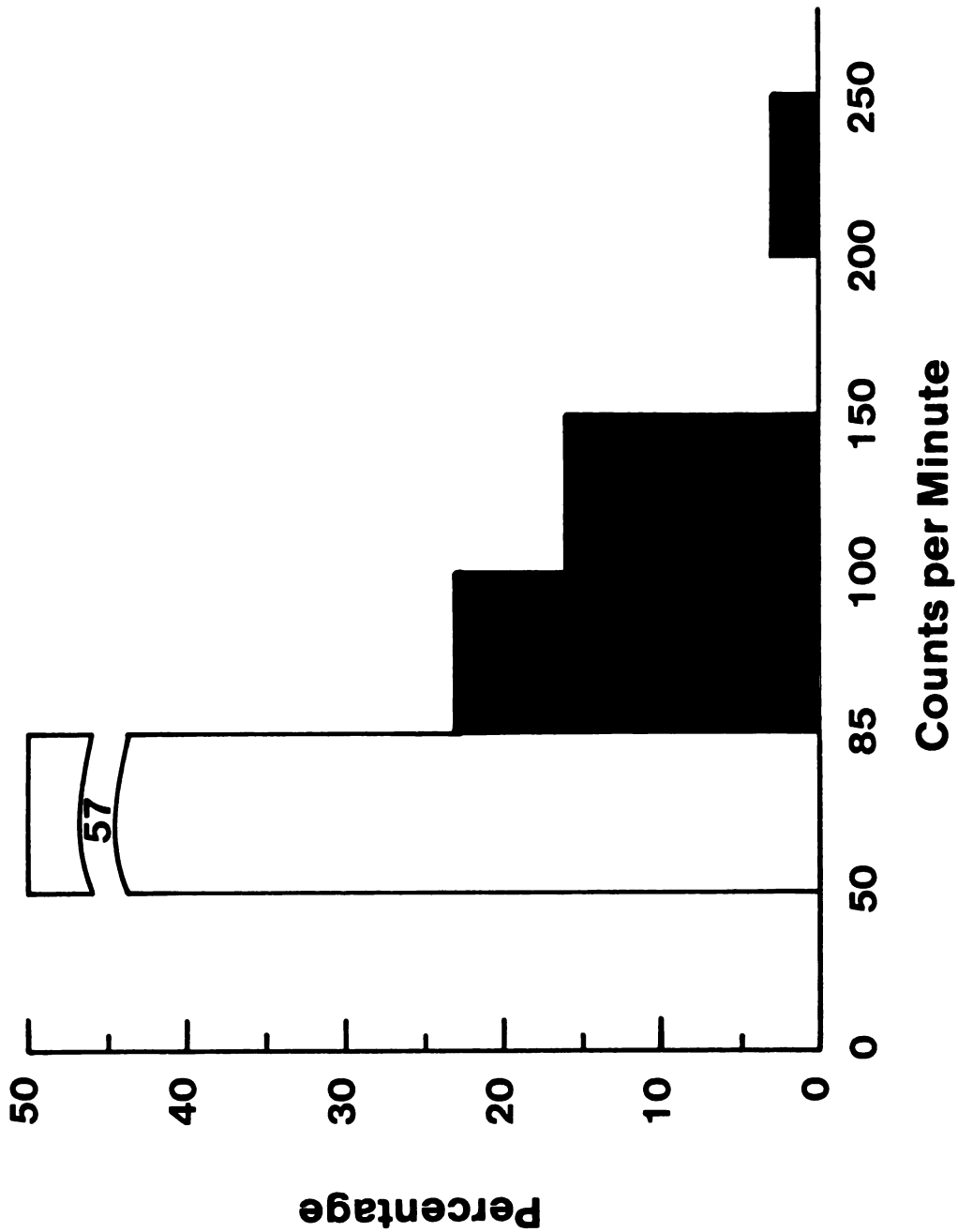
Percentage
50
40
30
20
10
0
57
0
50
85
100
150
200
250
Counts per Minute

Figure 8

Figure 9.--Percentage distribution of RIA counts per minute (cpm) of 30 individual late-instar and adult apterous blueberry aphids that fed for 24 hr on purified BBSSV in sachets.

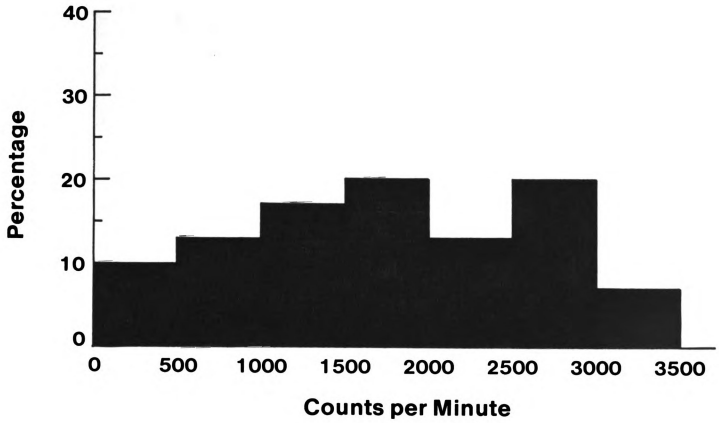
40
30
20
10
0
Percentage
0
500
1000
1500
2000
2500
3000
3500
Counts per Minute

Figure 9

Virus Transmission by Blueberry Aphids

Transmission of BBSSV to blueberry plants by blueberry aphids having an AAP of 24 hr occurred after an inoculation access period of 1 hr (Figures 10 and 11). Figure 10 shows the results of the first transmission test with 15 test plant replications per IAP treatment. Infection occurred with IAPs of 1 hr through 96 hr, but not at 6 hr.

The results of the second transmission test using a constant AAP of 24 hr and IAPs of 0, 1, 6, 12, 24, 48, 96, and 192 hr are shown in Figure 11. Blueberry plants were infected after all IAPs except for 48 hr in this experiment.

Source of Overwintering Blueberry Aphids

Apterous and alate blueberry aphids were first observed on caged source plants on 14 May 1982 (Figures 12 and 13), the same date that blueberry aphids were first observed on noncaged field plants. There were statistically significant differences ($P < 0.001$) in the mean aphid population numbers on the caged versus noncaged source plants (Table A-2). The caged aphid populations increased to greater numbers during the season than the uncaged aphid populations. However, both the caged and uncaged populations had the same seasonal patterns.

Alate Blueberry Aphid Activity

Alate Aphid Movement Outside the Field

Only four alatae were trapped outside of the blueberry field; only one caught on 29 May, 100 m east of the field was viruliferous.

Figure 10.--Transmission of blueberry shoestring virus (BBSSV) by blueberry aphids, experiment one. Aphids were allowed an acquisition access period of 24 hr on BBSSV-infected tissue. There were 15 test plant replications per inoculation access period treatment with 15 aphids transferred to each test plant.

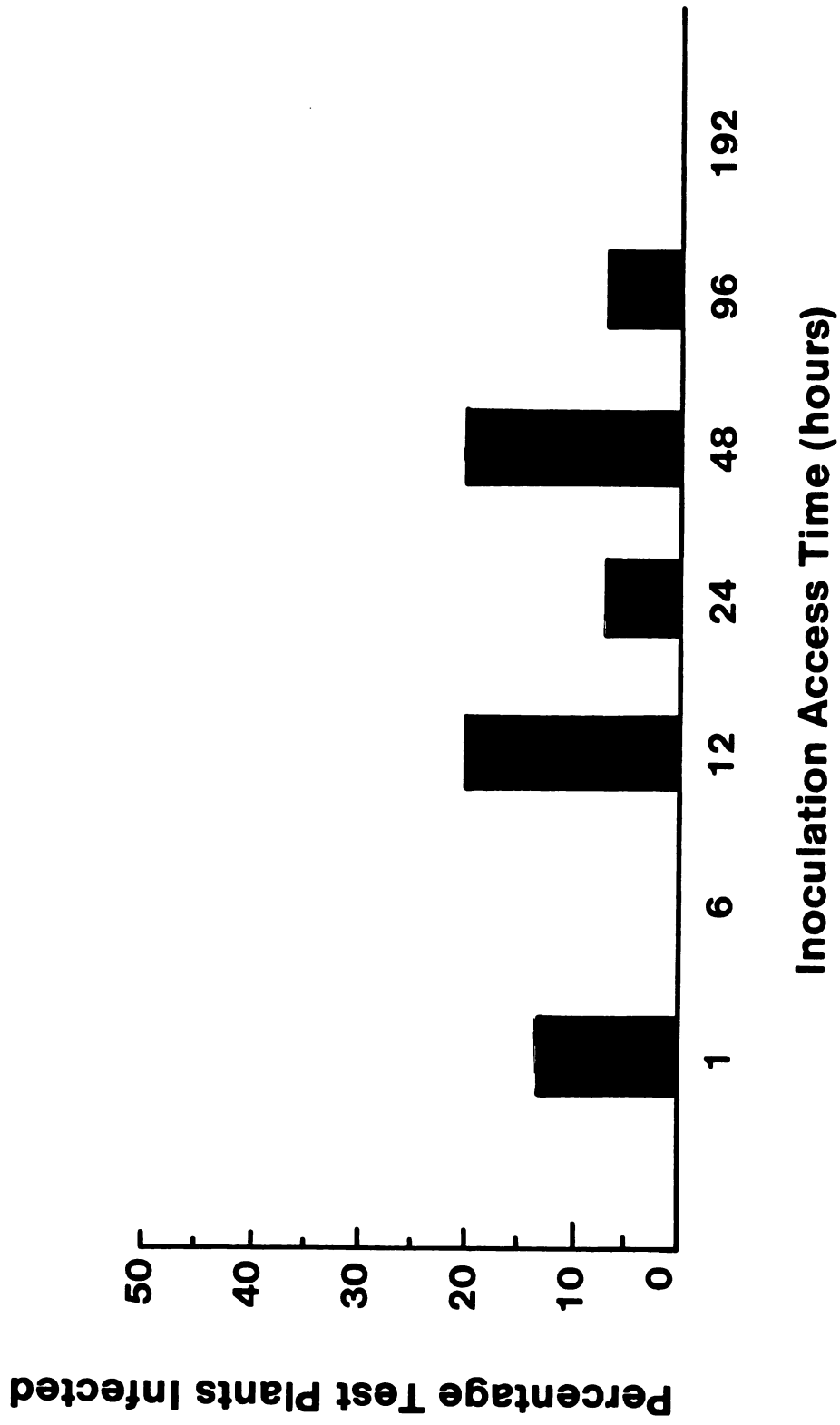
Percentage Test Plants Infected
0
10
20
30
40
50
1
6
12
24
48
96
192
Inoculation Access Time (hours)

Figure 10

Figure 11.--Transmission of blueberry shoestring virus (BBSSV) by blueberry aphids, experiment two. Aphids were allowed an acquisition access period of 24 hr on BBSSV-infected tissue. There were seven test plant replications per inoculation access period treatment with 15 aphids transferred to each test plant.

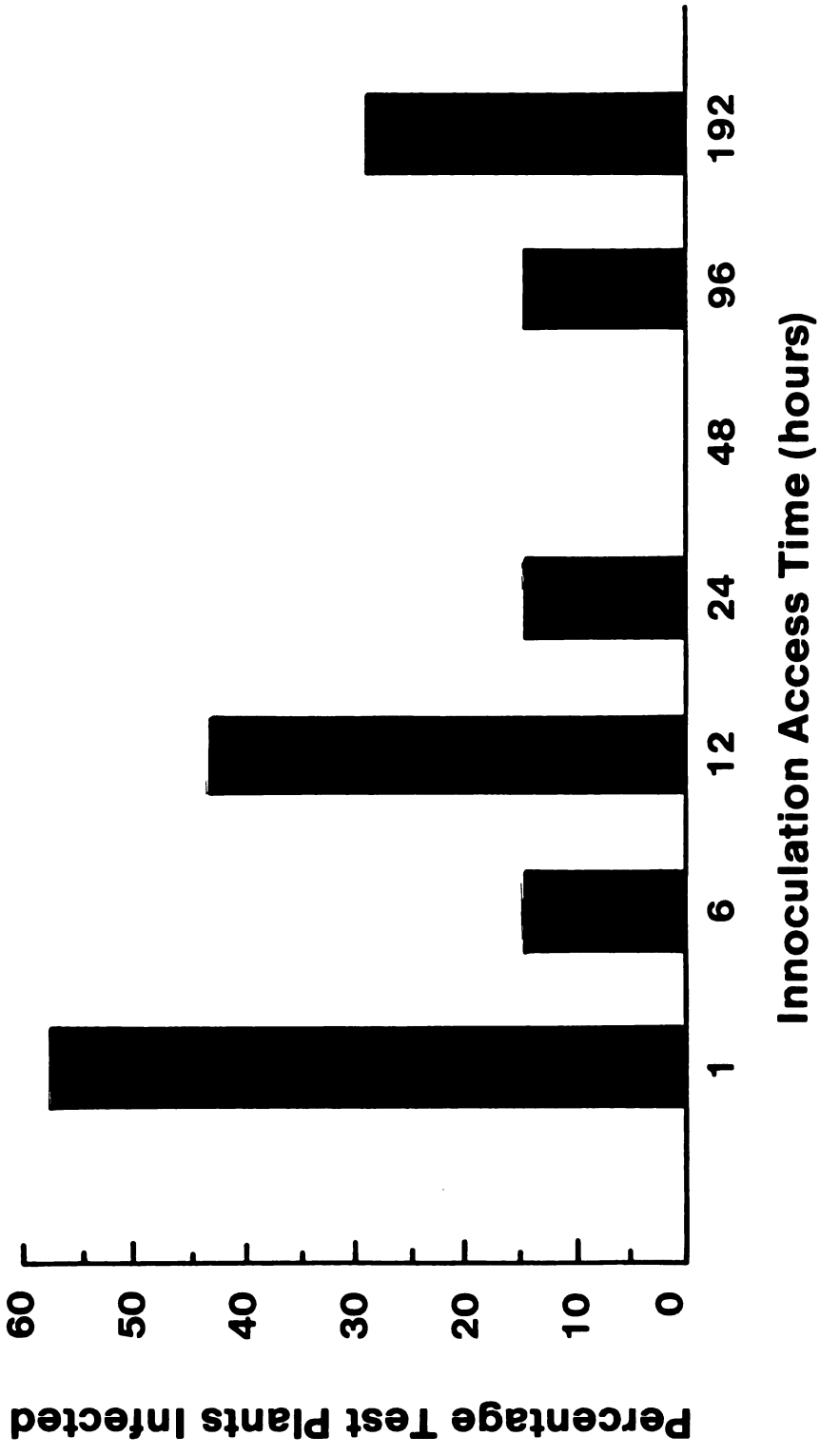
Percentage Test Plants Infected
0
10
20
30
40
50
60
1
6
12
24
48
96
192
Innoculation Access Time (hours)

Figure 11

Figure 12.--Seasonal apterous blueberry aphid populations on caged and uncaged BBSSV-infected source plants. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

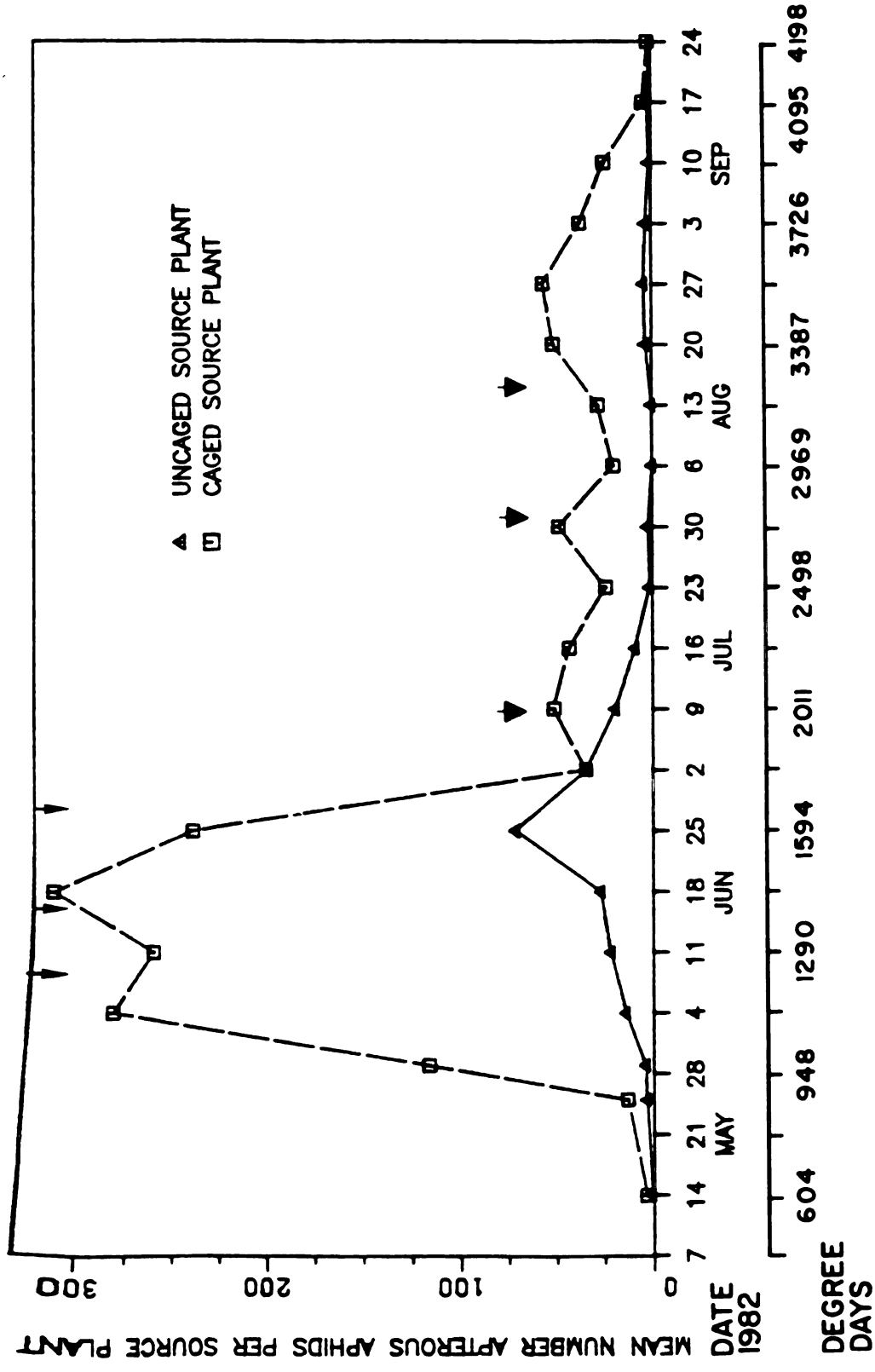
UNCAGED SOURCE PLANT
CAGED SOURCE PLANT
MEAN NUMBER APTEROUS APHIDS PER SOURCE PLANT
0
100
200
300
DATE 1982
7 14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY JUN JUL AUG SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 12

Figure 13.--Seasonal alate blueberry aphid populations on caged and uncaged BBSSV-infected source plants. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

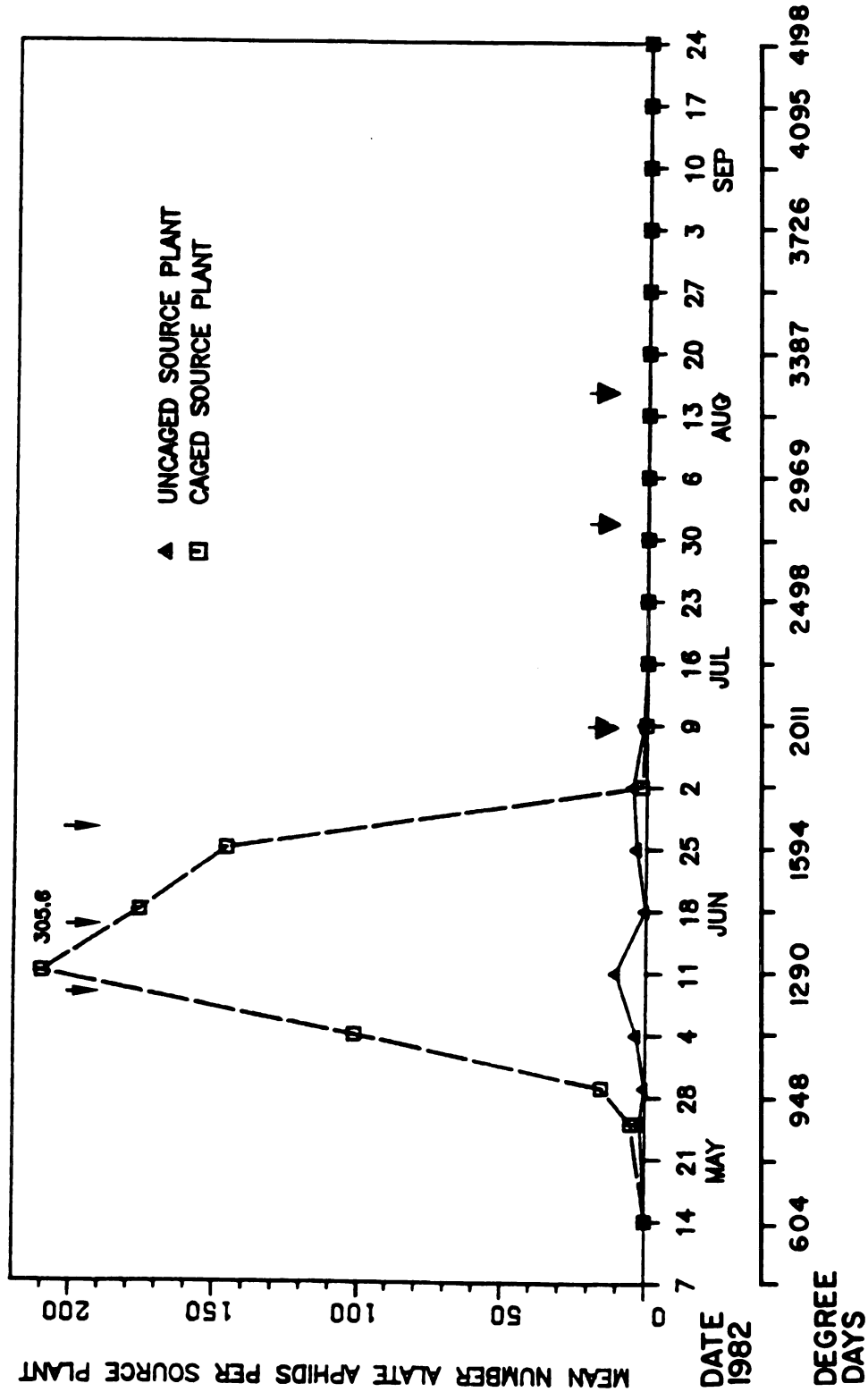
UNCAGED SOURCE PLANT
CAGED SOURCE PLANT
305.8
MEAN NUMBER ALATE APHIDS PER SOURCE PLANT
0
50
100
150
200
DATE 1982
7 14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY JUN JUL AUG SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 13

Alate Aphid Movement
Within the Field

The distribution of viruliferous blueberry aphids caught in yellow pan traps during 1982 is shown in Figure 14. In the figure each square represents the placement of yellow pan traps in the VenRoy blueberry field. Each letter adjacent to each square corresponds to the date an individual aphid was collected from the trap. A letter by the upper right corner of the square represents an aphid collected from the high (2 m) trap, while a letter by the lower right corner of the square represents an aphid caught in the lower (0.5 m) trap.

Most of the viruliferous aphids were caught early in the season--May through mid-June. A greater proportion of viruliferous aphids were collected from the low traps (26 of 35) versus the high traps (9 of 35). The northwest and central low traps caught 17 and 5 viruliferous blueberry aphids, respectively. The northeast and southeast high traps caught two and five viruliferous blueberry aphids, respectively, while the corresponding low traps did not catch any viruliferous aphids.

Although the yellow pan traps were set up to trap alate aphids, many nymphs and adult apterous aphids were collected from the traps as well. Figure 15 shows the numbers of apterous and alate blueberry aphids, irrespective of whether or not they were viruliferous, caught in all the yellow pan traps for each sampling date in the 1982 season. Most of the aphids were collected through mid-July. More than 30 apterous aphids were collected from the traps each sampling date from 11 June through 9 July; the greatest number of apterous aphids, 65, was collected on 2 July. The greatest number of alate aphids, 21, was

Figure 14.--Map of locations and dates of viruliferous blueberry aphids caught in yellow pan traps. Each square represents the location of two pan traps: one on a 2 m high platform and one on a 0.5 m box. Each letter represents one viruliferous aphid collected on the designated date. A letter at the upper right corner of the square indicates the aphid was caught in the high trap, while a letter at the lower right corner indicates the aphid was caught in the low trap. Eastmanville, MI. 1982.

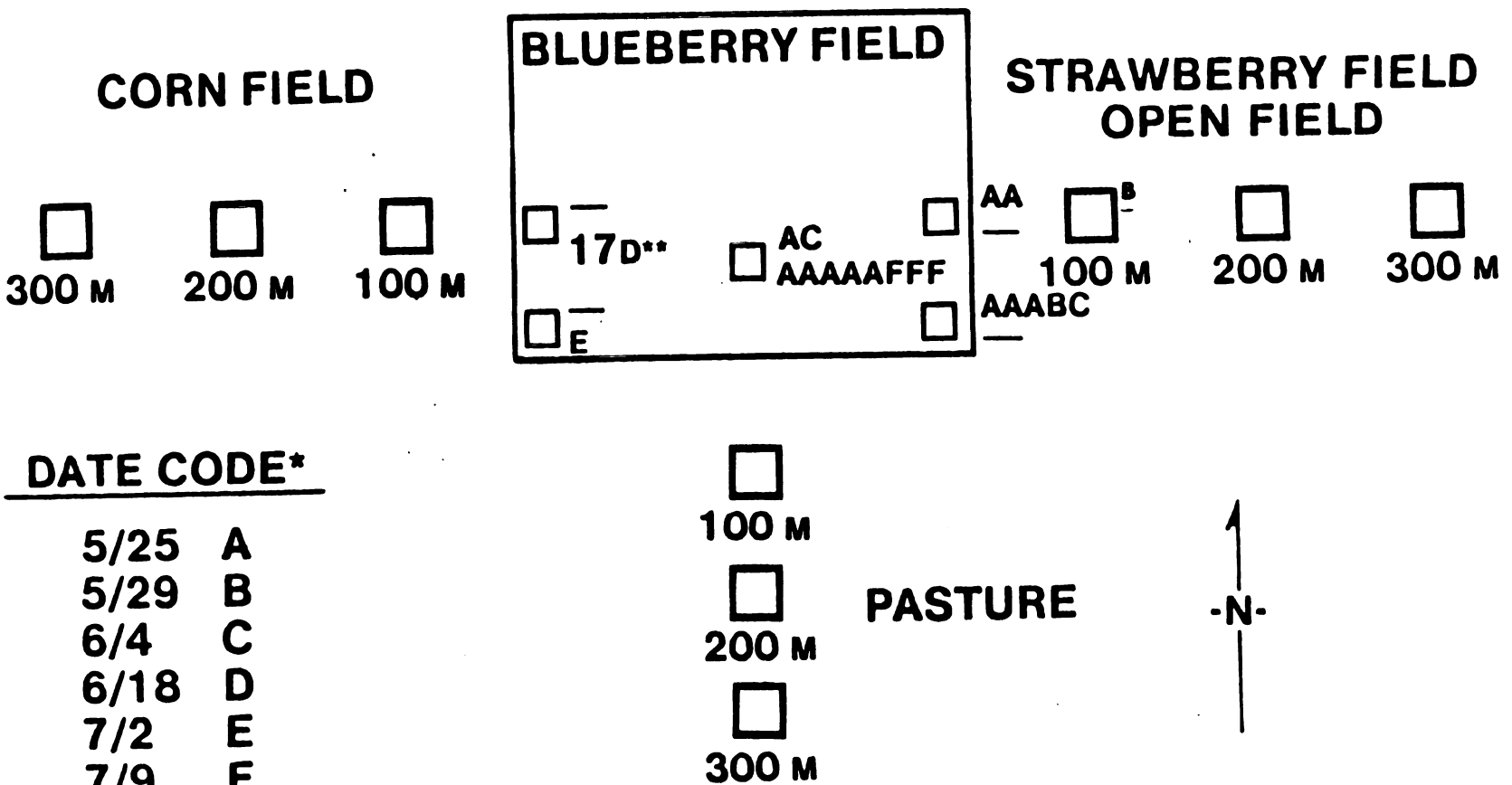
CORN FIELD
BLUEBERRY FIELD
STRAWBERRY FIELD
OPEN FIELD
300 M
200 M
100 M
17D**
AC
AAAAAFFF
E
AA
AAABC
B
100 M
200 M
300 M
DATE CODE*
5/25 A
5/29 B
6/4 C
6/18 D
7/2 E
7/9 F
100 M
200 M
300 M
PASTURE
-N-

Figure 14

Figure 15.--Seasonal distribution of apterous and alate blueberry aphids caught in 10 yellow pan traps. Each number represents the total number of apterous or alate aphids collected in the five low (0.5 m) and five high (2 m) traps within the field for each date regardless of whether or not the aphids were viruliferous. Eastmanville, MI. 1982.

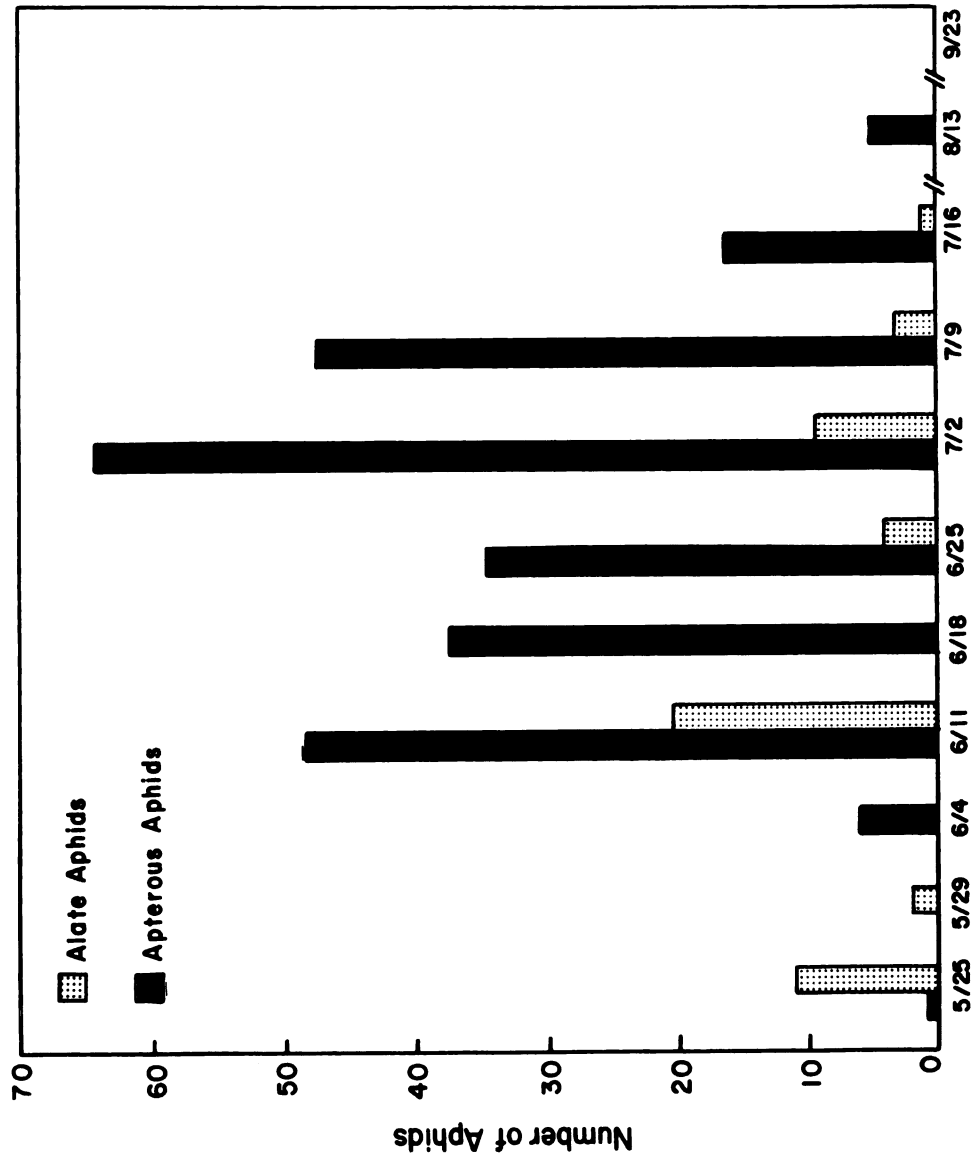
Alate Aphids
Apterous Aphids
Number of Aphids
0
10
20
30
40
50
60
70
5/25
5/29
6/4
6/11
6/18
6/25
7/2
7/9
7/16
8/13
9/23
Date

Figure 15

trapped 11 June. Fewer than 10 alatae were collected per sampling date after 11 June, and none were collected after 16 July.

Alate Aphids on Screen Cages

Of the 500 alate blueberry aphids collected from screen cage walls enclosing BBSSV-infected source bushes and assayed for BBSSV, 14 were viruliferous (Table 1). Overall, 2.8% of these alate blueberry aphids tested were viruliferous. This figure indicates the proportion of viruliferous aphids that may fly to other bushes.

Table 1.--Alate blueberry aphids collected from screen cage walls enclosing BBSSV-infected source plants.

Sampling Date	Number of Alatae Viruliferous	Number of Alatae Assayed[a]	Percent Alatae Viruliverous
11 June	2	80	2.5
18 June	1	107	0.9
25 June	2	44	4.5
26 June	3	106	2.8
26 June	6	163	3.7
Total	14	500	2.8

[a]Aphids were individually tested for presence of BBSSV by RIA.

Seasonal Apterous Blueberry Aphid Populations and Bush-to-Bush Movement

Source Plants

Figure 12 shows the seasonal populations of apterous aphids on BBSSV-infected source plants. The points represent the mean numbers of apterous aphids counted for seven caged or seven noncaged source plants.

The apterae populations on the caged source plants were much greater over the season than the corresponding populations on the noncaged source plants. The enclosing screen cages provided protection against aphid mortality factors such as rain, wind, parasites, and predators.

Although the population numbers of the apterae on the caged versus noncaged source plants were significantly different ($P < 0.001$), the populations followed the same general seasonal pattern. The mean numbers of apterae per source plant were maximum the last part of June: 320 apterae on 18 June and 71 apterae on 25 June for caged and noncaged source plants, respectively. The populations then decreased to a minimum during late July and early August. From mid- to late August there was a slight increase in the mean numbers of apterae on source plants which subsequently decreased and remained very low through September when the experiments were terminated.

Trap Plants

The mean numbers of apterous aphids per trap plant touching and not touching source bushes are shown in Figures 16 and 17, respectively. As with the apterous populations on the source plants,

Figure 16.--Seasonal distribution of apterous blueberry aphids on blueberry trap plants touching BBSSV-infected source plants. Trap plants were or were not enclosed in aphid-proof cages with the source plants. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

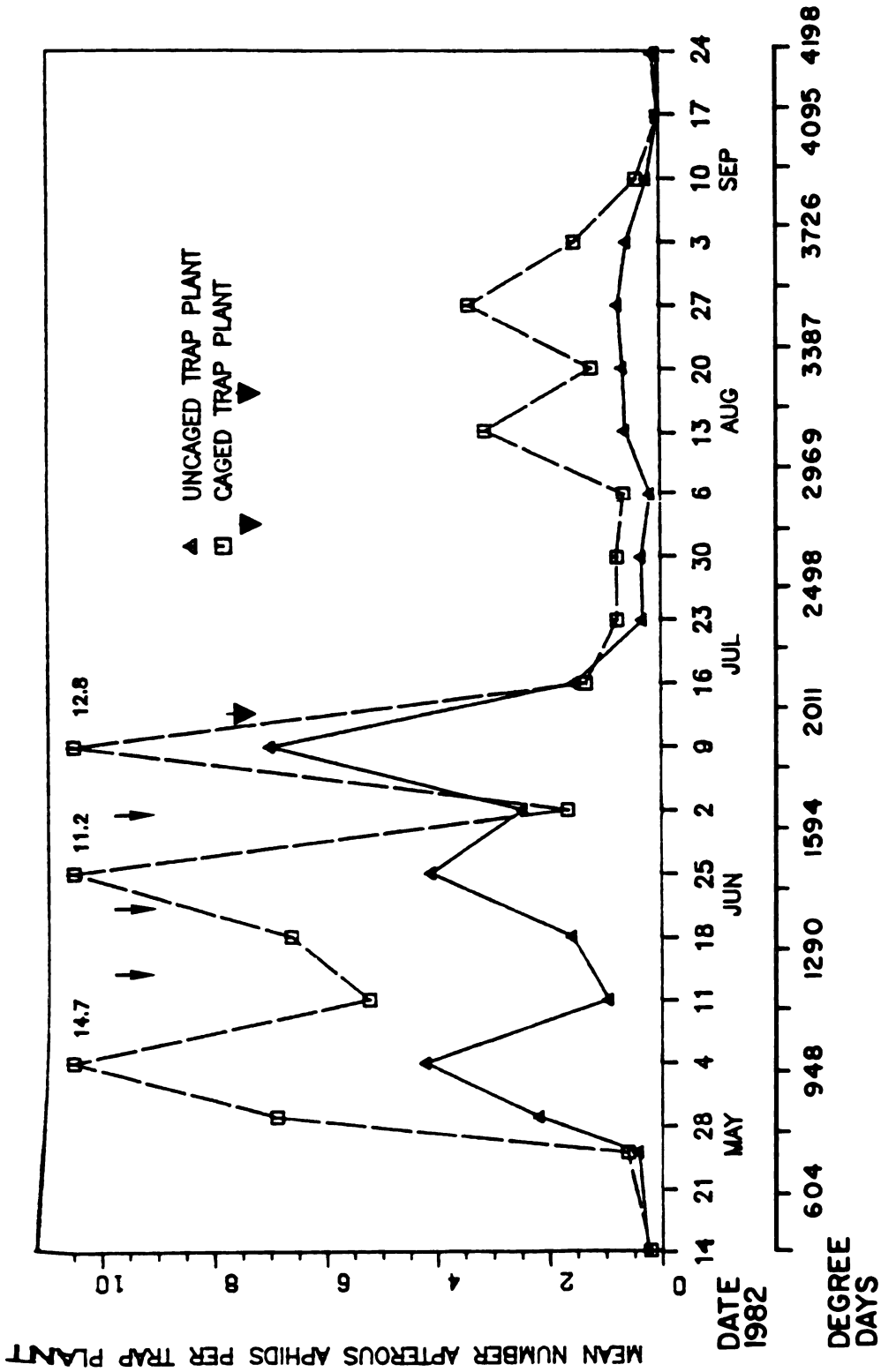
MEAN NUMBER APTEROUS APHIDS PER TRAP PLANT
0
2
4
6
8
10
UNCAGED TRAP PLANT
CAGED TRAP PLANT
14.7
11.2
12.8
14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY
JUN
JUL
AUG
SEP
DATE 1982
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198
DEGREE DAYS

Figure 16

Figure 17.--Seasonal distribution of apterous blueberry aphids on blueberry trap plants not touching BBSSV-infected source plants. Trap plants were or were not enclosed in aphid-proof cages with the source plants. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

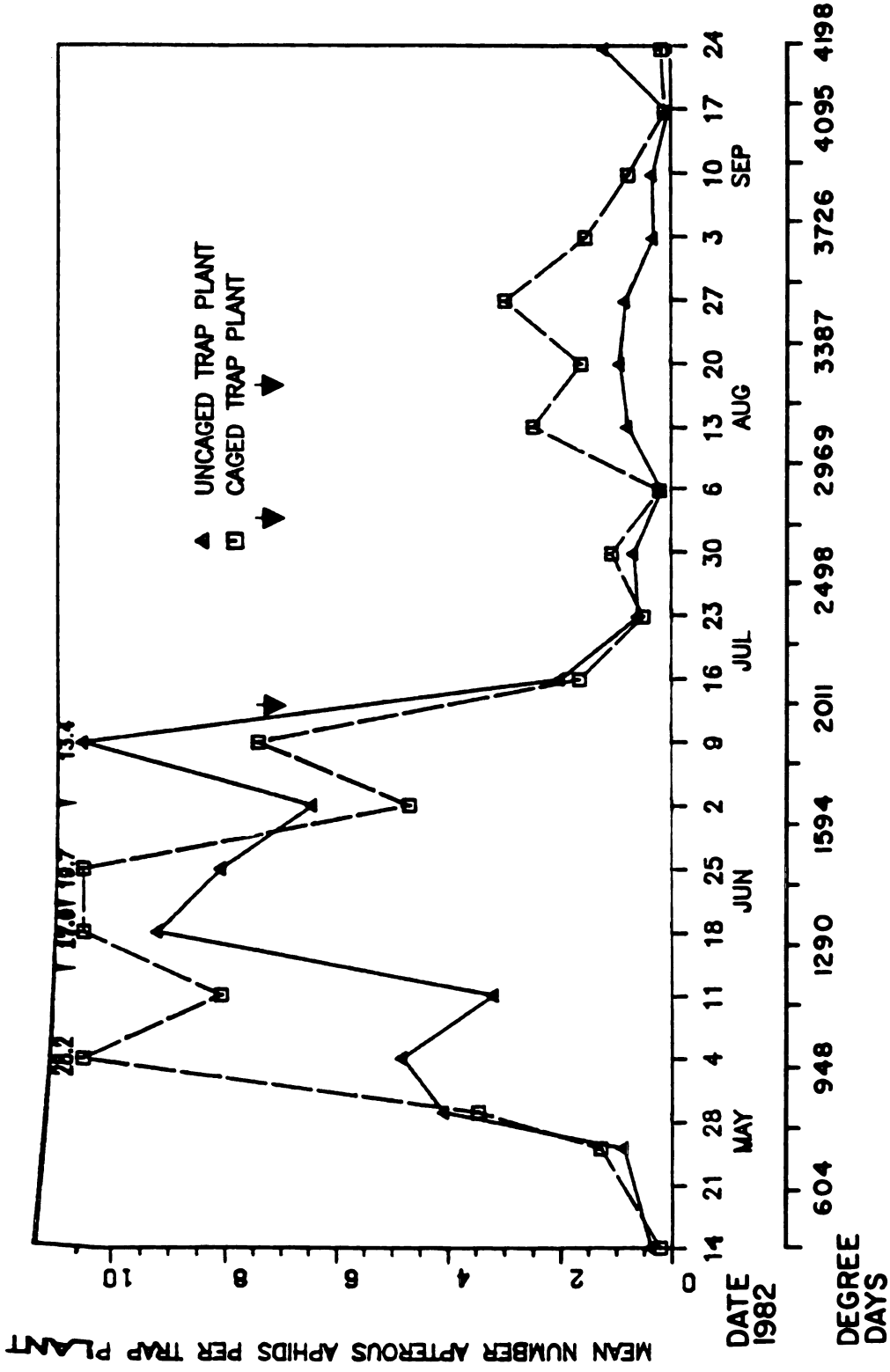
MEAN NUMBER APTEROUS APHIDS PER TRAP PLANT
0
2
4
6
8
10
UNCAGED TRAP PLANT
CAGED TRAP PLANT
28.2
13.4
DATE 1982
14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY
JUN
JUL
AUG
SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 17

those populations on the plants enclosed within cages were significantly greater ($P < 0.01$) than those on plants not within the cages and exposed to the natural environment. These aphid populations also followed the same seasonal fluctuations. The populations on the trap plants were very high the first half of the growing season, through the first week of July. The populations were low during late July and then increased again during August before tapering to the low mean apterae numbers found in autumn. The apterae population pattern had two peaks: one very high peak early in the season when the plants were rapidly growing, and another slight peak during August when the plants had new growth after fruiting.

The relative decreases in apterae populations found on the trap plants on 11 June, 2 July, 16 July, and 20 August (Figures 16 and 17) were due to insecticide applications. These decreases in populations after pesticide applications are not as apparent on the source plant apterae populations (Figure 12).

Viruliferous Apterous Blueberry Aphids

Source Plants

After the aphid populations were counted, samples were collected and tested for presence of BBSSV using RIA. The incidence of viruliferous apterous aphids on caged and noncaged source plants is presented in Figure 18. There were no significant differences ($P < 0.05$) in the percentage of viruliferous aphids on caged versus noncaged source plants. The data points for 14 May through 4 June are the results of aphids tested for BBSSV in groups of five. Thereafter, apterae were individually assayed for BBSSV. Through 9 July the mean

Figure 18.--Seasonal distribution of viruliferous apterous aphids on caged and uncaged BBSSV-infected source plants. Degree day base 38 F. Eastmanville, MI. 1982.

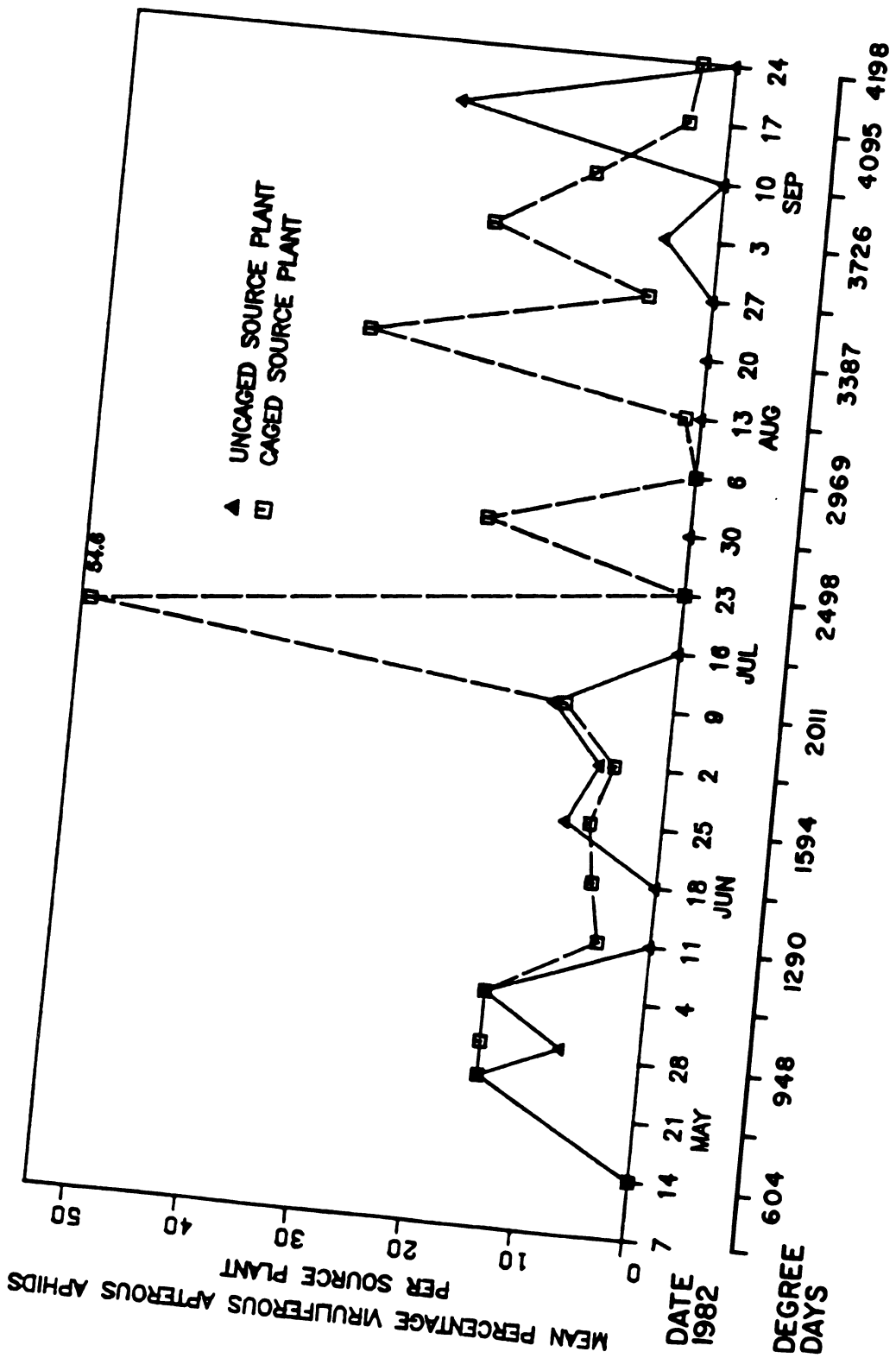
MEAN PERCENTAGE VIRULIFEROUS APTEROUS APHIDS PER SOURCE PLANT
0
10
20
30
40
50
84.6
UNCAGED SOURCE PLANT
CAGED SOURCE PLANT
DATE 1982
7 14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY
JUN
JUL
AUG
SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 18

percentages of viruliferous apterous aphids on caged and noncaged source plants were similar. Between 16 July and the end of September these percentages varied widely. The large differences in mean percentage viruliferous apterae through the season may have been due to the sample size variation. After 9 July there were very few apterae on the source plants, and there were even fewer apterae that could be collected and assayed. The smaller sample numbers may have resulted in greater differences in the proportions of virus-carrying aphids.

Trap Plants

The mean percentages of viruliferous apterous aphids on trap plants touching and not touching source plants are presented in Figures 19 and 20, respectively. Aphids were tested for BBSSV in batches of five for the data points of the dates 14 May through 4 June. Apterous aphids were individually tested after 4 June.

Most of the mean percentages of viruliferous apterous aphids on trap plants were less than 20%; however, these percentages fluctuated throughout the season. This was probably due to the small sample sizes mentioned earlier for the source plants. In addition, the very high percentages found on 16 July for the source plants (Figure 18) and trap plants touching and not touching the source plants (Figures 19 and 20), respectively, may be explained by spurious assay results rather than deviations in field biology.

Figure 19.--Seasonal distribution of viruliferous apterous aphids on blueberry trap plants touching BBSSV-infected source plants. Trap plants were or were not enclosed in aphid-proof cages with the source plants. Degree day base 38 F. Eastmanville, MI. 1982.

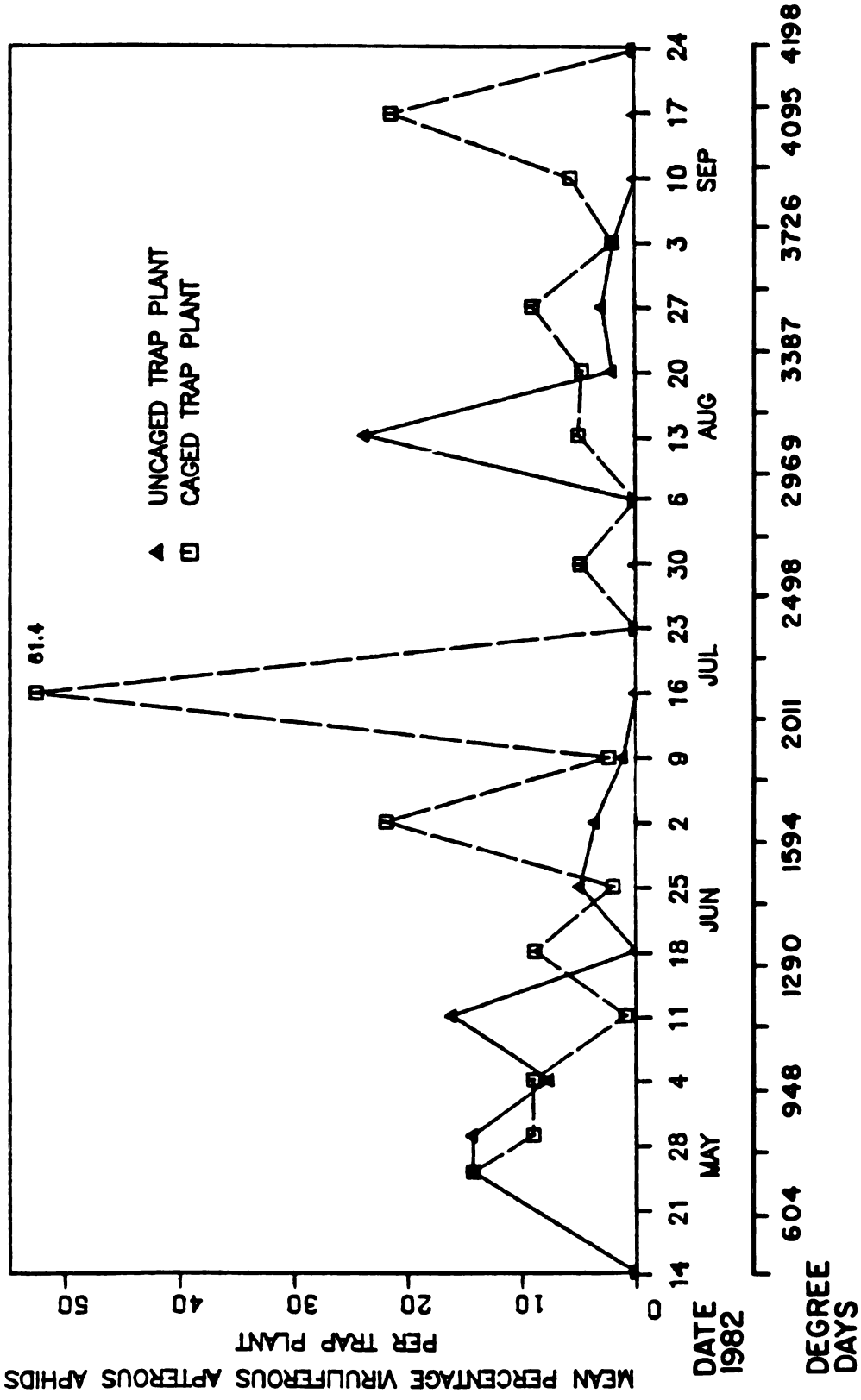
MEAN PERCENTAGE VIRULIFEROUS APTEROUS APHIDS PER TRAP PLANT
0
10
20
30
40
50
61.4
UNCAGED TRAP PLANT
CAGED TRAP PLANT
DATE 1982
14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY
JUN
JUL
AUG
SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 19

Figure 20.--Seasonal distribution of viruliferous apterous aphids on blueberry trap plants not touching BBSSV-infected source plants. Trap plants were or were not enclosed in aphid-proof cages with the source plants. Degree day base 38 F. Eastmanville, MI. 1982.

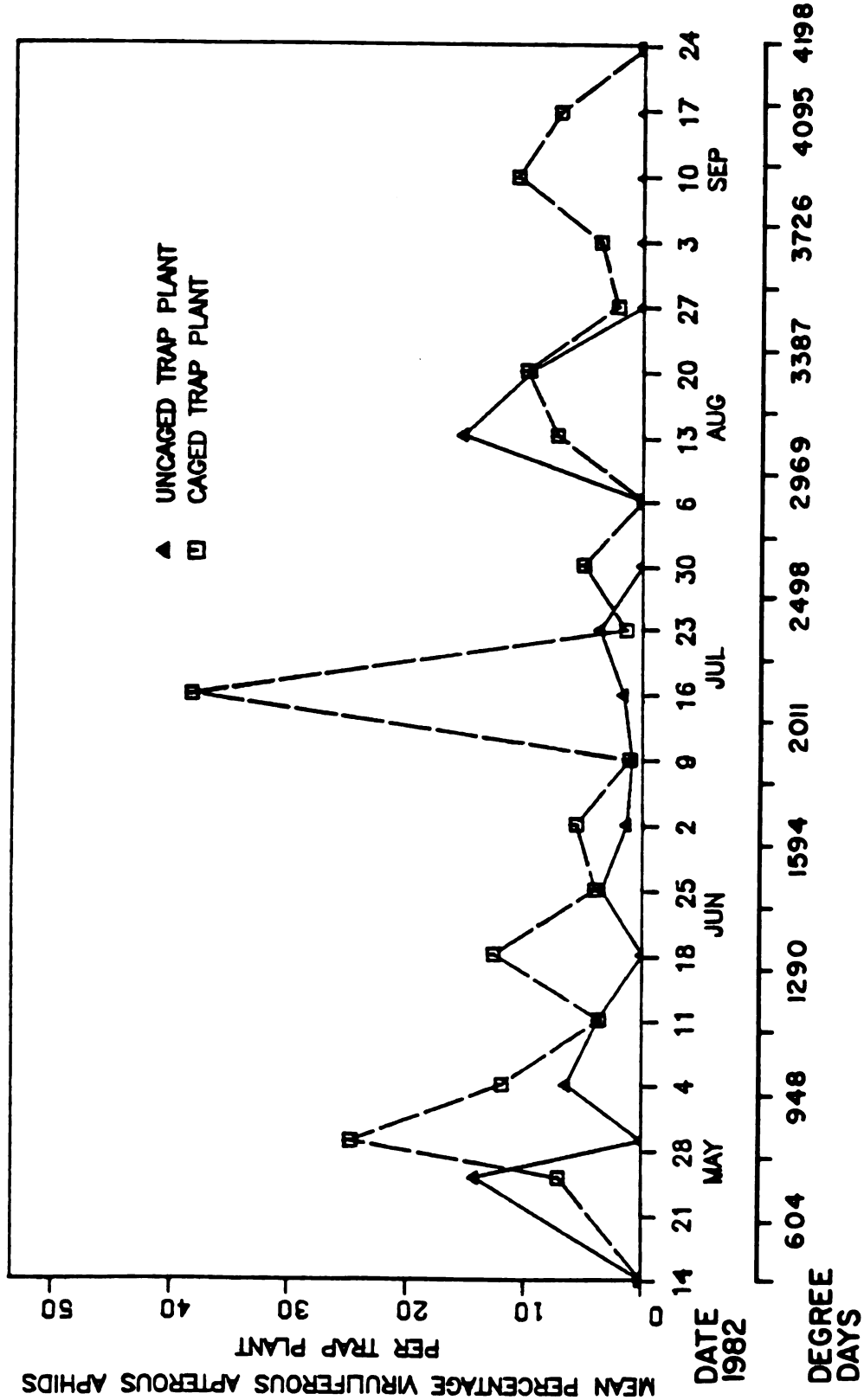
MEAN PERCENTAGE VIRULIFEROUS APTEROUS APHIDS PER TRAP PLANT
0
10
20
30
40
50
UNCAGED TRAP PLANT
CAGED TRAP PLANT
DATE 1982
14 21 28 MAY 4 11 18 25 JUN 2 9 16 23 30 JUL 6 13 20 27 AUG 3 10 17 24 SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 20

Seasonal Alate Blueberry Aphid Populations

Source Plants

The mean population numbers of alate blueberry aphids counted on caged and noncaged source plants is shown in Figure 13. No alatae were found on 14 May. The caged alate population logarithmically increased to a maximum mean number of 305.6 alatae per source bush on 11 June. This population then gradually decreased over the next 2 weeks before sharply declining prior to 2 July. This sharp drop in the caged alatae population may have been due either to natural population dynamics, the insecticide applied in the field on 28 June, or both. The greatest mean number of alatae counted on noncaged source plants was also on 11 June. No alatae were observed on any of the source plants after 16 July.

Trap Plants

Figures 21 and 22 show the mean alatae populations on trap plants. Populations were greatest from 29 May through 25 June. Except for a mean number of 0.11 alatae (four aphids for 35 trap plants not touching source plants) found on 6 August, no alatae were found after 23 July. The maximum mean number of alatae on caged touching trap plants was 14.9 on 18 June, while the maximum mean number for caged nontouching trap plants was 5 on 25 June. For the noncaged trap plants there were no definite maximum peak populations; the mean numbers were never greater than one alate aphid per trap plant.

Figure 21.--Seasonal distribution of alate blueberry aphids on blueberry trap plants touching BBSSV-infected source plants. Trap plants were or were not enclosed within aphid-proof cages with the source plants. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

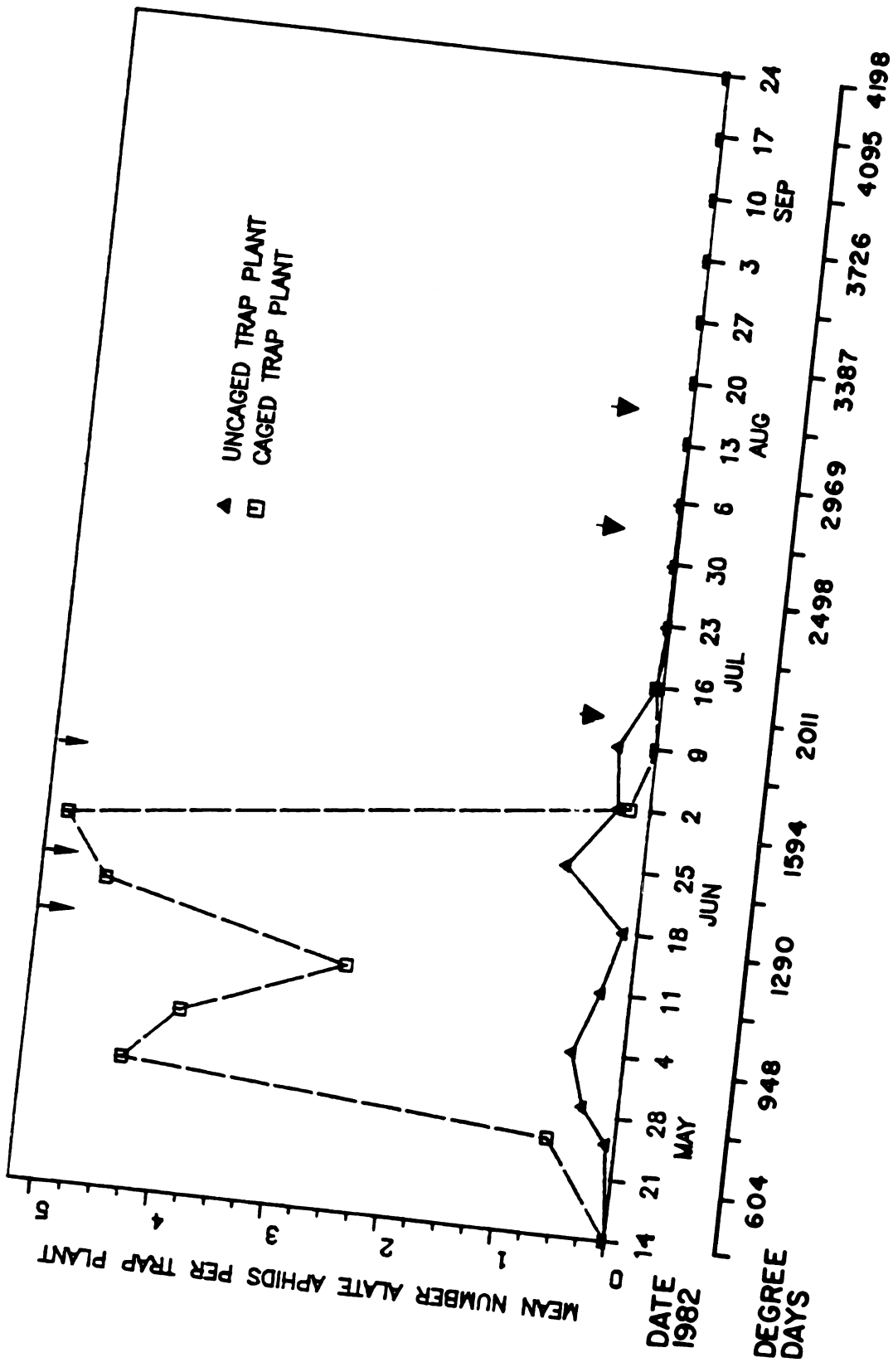
UNCAGED TRAP PLANT
CAGED TRAP PLANT
MEAN NUMBER ALATE APHIDS PER TRAP PLANT
0
1
2
3
4
5
DATE 1982
14 21 28 MAY 4 11 18 25 JUN 2 9 16 23 30 JUL 6 13 20 27 AUG 3 10 17 24 SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 21

Figure 22.--Seasonal distribution of alate blueberry aphids on blueberry trap plants not touching BBSSV-infected source plants. Trap plants were or were not enclosed in aphid-proof cages with the source plants. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

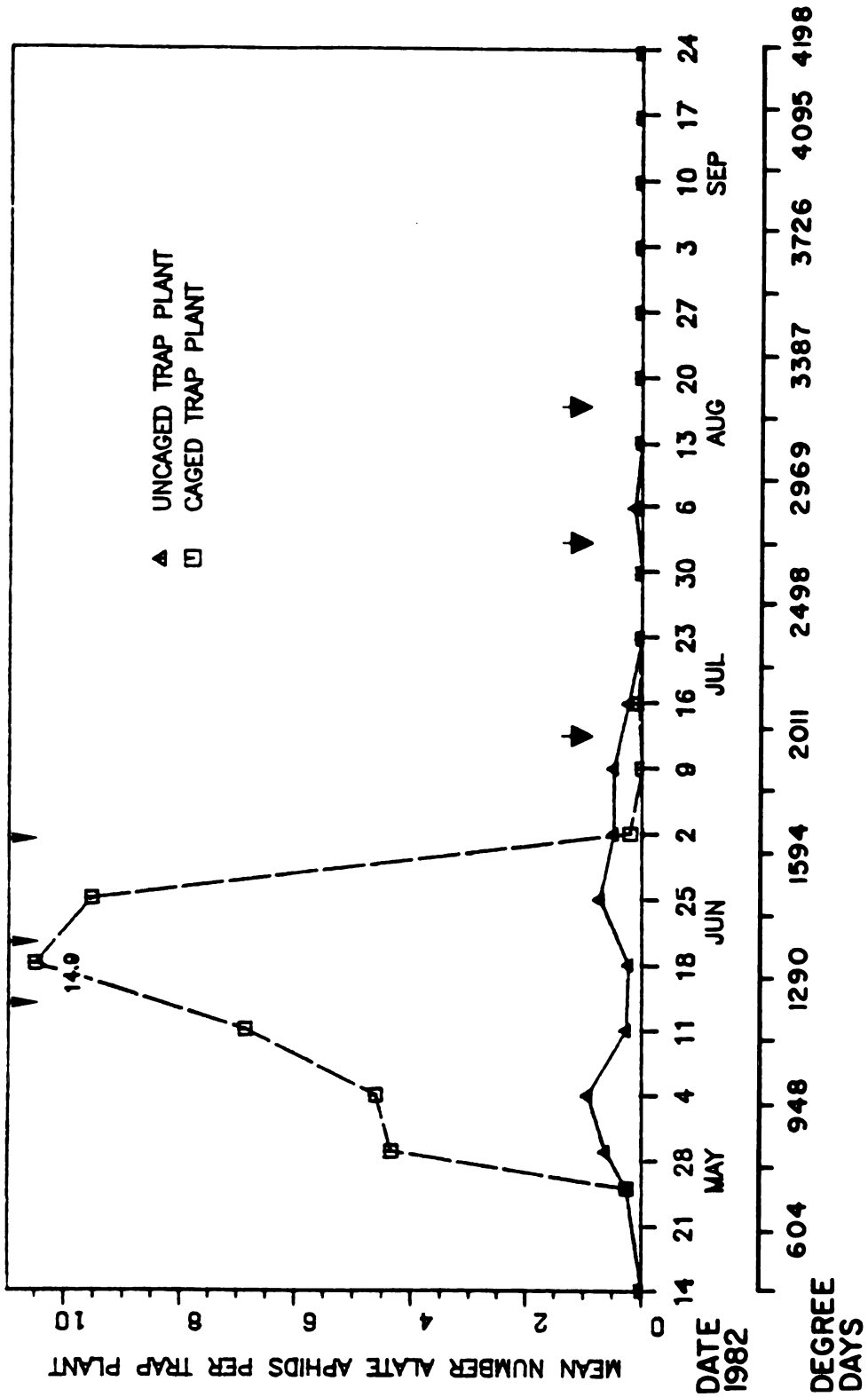
UNCAGED TRAP PLANT
CAGED TRAP PLANT
14.0
MEAN NUMBER ALATE APHIDS PER TRAP PLANT
0
2
4
6
8
10
DATE 1982
14 21 28 MAY 4 11 18 25 JUN 2 9 16 23 30 JUL 6 13 20 27 AUG 3 10 17 24 SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 22

Viruliferous Alatae

Source Plants

Viruliferous alatae on source plants were first detected 25 May (Figure 23). The mean percentage of the viruliferous alate aphids for noncaged source plants was highest the first date of detection, 25 May. For caged source plants, the maximum (51.6%) was reached the next sampling date--29 May. On other sampling dates the mean percentages of viruliferous alatae on caged or noncaged source plants ranged from 3.6 to 25%. No viruliferous alatae were detected on source plants after 2 July.

Trap Plants

The mean percentages of viruliferous alate aphids on touching and nontouching trap plants are presented in Figures 24 and 25, respectively. Virus-carrying alatae were first detected 25 May, the first day alatae were observed. These mean percentages were greatest on 25 May for noncaged trap plants, and on 4 June, for the caged trap plants.

Viruliferous alatae were collected from noncaged touching (Figure 24) and nontouching (Figure 25) trap plants only during 2- and 3-week periods, respectively, early in the season. Viruliferous alatae from caged trap plants (Figures 24 and 25) were detected over a longer period (5 weeks).

Quantity of BBSSV in Individual Aphids

The quantity of BBSSV in individual blueberry aphids collected from the trap plants and BBSSV-infected source plants is shown in

Table 2. Although most of the virus-carrying aphids contained relatively small quantities of BBSSV (less than 1.5 ng), quantities of BBSSV greater than 100 ng were detected in both apterous and alate individuals on trap plants as well as on source plants. The quantity of BBSSV detected in individual aphids for each sampling date varied greatly.

Table 2.--The distribution of quantities of blueberry shoestring virus (BBSSV) in individual viruliferous blueberry aphids collected from a field with BBSSV-infected source plants and healthy trap plants. Aphids were tested individually for the presence of BBSSV with RIA. Eastmanville, MI, 1982.[a]

Quantity of BBSSV per Aphid (ng)	Number of Viruliferous Aphids	
	Apterous Aphids	Alate Aphids
< 0.5	18	3
> 0.5 to 1.5	28	8
> 1.5 to 5	6	6
> 5 to 15	11	5
> 15 to 50	2	2
> 50 to 100	3	0
> 100	3	2

[a]Values are based on an RIA standard curve developed using purified BBSSV as test antigen.

Seasonal Trap Plant Infection

The percentages of noncaged trap plants touching and not touching infected source plants is presented in Figure 26. There were no significant differences in infection rate between trap plants touching

Figure 23.--Seasonal distribution of viruliferous alate blueberry aphids on caged and noncaged BBSSV-infected source plants. Degree day base 38 F. Eastmanville, MI. 1982.

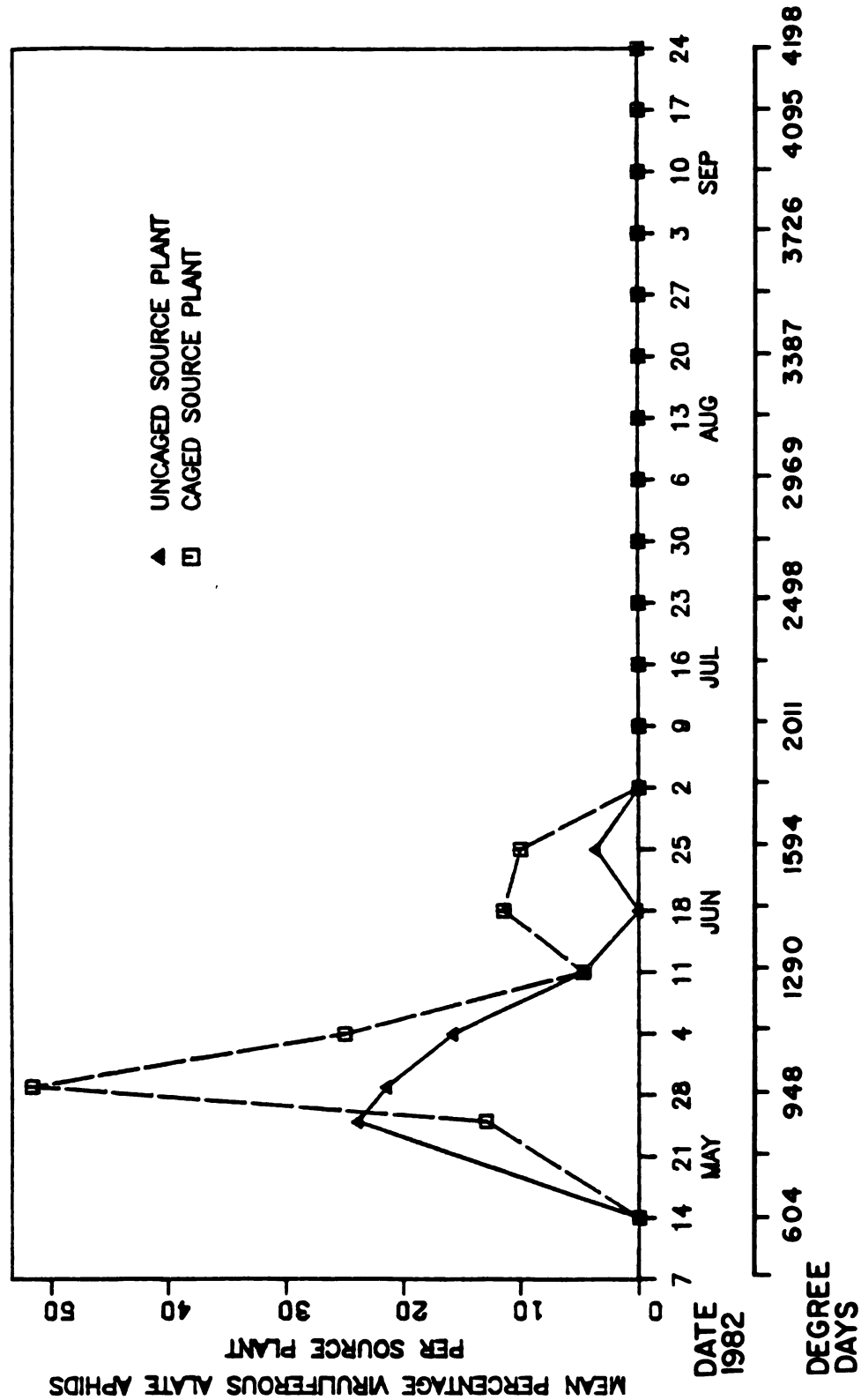
UNCAGED SOURCE PLANT
CAGED SOURCE PLANT
MEAN PERCENTAGE VIRULIFEROUS ALATE APHIDS PER SOURCE PLANT
0
10
20
30
40
50
DATE 1982
7
14
21
MAY
28
4
11
18
JUN
25
2
9
16
JUL
23
30
6
13
AUG
20
27
3
10
SEP
17
24
DEGREE DAYS
604
948
1290
1594
2011
2498
2969
3387
3726
4095
4198

Figure 23

Figure 24.--Seasonal distribution of viruliferous alate blueberry aphids on trap plants touching BBSSV-infected source plants. Trap plants were or were not enclosed within aphid-proof cages with source plants. Degree day base 38 F. Eastmanville, MI. 1982.

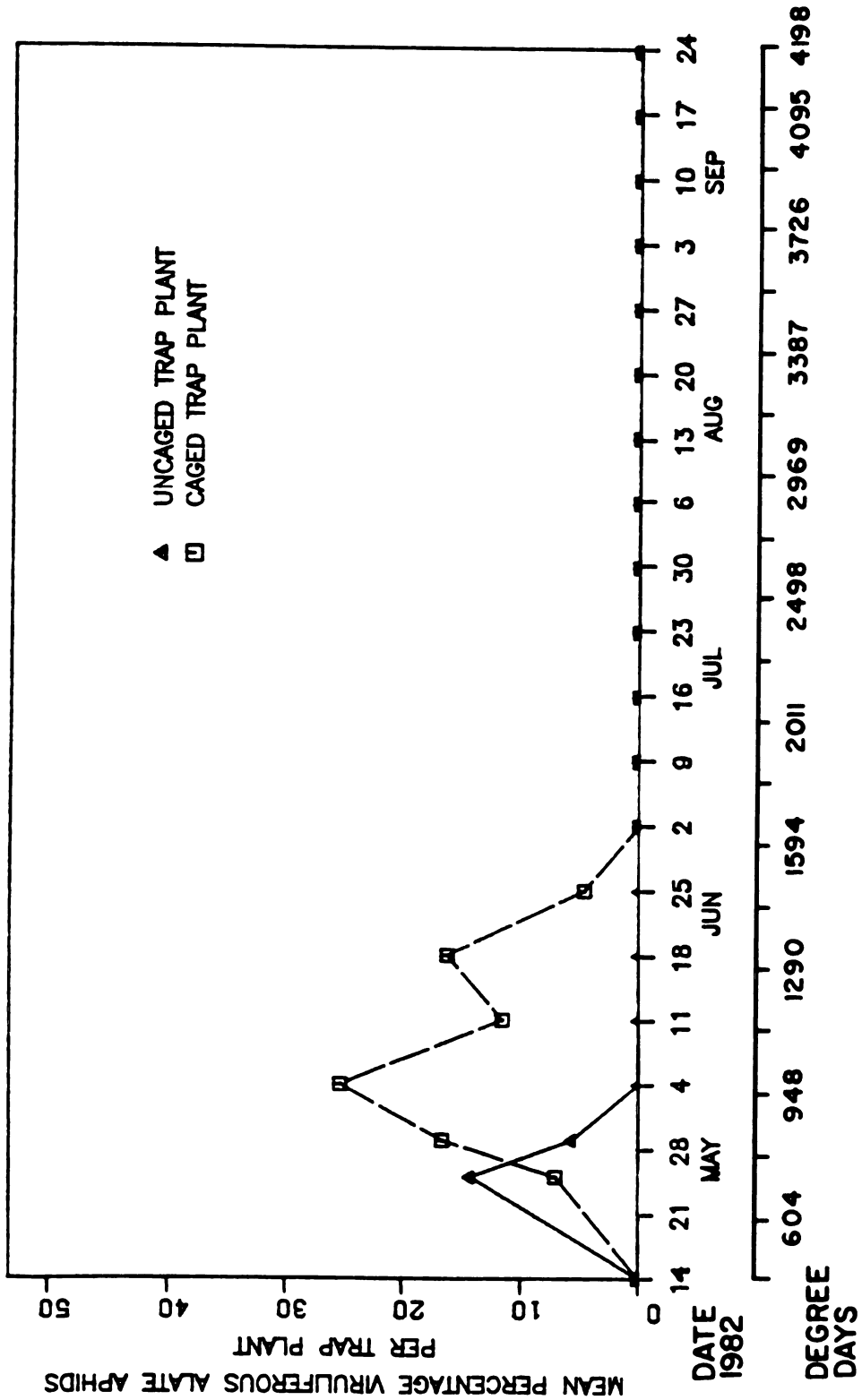
MEAN PERCENTAGE VIRULIFEROUS ALATE APHIDS PER TRAP PLANT
0
10
20
30
40
50
UNCAGED TRAP PLANT
CAGED TRAP PLANT
DATE 1982
14 21 28 MAY 4 11 18 25 JUN 2 9 16 23 30 JUL 6 13 20 27 AUG 3 10 17 24 SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 24

Figure 25.--Seasonal distribution of viruliferous alate blueberry aphids on trap plants not touching BBSSV-infected source plants. Trap plants were or were not enclosed within aphid-proof cages with source plants. Degree day base 38 F. Eastmanville, MI. 1982.

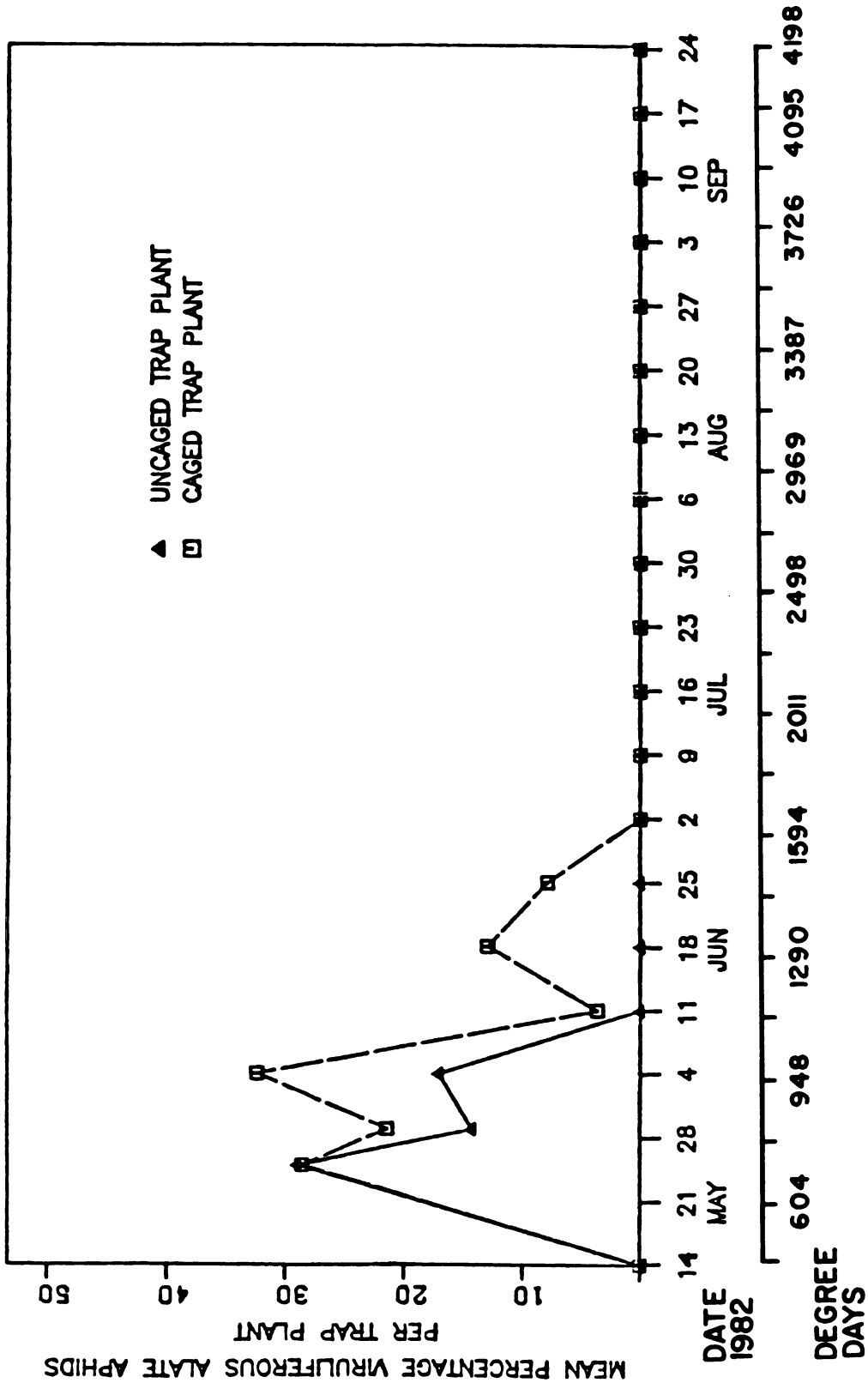
MEAN PERCENTAGE VIRULIFEROUS ALATE APHIDS PER TRAP PLANT
10
20
30
40
50
UNCAGED TRAP PLANT
CAGED TRAP PLANT
DATE 1982
14 21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY JUN JUL AUG SEP
DEGREE DAYS
604 948 1290 1594 2011 2498 2969 3387 3726 4095 4198

Figure 25

Figure 26.--Seasonal blueberry trap plant infection after 4-week exposure period to BBSSV-infected source plants within the field. Trap plants were not caged with source plants. Eastmanville, MI. 1982.

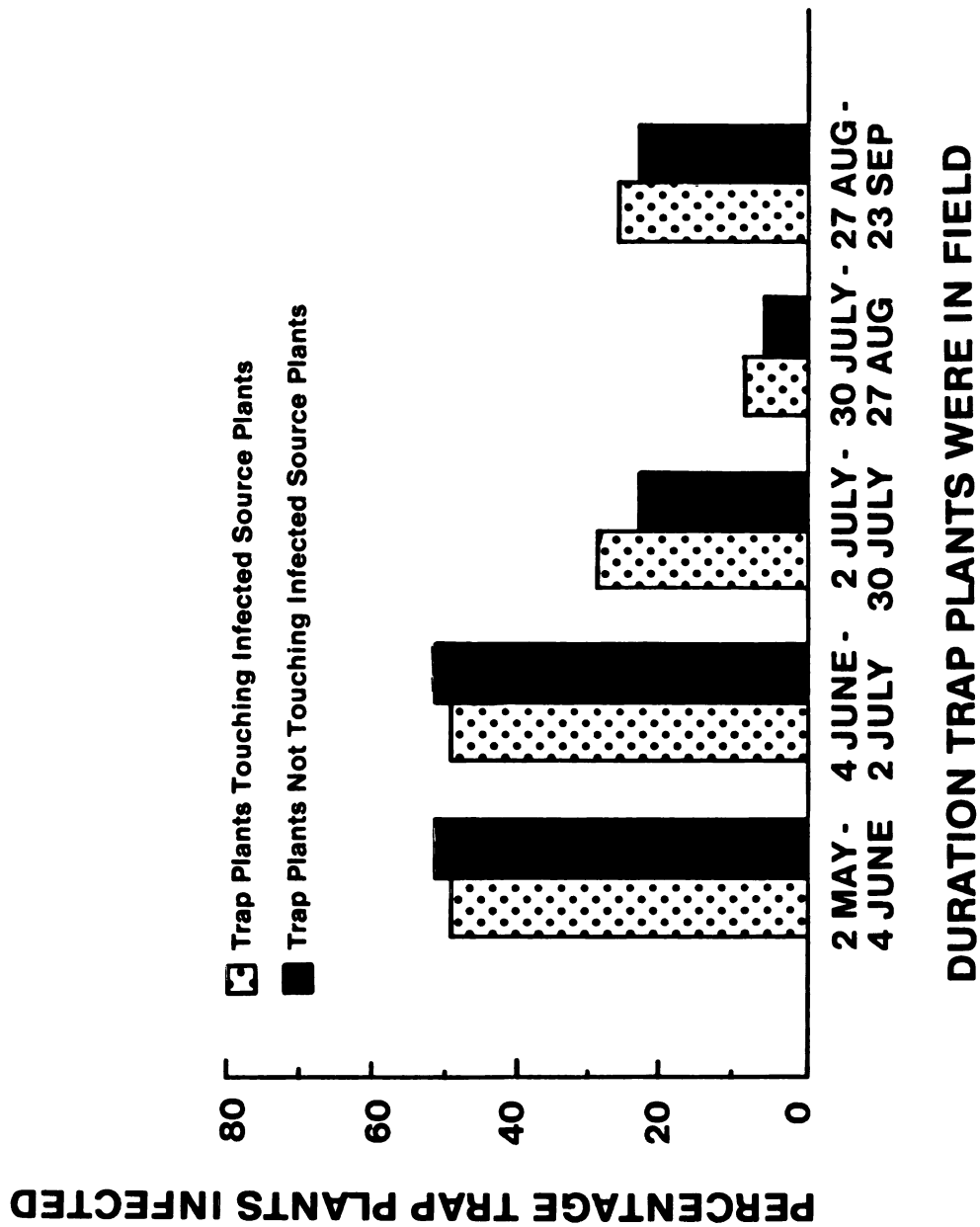
PERCENTAGE TRAP PLANTS INFECTED
80
60
40
20
0
Trap Plants Touching Infected Source Plants
Trap Plants Not Touching Infected Source Plants
2 MAY- 4 JUNE
4 JUNE- 2 JULY
2 JULY- 30 JULY
30 JULY- 27 AUG
27 AUG- 23 SEP
DURATION TRAP PLANTS WERE IN FIELD

Figure 26

and not touching source plants. The aphids were apparently able to move to and transmit virus to trap plants adjacent to touching plants regardless of physical contact between the bushes.

The greatest amount of BBSSV transmission occurred early in the season in May and June. During these infection periods 51% of the nontouching trap plants became infected, while 49% of the touching trap plants became infected. As the season progressed through July and August there was less BBSSV infection. In August only 9% and 6% of the touching and nontouching trap plants, respectively, became infected. The increase of trap plant infection during September corresponds to the slight resurgence in apterous aphid populations during this time. There was no statistically significant difference ($P < 0.001$) between touching and nontouching trap plant infection over time.

Figure 27 shows the relationship between noncaged apterous aphid populations on source and trap plants and noncaged trap plant infection through the season. The percentage of trap plant infection seems to correspond with the size of the aphid populations. More infection occurred when the populations were high. Conversely, little infection occurred when the populations were low, as was found in mid-August.

Figure 27.--The relationship between the incidence of BBSSV-infected trap plants and the mean numbers of apterous blueberry aphids on noncaged source plants and trap plants. Trap plants were touching or not touching the source plants for 4-week intervals. Degree day base 38 F. Narrow arrows indicate insecticide spray application of Guthion 2 SC (2 lb ai/gallon), 1 pt/acre, by air blast sprayer. Wide arrows indicate application of Aqua Malathion (8 lb ai/gallon), 2 pt/acre, by air blast sprayer. Eastmanville, MI. 1982.

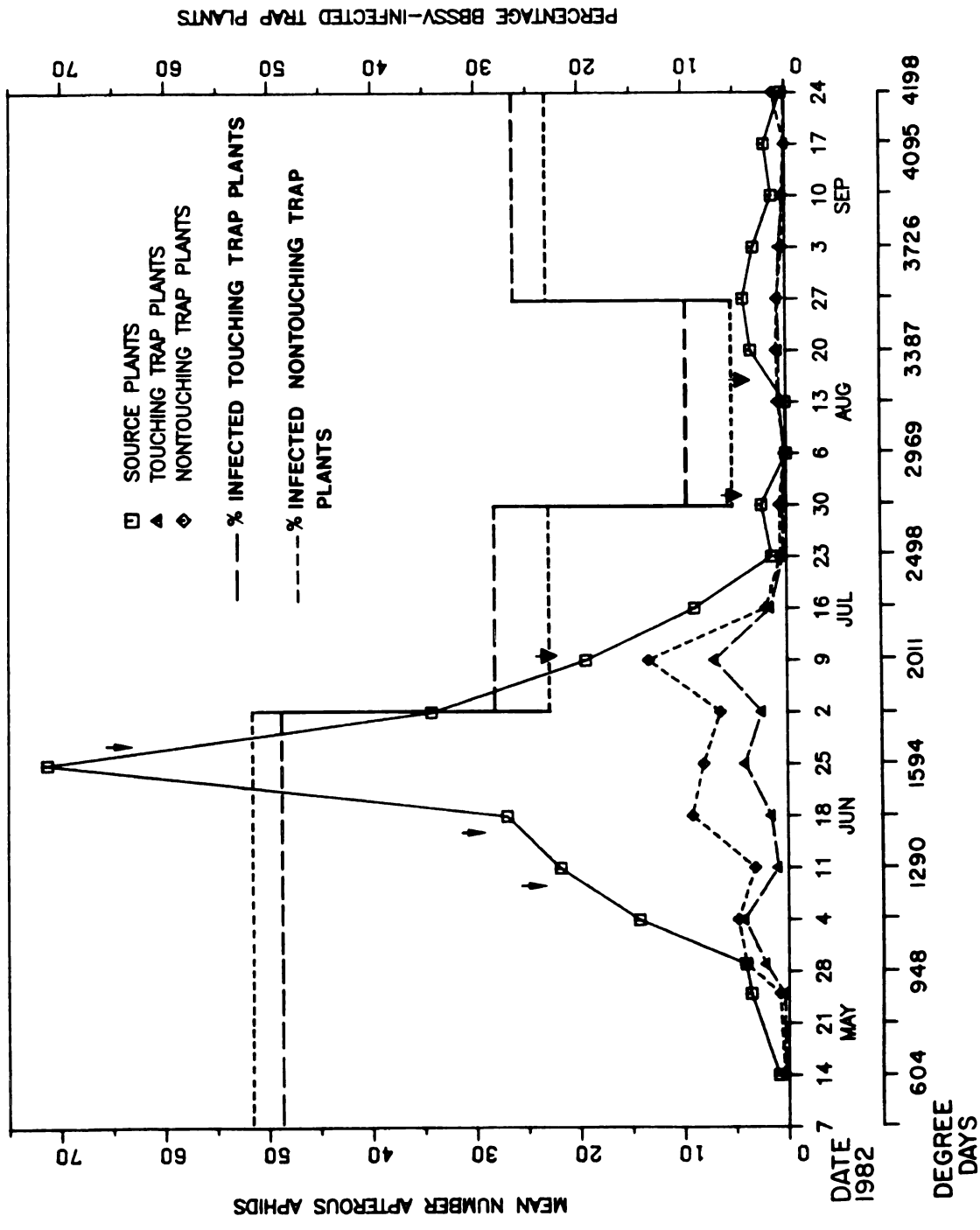
PERCENTAGE BBSSV-INFECTED TRAP PLANTS
MEAN NUMBER APTEROUS APHIDS
SOURCE PLANTS
TOUCHING TRAP PLANTS
NONTOUCHING TRAP PLANTS
% INFECTED TOUCHING TRAP PLANTS
% INFECTED NONTOUCHING TRAP PLANTS
DATE 1982
DEGREE DAYS
MAY
JUN
JUL
AUG
SEP

Figure 27

DISCUSSION

RIA was able to detect BBSSV in individual aphids collected from the field as well as aphids that had fed on very high concentrations of purified BBSSV preparations. ELISA and immunosorbent electron microscopy (ISEM) are not as sensitive as RIA in their ability to detect BBSSV in aphids (Gillett et al., 1983). ISEM is not amenable to processing large batches of samples since each sample must be individually scanned and visualized. In addition, icosahedral virions are difficult to distinguish among the insect proteins and debris. ELISA is a sensitive and quick assay easily adapted to processing large quantities of samples. Likewise, RIA is sensitive and quick with basically the same assay procedures as ELISA; however, with RIA, one must work with radioactive material. In addition, ^{125}I-gamma globulin cannot be stored as long as the enzyme conjugate used with ELISA because of the 60-day half-life of the radionuclide. In spite of the drawbacks of RIA over ELISA, RIA was used to detect BBSSV in blueberry aphids because it is more sensitive. The difference in sensitivity may be explained by the greater spatial impairment caused by conjugation of the enzyme to the antibody, compared to the radioactive iodine, which may reduce the binding ability of the antibody (Koenig, 1978).

The quantities of BBSSV acquired in any AAP by individual blueberry aphids varied greatly. Of the individuals that fed on BBSSV-infected tissue for a 24 hr AAP, 57% did not acquire detectable

quantities of virus. Quantities of 1 ng or less were detected in the virus-carrying individuals that had fed on infected tissue. This low quantity of virus uptake may be explained by an uneven distribution of BBSSV in the plant tissue. Hartmann et al. (1972) found an abundance of VLPs in the leaf epidermal cells, but varying amounts in the cytoplasm-containing cells of the xylem tissue.

Individual aphids that fed on purified preparations of BBSSV in sachets also showed large variability in virus uptake. These aphids, however, had access to homogeneous concentrations of virus versus the uneven distribution found in plants. Variability in this case suggests differences in individual aphid feeding behavior.

The variability found in the two virus transmission tests may be explained partly by the inefficiency of aphid transmission. Less than 50% of the aphids allowed to feed on BBSSV-infected plant tissue acquired detectable quantities of virus. These aphids ingested less than 1 ng of virus. The percentage of plants infected was greater for the second transmission test. Faster growing, more succulent test plants which are more susceptible to virus infection were used.

The virus transmission tests did indicate that transmission can occur with AAPs of 24 hr and an IAP of 1 hr. This suggests a semi-persistent virus-vector relationship.

Using autoradiography, Petersen et al. (1982) showed that BBSSV progressively moves through the alimentary canal of the aphid with increasing AAPs. In addition, using ferritin-labeled antibody techniques, BBSSV was shown to be associated with the aphid salivary glands (M. Petersen, unpublished data).

Although RIA, autoradiography, and ferritin-labeled antibody techniques are effective methods of detecting virus in individual aphids, they only indicate the presence of virus. The presence of virus does not necessarily mean that the virus can or will be transmitted. Information about virus-carrying aphids must still be related to the capacity to transmit. Transmission tests remain the most reliable means of distinguishing between vectors and nonvectors (Raccah, 1983).

In field studies, large populations of blueberry aphids were found on caged source bushes. This indicates that blueberry aphids do indeed overwinter within the blueberry field in association with blueberry bushes and have monoecious aphid life cycle characteristics. These findings are in agreement with those of Elsner (1982), who found oviparous female blueberry aphids and eggs in late autumn on basal blueberry shoots. This is important as no time delay is necessary for aphids to migrate into the field before BBSSV can be spread.

Throughout the season there were significantly greater populations of apterae and alatae on caged versus noncaged plants. This probably resulted from the protection that the screen cages provided the aphids against wind, rain, and predators. Aphid populations within the cages were an indication of the potential number of aphids possible since they were protected from mortality factors. In addition, the alatae on caged plants also represented the potential number of alatae capable of migrating. Alatae found on noncaged source plants are aphids which are not in transit; that is, they have yet to migrate or have already migrated. By comparing the numbers of alatae on caged versus noncaged

source plants, one can gain an idea of the potential alatae "pressure" for migration to other bushes.

The yellow pan traps may not have been optimal traps for monitoring the alate populations. The number of alate caught in yellow pan traps was low even when there were large populations of alatae on source plants. Elsner (1982) also had low numbers of trap catches with the same type of traps. He suggested that there was competition between the blueberry leaves and traps as attractive stimuli to the aphids (Elsner, 1982). In addition, over the season, aphids may have been dislodged from the bushes and fallen into the traps because of wind or rain. The apterous aphids trapped on 13 August may have been an example of this. These aphids were trapped during a very windy and rainy period when aphid populations were relatively low.

The small number of alatae (four) caught in traps outside of the field implied that there was little movement of alatae outside of the field and that transmission of BBSSV from field to field by flying aphids is not very likely. This corroborated the conclusions of an analysis-of-disease-incidence study by Lesney et al. (1978). The study determined that the inoculum source was within the field rather than introduced from outside of the field. In addition, Elsner (1982) found very few blueberry aphids outside of blueberry fields even when acceptable alternate hosts were present. On the other hand, the relative ineffectiveness of the traps for monitoring alate populations within the field indicated that the traps outside of the field may likewise be ineffective.

Alatae were found only during the first 9 weeks of the growing season, while apterae were found throughout the season. Therefore, alatae were only available for potential long-distance virus transmission during the first part of the season.

The mean percentage of viruliferous apterae usually ranged between 5% and 15% throughout the season. Since aphid populations were greatest early in the season, the potential numbers of viruliferous aphids were also greatest during this time. At this time, based on observation, there seemed to be a greater proportion of symptomatic tissue to healthy leaf tissue; leaves and shoots were very succulent and rapidly growing--the type of tissue that blueberry aphids prefer. The greater proportion of succulent infected tissue to healthy tissue early in the season increases the probability that aphids will acquire virus. In addition, the large aphid populations lead to crowding, which stimulates aphid movement to other blueberry plants. Finally, viruliferous aphids feeding on new tissue are probably more likely to transmit virus because rapidly growing plant tissue is more susceptible to plant virus infection.

There were no statistically significant differences ($P < 0.001$) in apterae or alatae populations on touching versus nontouching trap plants over the season. This indicated that although aphids moved to touching or "bridged" plants they also moved easily to nontouching plants. Aphids commonly walked to nearby bushes, but also may have been knocked off the source bushes onto nearby trap plants. Pruning bushes so that they do not overlap would not be an effective method of control.

Trap plant infection occurred throughout the entire growing season. The greatest incidence of trap plant infection was during the first two time periods of exposure from May through July. This was to be expected since it was during this period that the greatest aphid populations were present. The decrease in percentage infection in August likewise corresponded to a drop in aphid population. More than 30% of the trap plants were infected at the end of the season in September when there were very few aphids present, however. This may be because the trap plants were 2-year-old plants that were in better growing condition at the end of the season than the source plants. Aphids would have been more attracted to the more succulent growing trap plant material than the source plants planted in the field. It was likely that the attractiveness of the growing trap plants over the field source plants resulted in a higher percentage of BBSSV-infected plants in September than was expected.

Results from this research suggest a semi-persistent virus-vector relationship. Therefore, insecticides should be effective in controlling the spread of BBSSV in a BBSSV-infected field. If no shoestring disease was present in the field, a minimal spray program for aphids that allows natural predators and parasites to control aphids would be sufficient. However, if shoestring disease was present, a well-timed spray program would be necessary to control aphid populations to prevent further spread of the disease. Aphid population and seasonal trap plant infection data provide information for timing the insecticide sprays. Efficacious insecticides should be applied beginning early in the growing season to keep aphid populations at low levels.

At harvest the populations should be at minimal levels since aphids are carried down the row in mechanical harvesters (M. Whalon, 1981). Growers should wash out the harvesters before moving them to another field.

An ideal control strategy is the use of blueberry bushes that are resistant to the virus or the aphid. The highbush blueberry cv. Bluecrop has already been identified as having field resistance to blueberry shoestring disease (D. Ramsdell & J. Hancock, unpublished data).

Finally, information from this research may be used in the development of a virus-vector-plant model. Such a model is already being constructed (R. Kriegel, unpublished data). The model may be used eventually as a management tool for controlling blueberry shoestring disease. However, more information such as individual vector efficiency and virus titer in the plant must be determined and incorporated into the model before it can be fully implemented.

APPENDICES

APPENDIX A

1982 BLUEBERRY APHID POPULATION AND PERCENTAGE VIRULIFEROUS DATA

Weather data sources:

Degree day accumulation from 1 January to 31 March was estimated from NOAA data for Grand Haven. Degree day accumulation from 1 April to 23 September was obtained from the Agricultural Weather Observation Station at Allendale, MI.

Table A-1.--Apterous blueberry aphid populations on blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Date Sampled	Degree Day Accumulation[a]	Mean[b]		Standard Deviation	
		Cage[c]	No Cage[d]	Cage	No Cage
14 May	604	3.6	.9	2.4	1.9
25 May	874	13.4	3.6	16.8	5.0
29 May	978	116.1	4.1	128.6	3.8
4 June	1112	280.0	14.3	380.1	11.1
11 June	1290	259.0	21.9	118.2	14.5
18 June	1442	320.1	27.0	273.5	29.0
25 June	1594	238.4	71.3	249.6	47.6
2 July	1778	34.6	34.3	28.3	21.1
9 July	2011	51.1	19.4	42.5	12.8
16 July	2249	43.1	8.9	27.8	13.9
23 July	2498	24.1	1.4	29.7	1.6
30 July	2735	48.3	2.4	35.4	2.1
6 August	2969	20.1	0.0	23.8	0.0
13 August	3169	28.0	.1	20.5	.4
20 August	3387	51.0	3.4	40.6	3.6
27 August	3569	56.4	4.1	40.4	4.1
3 September	3726	37.1	3.1	9.9	2.9
10 September	3884	24.4	1.3	15.4	1.4
17 September	4096	4.3	2.0	3.3	1.5
23 September	4185	1.7	.4	3.0	1.1

[a]Degree day (base 38 F) accumulation from 1 January 1982.

[b]There were seven source plant replicates per treatment.

[c]Source plants were individually enclosed within aphid-proof screen cages before budbreak to observe if aphids overwintered within the field.

[d]Source plants were not enclosed within cages.

Table A-2.--Analysis of variance of apterous blueberry aphid populations on blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	617956.13	82.85	0.0000
Cage	1	358429.73	48.06	0.0000
Error (1)	12	7458.43		
Time	19	45305.32	5.52	0.0000
Time x Cage Interaction	19	30141.92	3.67	0.0000
Error (2)	228	8203.95		

Table A-3.--Apterous blueberry aphid populations on blueberry trap plants touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Cage[d]	No Cage[e]	Cage	No Cage
14 May	604	1.1	1.3	1.2	1.6
25 May	874	3.1	2.1	4.7	3.1
29 May	978	34.4	10.9	42.1	8.4
4 June	1112	73.6	21.0	45.0	18.6
11 June	1290	26.1	4.7	19.0	4.2
18 June	1442	33.1	7.9	20.2	7.2
25 June	1594	56.0	20.4	29.2	10.3
2 July	1778	8.3	12.3	8.1	3.0
9 July	2011	64.0	34.9	75.3	26.2
16 July	2249	6.7	7.7	4.0	5.4
23 July	2498	3.9	1.6	5.2	1.4
30 July	2735	3.9	1.7	3.8	1.7
6 August	2969	3.3	.9	2.2	.7
13 August	3169	15.6	3.1	14.3	2.7
20 August	3387	6.1	3.3	5.6	2.4
27 August	3569	17.0	3.7	10.5	4.1
3 September	3726	7.6	2.9	2.9	2.7
10 September	3884	2.1	1.1	2.0	1.5
17 September	4096	.1	.1	.4	.4
23 September	4185	.1	.6	.4	.8

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of apterous blueberry aphid populations on five blueberry trap plants touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-4.--Analysis of variance of apterous blueberry aphid populations on blueberry trap plants touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	45237.43	169.05	0.0000
Cage	1	8792.00	32.85	0.0001
Error (1)	12	267.60		
Time	19	3324.64	10.16	0.0000
Time x Cage Interaction	19	813.63	2.49	0.0008
Error (2)	228	327.20		

Table A-5.--Apterous blueberry aphid populations on blueberry trap plants not touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Cage[d]	No Cage[e]	Cage	No Cage
14 May	604	1.1	1.9	1.7	3.3
25 May	874	6.4	4.3	13.8	5.5
29 May	978	17.3	20.3	11.4	11.1
4 June	1112	141.4	24.0	151.1	15.1
11 June	1290	40.3	15.9	34.9	11.0
18 June	1442	84.9	45.9	62.5	47.5
25 June	1594	95.3	40.3	44.8	24.5
2 July	1778	23.4	32.1	17.9	14.6
9 July	2011	37.9	66.9	24.1	38.7
16 July	2249	8.3	10.0	3.5	5.5
23 July	2498	2.6	3.0	3.6	4.9
30 July	2735	5.4	3.4	4.8	6.1
6 August	2969	1.1	.9	1.1	.4
13 August	3169	12.4	3.9	9.9	2.7
20 August	3387	8.1	4.6	7.7	4.1
27 August	3569	14.9	4.1	11.6	5.1
3 September	3726	7.7	1.6	5.5	1.1
10 September	3884	3.9	1.7	3.4	2.9
17 September	4096	.6	.3	1.1	.5
23 September	4185	.9	.6	.7	1.1

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of apterous blueberry aphid populations on five blueberry trap plants not touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants not touching source plants were enclosed with their respective source plants within aphid-proof cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-6.--Analysis of variance of apterous blueberry aphid populations on blueberry trap plants not touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	111800.09	96.19	0.0000
Cage	1	9131.43	7.85	0.0160
Error (1)	12	1162.28		
Time	19	9392.16	10.34	0.0000
Time x Cage Interaction	19	3224.61	3.55	0.0000
Error (2)	228	908.57		

Table A-7.--The proportion of viruliferous apterous blueberry aphids on blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. Eastmanville, MI, 1982.

Date Sampled	Degree Day Accumulation[a]	Mean[b]		Standard Deviation	
		Cage[c]	No Cage[d]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	.1	.1	.4	.4
29 May	978	.1	.1	.4	.2
4 June	1112	.1	.1	.4	.4
11 June	1290	.1	0.0	.1	0.0
18 June	1442	.1	0.0	.1	0.0
25 June	1594	.1	.1	.1	.2
2 July	1778	0.0	.1	.1	.1
9 July	2011	.1	.1	.1	.1
16 July	2249	.5	0.0	.4	0.0
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	.2	0.0	.2	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	.3	0.0	.2	0.0
27 August	3569	.1	0.0	.1	0.0
3 September	3726	.2	0.0	.1	.1
10 September	3884	.1	0.0	.2	0.0
17 September	4096	0.0	.2	.1	.3
23 September	4185	0.0	0.0	.1	0.0

[a]Degree day (base 38 F) accumulation from 1 January 1982.

[b]There were seven source plant replicates per treatment.

[c]Source plants were individually enclosed within aphid-proof screen cages before budbreak to observe if aphids overwintered within the field.

[d]Source plants were not enclosed within cages.

Table A-8.--Analysis of variance of the proportion of viruliferous apterous blueberry aphids on blueberry shoestring virus-infected source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	1.69	44.01	0.0000
Cage	1	.31	7.96	0.0154
Error (1)	12	.04		
Time	19	.07	2.19	0.0037
Time x Cage Interaction	19	.88	2.46	0.0099
Error (2)	228	.03		

Table A-9.--The proportion of viruliferous apterous blueberry aphids on blueberry trap plants touching blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Cage[d]	No Cage[e]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	0.0	.4	0.0	.9
29 May	978	0.0	0.0	.1	0.0
4 June	1112	.1	0.0	.2	.1
11 June	1290	0.0	0.0	.1	0.0
18 June	1442	0.0	0.0	0.0	0.0
25 June	1594	0.0	0.0	0.0	.1
2 July	1778	.1	0.0	.2	.1
9 July	2011	.1	0.0	.1	.1
16 July	2249	.6	0.0	.3	0.0
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	.2	0.0	.3	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	.7	0.0	1.1	0.0
27 August	3569	0.0	0.0	.1	0.0
3 September	3726	.2	0.0	.1	0.0
10 September	3884	.6	0.0	1.1	0.0
17 September	4096	0.0	0.0	0.0	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of the proportion of viruliferous apterous blueberry aphids on five blueberry trap plants touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-10.--Analysis of variance of the proportion of viruliferous apterous blueberry aphids on blueberry trap plants touching blueberry shoestring virus-infected source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	1.73	23.63	0.0004
Cage	1	.80	10.95	0.0062
Error (1)	12	.87		
Time	19	.18	1.94	0.0122
Time x Cage Interaction	19	.22	2.42	0.0011
Error (2)	228	.09		

Table A-11.--The proportion of viruliferous apterous blueberry aphids on blueberry trap plants not touching blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Caged[d]	No Cage[e]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	.1	.1	.2	.4
29 May	978	.2	0.0	.4	0.0
4 June	1112	.1	.1	.2	.1
11 June	1290	0.0	0.0	.1	0.0
18 June	1442	.1	0.0	.1	0.0
25 June	1594	0.0	0.0	.1	0.0
2 July	1778	.1	0.0	.1	0.0
9 July	2011	0.0	0.0	0.0	0.0
16 July	2249	.4	0.0	.3	0.0
23 July	2498	0.0	0.0	0.0	.1
30 July	2735	.1	0.0	.1	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	.1	.2	.1	.3
20 August	3387	.1	.1	.1	.2
27 August	3569	0.0	0.0	.1	0.0
3 September	3726	0.0	0.0	.1	0.0
10 September	3884	.1	0.0	.2	0.0
17 September	4096	.1	0.0	.2	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of the proportion of viruliferous apterous blueberry aphids on five blueberry trap plants not touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-12.--Analysis of variance of the proportion of viruliferous apterous blueberry aphids on blueberry trap plants not touching blueberry shoestring virus-infected source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	.82	47.07	0.0000
Cage	1	.16	9.34	0.0100
Error (1)	12	.02		
Time	19	.04	2.01	0.0089
Time x Cage Interaction	19	.04	1.95	0.0119
Error (2)	228	.02		

Table A-13.--Alate blueberry aphid populations on blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Date Sampled	Degree Day Accumulation[a]	Mean[b]		Standard Deviation	
		Cage[c]	No Cage[d]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	5.1	1.9	11.1	2.6
29 May	978	15.4	.4	9.7	.8
4 June	1112	101.3	3.3	91.7	3.7
11 June	1290	305.6	10.4	79.1	6.7
18 June	1442	175.0	.3	121.4	.8
25 June	1594	145.9	3.3	92.3	2.8
2 July	1778	1.6	4.7	3.7	2.8
9 July	2011	.3	1.1	.8	1.1
16 July	2249	0.0	0.0	0.0	0.0
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	0.0	0.0	0.0	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	0.0	0.0	0.0	0.0
27 August	3569	0.0	0.0	0.0	0.0
3 September	3726	0.0	0.0	0.0	0.0
10 September	3884	.1	0.0	.4	0.0
17 September	4096	0.0	0.0	0.0	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Degree day (base 38 F) accumulation from 1 January 1982.

[b]There were seven source plant replicates per treatment.

[c]Source plants were individually enclosed within aphid-proof screen cages before budbreak to observe if aphids overwintered within the field.

[d]Source plants were not enclosed within cages.

Table A-14.--Analysis of variance of alate blueberry aphid populations on blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	105574.89	118.85	0.0000
Cage	1	92202.00	103.80	0.0000
Error (1)	12	888.28		
Time	19	24737.48	25.78	0.0000
Time x Cage Interaction	19	22440.24	23.39	0.0000
Error (2)	228	959.49		

Table A-15.--Alate blueberry aphid populations on blueberry trap plants touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Cage[d]	No Cage[e]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	3.0	.4	5.1	.8
29 May	978	21.7	1.6	29.6	1.3
4 June	1112	19.4	2.3	13.0	1.1
11 June	1290	12.4	1.3	13.4	1.4
18 June	1442	23.3	.6	11.2	.5
25 June	1594	25.1	3.4	24.6	2.6
2 July	1778	.9	1.4	1.9	1.3
9 July	2011	0.0	1.7	0.0	1.1
16 July	2249	.3	.3	.8	.5
23 July	2498	0.0	.1	0.0	.4
30 July	2735	0.0	.1	0.0	.4
6 August	2969	0.0	.1	0.0	.4
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	0.0	0.0	0.0	0.0
27 August	3569	0.0	0.0	0.0	0.0
3 September	3726	0.0	0.0	0.0	0.0
10 September	3884	0.0	0.0	0.0	0.0
17 September	4096	0.0	0.0	0.0	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of alate blueberry aphid populations on five blueberry trap plants touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-16.--Analysis of variance of alate blueberry aphid populations on blueberry trap plants touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	2502.53	19.23	0.0009
Cage	1	1504.29	11.56	0.0053
Error (1)	12	130.09		
Time	19	348.66	7.50	0.0000
Time x Cage Interaction	19	258.30	5.59	0.0000
Error (2)	228	45.87		

Table A-17.--Alate blueberry aphid populations on blueberry trap plants not touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Cage[d]	No Cage[e]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	1.3	1.3	2.4	1.9
29 May	978	21.7	3.1	37.6	1.8
4 June	1112	23.0	4.6	16.4	3.3
11 June	1290	34.3	1.3	39.4	1.0
18 June	1442	74.3	1.1	33.5	.7
25 June	1594	47.7	3.6	35.7	4.2
2 July	1778	1.0	3.4	1.4	1.5
9 July	2011	0.0	2.4	0.0	1.4
16 July	2249	.3	1.1	.5	.9
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	0.0	0.0	0.0	0.0
6 August	2969	0.0	.6	0.0	.8
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	0.0	0.0	0.0	0.0
27 August	3569	0.0	0.0	0.0	0.0
3 September	3726	0.0	0.0	0.0	0.0
10 September	3884	0.0	0.0	0.0	0.0
17 September	4096	0.0	0.0	0.0	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of alate blueberry aphic populations on five blueberry trap plants not touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants not touching source plants were enclosed with their respective source plants within aphid-proof cates.

[e]Trap plants and source plants were not enclosed within cages.

Table A-18.--Analysis of variance of alate blueberry aphid populations on blueberry trap plants not touching blueberry shoestring virus-infected blueberry source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	8870.63	40.36	0.0000
Cage	1	5796.70	26.37	0.0002
Error (1)	12	219.79		
Time	19	1557.55	11.38	0.0000
Time x Cage Interaction	19	1367.72	9.99	0.0000
Error (2)	228	136.90		

Table A-19.--The proportion of viruliferous alate blueberry aphids on blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. Eastmanville, MI, 1982.

Date Sampled	Degree Day Accumulation[a]	Mean[b]		Standard Deviation	
		Cage[c]	No Cage[d]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	.1	.2	.2	4
29 May	978	.5	.2	.3	.4
4 June	1112	.1	0.0	.2	0.0
11 June	1290	0.0	0.0	.1	.1
18 June	1442	.1	0.0	.2	0.0
25 June	1594	.1	0.0	.2	.1
2 July	1778	0.0	0.0	0.0	0.0
9 July	2011	0.0	0.0	0.0	0.0
16 July	2249	0.0	0.0	0.0	0.0
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	0.0	0.0	0.0	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	0.0	0.0	0.0	0.0
27 August	3569	0.0	0.0	0.0	0.0
3 September	3726	0.0	0.0	0.0	0.0
10 September	3884	0.0	0.0	0.0	0.0
17 September	4096	0.0	0.0	0.0	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Degree day (base 38 F) accumulation from 1 January 1982.

[b]There were seven source plant replicates per treatment.

[c]Source plants were individually enclosed within aphid-proof screen cages before budbreak to observe if aphids overwintered within the field.

[d]Source plants were not enclosed within cages.

Table A-20.--Analysis of variance of the proportion of viruliferous alate blueberry aphids on blueberry shoestring virus-infected source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	.70	43.27	0.0000
Cage	1	.04	2.71	0.1255
Error (1)	12	.01		
Time	19	.13	7.02	0.0000
Time x Cage Interaction	19	.02	1.18	0.2768
Error (2)	228	.02		

Table A-21.--The proportion of viruliferous alate blueberry aphids on blueberry trap plants not touching blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Caged[d]	No Cage[e]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	.1	.1	.4	.4
29 May	978	.1	.1	.4	.2
4 June	1112	.1	.1	.4	.4
11 June	1290	0.0	0.0	.1	0.0
18 June	1442	.1	0.0	.1	0.0
25 June	1594	.1	.1	.1	.2
2 July	1778	0.0	.1	.1	.1
9 July	2011	.1	.1	.1	.1
16 July	2249	0.0	0.0	0.0	0.0
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	.2	0.0	.2	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	.3	0.0	.2	0.0
27 August	3569	.1	0.0	.1	0.0
3 September	3726	.2	0.0	.1	.1
10 September	3884	.1	0.0	.2	0.0
17 September	4096	0.0	.2	.1	.3
23 September	4185	0.0	0.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of the proportion of viruliferous alate blueberry aphids on five blueberry trap plants not touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-22.--Analysis of variance of the proportion of viruliferous alate blueberry aphids on blueberry trap plants not touching blueberry shoestring virus-infected source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	1.13	46.98	0.0000
Cage	1	.06	4.09	0.0660
Error (1)	12	.02		
Time	19	.04	1.54	0.0746
Time x Cage Interaction	19	.03	1.18	0.2726
Error (2)	228	.03		

Table A-23.--The proportion of viruliferous alate blueberry aphids and blueberry trap plants touching blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. Eastmanville, MI, 1982.

Date Sampled[a]	Degree Day Accumulation[b]	Mean[c]		Standard Deviation	
		Cage[d]	No Cage[e]	Cage	No Cage
14 May	604	0.0	0.0	0.0	0.0
25 May	874	.3	.2	.5	.4
29 May	978	.2	.1	.4	.4
4 June	1112	.3	.2	.3	.3
11 June	1290	0.0	0.0	.1	0.0
18 June	1442	.1	0.0	.1	0.0
25 June	1594	.1	0.0	.1	0.0
2 July	1778	0.0	0.0	0.0	0.0
9 July	2011	0.0	0.0	0.0	0.0
16 July	2249	0.0	0.0	0.0	0.0
23 July	2498	0.0	0.0	0.0	0.0
30 July	2735	0.0	0.0	0.0	0.0
6 August	2969	0.0	0.0	0.0	0.0
13 August	3169	0.0	0.0	0.0	0.0
20 August	3387	0.0	0.0	0.0	0.0
27 August	3569	0.0	0.0	0.0	0.0
3 September	3726	0.0	0.0	0.0	0.0
10 September	3884	0.0	0.0	0.0	0.0
17 September	4096	0.0	0.0	0.0	0.0
23 September	4185	0.0	0.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 7 May to 4 June, 4 June to 2 July, 2 July to 30 July, 30 July to 27 August, and 27 August to 23 September.

[b]Degree days (base 38 F) accumulated from 1 January 1982.

[c]Mean sums of the proportion of viruliferous alate blueberry aphids on five blueberry trap plants touching source plants. There were seven source plant replicates per treatment.

[d]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[e]Trap plants and source plants were not enclosed within cages.

Table A-24.--Analysis of variance of the proportion of viruliferous alate blueberry aphids on blueberry trap plants touching blueberry shoestring virus-infected source plants in the field. Eastmanville, MI, 1982.

Source	Degrees of Freedom	Mean Square	F	Significant Level of F Value
Mean	1	.43	21.17	0.0006
Cage	1	.06	2.72	0.1247
Error (1)	12	.02		
Time	19	.09	4.12	0.0000
Time x Cage Interaction	19	.01	.38	0.9920
Error (2)	228	.02		

APPENDIX B

1981 FIELD STUDIES OF THE SEASONAL POPULATIONS OF *ILLINOIA PEPPERI* AND THE SPREAD OF BLUEBERRY SHOESTRING VIRUS

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1981 FIELD STUDIES OF THE SEASONAL POPULATIONS OF *ILLINOIA PEPPERI* AND THE SPREAD OF BLUEBERRY SHOESTRING VIRUS

RESULTS

Blueberry Aphid Populations and BBSSV Infection Periods

Source plants and trap plants were used to study the bush-to-bush movement of blueberry shoestring virus (BBSSV)-carrying aphids and BBSSV-infection periods. Field plots were set up at the Gordon DeVries farm, West Olive, Ottawa County, MI. The plots consisted of highbush blueberry bushes, cv. Jersey, approximately 25 years old, planted on a 10 ft by 3 ft spacing. The field was mapped for BBSSV infection by noting symptomatic bushes with red streaking on 1- and 2-year-old shoots before budbreak. Flower buds from symptomless bushes were sampled during budswell and assayed for BBSSV with ELISA to determine if the bushes were infected but symptomless with BBSSV.

Twenty BBSSV-infected bushes (hereafter referred to as source bushes) in the field were selected for use in these studies. The 20 bushes consisted of 10 symptomatic bushes and 10 infected but symptomless bushes. The source bushes were uniformly pruned to the same size and number of shoots. To determine whether or not blueberry aphids overwinter on blueberry bushes, five of the symptomatic source

bushes and five of the symptomless bushes were each enclosed within cages as previously described in the main body of the thesis. The remaining five each symptomatic and symptomless source bushes remained not caged.

Two-year-old blueberry cv. Jersey plants in pots served as trap plants. The trap plants were tested for BBSSV infection with ELISA and sprayed with 0.5% DDVP as previously described prior to use in the field. Groups of 10 trap plants were placed around each of the 20 source plants so that five trap plants and the source plant touched (touching trap plants) and the other five trap plants did not touch the source plant (nontouching trap plants).

The trap plants were exposed to the source plants for 4-week intervals in 1981: 5 May to 2 June, 2 June to 30 June, 30 June to 28 July, 28 July to 25 August, and 25 August to 22 September. After each exposure period the trap plants were sprayed with DDVP and kept in isolation outside at Michigan State University. After a winter dormant period, leaf samples from the trap plants were collected and tested for BBSSV-infection with ELISA.

Apterous and alate blueberry aphid populations on the source plants and trap plants were monitored weekly. Blueberry aphid samples were collected and kept in ELISA extraction buffer at 0-4 C until processed for the presence of BBSSV with RIA. Apterous aphid samples were tested in groups of five.

Alate Dispersal Studies

Yellow pan traps were used to monitor the movement of alate blueberry aphids. The yellow pan traps were placed on 0.5 m boxes (low

traps) and on 2 m high wooden platforms (high traps) in the corners and center of a section of the Frank VenRoy blueberry field in Eastmanville, Ottawa County, MI. The traps were checked twice weekly for blueberry aphids. Blueberry aphids collected from the traps were tested individually for BBSSV with RIA.

To study the movement of blueberry aphids outside of the field, yellow pan traps were placed on 2 m high platforms 100, 200, and 300 m east, west, and south of the VenRoy field. The VenRoy blueberry field was ideal for this study since there were no adjacent blueberry fields that could also be sources of blueberry aphids. These traps were also monitored twice weekly for blueberry aphids.

RESULTS AND DISCUSSION

Blueberry Aphid Populations and BBSSV Infection Periods

Due to insecticide spraying by the grower, there was a negligible amount of blueberry aphids on the source plants by mid-June. It could not be determined if the lack of aphids on the caged source plants was due to the insecticide sprays or if there were no overwintering aphids on the bushes. Because blueberry aphids were necessary for this study, blueberry aphids from a cv. Bluetta field with no history of BBSSV-infection were planted on the source bushes on 30 June 1981.

The populations of apterous blueberry aphids on the source plants are shown in Table B-1. There were more apterae on the caged source plants versus the noncaged source plants due to the protection provided against aphid mortality factors previously discussed. The populations

were greatest in mid-August and then decreased to negligible numbers at the end of September. The apterous aphid populations on touching and nontouching trap plants (Tables B-2 and B-3) showed a similar pattern.

The proportion of viruliferous apterous aphids on source plants is shown in Table B-4. Viruliferous apterous aphids were consistently detected from 28 July through 11 August. There were few viruliferous aphids after mid-August. Tables B-5 and B-6 show the proportion of viruliferous aphids on touching and nontouching trap plants, respectively. Viruliferous apterous aphids were found consistently through 18 August. On the last sampling date, 22 September, there were relatively high proportions of viruliferous apterae. This may have been due to an unusually sensitive RIA test batch.

Alate blueberry aphids were observed through most of the 1981 season. Alatae were observed on both caged and noncaged source bushes through 11 August, while alatae were observed on touching and nontouching trap plants as late as 1 September.

There was no BBSSV-infection of trap plants surrounding noncaged source plants through June. For the 30 June to 28 July exposure period, 2 of 50 touching and 2 of 50 nontouching trap plants became infected with BBSSV. The next exposure period for 28 July to 25 August resulted in 1 of 50 touching and 3 of 50 nontouching BBSSV-infected trap plants. Only one touching and one nontouching trap plant became infected with BBSSV during the final exposure period from 25 August to 23 September.

Although few trap plants became infected with BBSSV, the infection periods corresponded to the aphid population. Just as there were no

blueberry aphids present through June, there was no BBSSV infection of any of the trap plants surrounding source plants without cages. It appeared that trap plants not touching source plants were as likely to become infected as trap plants touching source plants. However, the effect of trap plants touching or not touching adjacent BBSSV-infected source plants could not be positively determined, due to the small number of infected trap plants in this study.

Alate Dispersal Studies

Only five blueberry aphids were trapped by yellow pan traps. Of these five, only one was an alate blueberry aphid, caught in the high trap in the northwest corner of the field on 10 June. There were two apterous blueberry aphids caught on 18 July in the high traps in the middle and southeast corner of the field. Two apterous blueberry aphids were trapped 21 July--one from the high southeast trap and the other in the low trap in the center of the field.

No blueberry aphids were caught in the yellow pan traps outside of the field. Compared to the 1982 results in the main body of the thesis, these 1981 dispersal results may have indicated yearly variation in the number of aphids present in the field. Elsner (1982) reported yearly differences in the numbers of blueberry aphids caught in yellow pan traps similar to that found with these (1981 versus 1982) dispersal studies. By itself the lack of blueberry aphids found in the traps outside of the field in 1981 indicated that the importance of long-distance movement of blueberry aphids is not known. However, this information in addition to the 1982 results indicated that there

probably was little movement of blueberry aphids outside of the field as previously discussed in the main body of the thesis.

Table B-1.--Apterous blueberry aphid populations on blueberry shoestring virus-infected blueberry source plants in the field. West Olive, MI, 1981.

Date Sampled	Symptomatic Source Plant[a]		Symptomless Source Plant	
	Cage[b]	No Cage[c]	Cage	No Cage
14 July	62.4	58.0	23.0	39.0
21 July	133.6	68.4	123.0	58.0
28 July	220.0	238.0	62.0	50.0
4 August	390.0	328.0	186.0	316.0
11 August	300.0	221.0	332.0	214.0
18 August	118.0	31.0	135.0	44.0
24 August	151.0	19.0	122.0	19.0
1 September	40.0	10.0	205.0	10.0
8 September	3.8	2.0	42.0	15.0
15 September	1.0	0.0	.4	0.0
22 September	0.0	0.0	0.0	.4

[a]Means of five source plant replicates per treatment.

[b]Source plants were individually enclosed within aphid-proof screen cages before budbreak to observe if aphids overwintered within the field.

[c]Source plants were not enclosed within cages.

Table B-2.--Apterous blueberry aphid populations on blueberry trap plants touching blueberry shoestring virus-infected blueberry source plants in the field. West Olive, MI, 1981.

Date Sampled[a]	Symptomatic Source Plant[b]		Symptomless Source Plant	
	Cage[c]	No Cage[d]	Cage	No Cage
14 July	13.4	5.6	1.4	14.6
21 July	22.2	38.0	14.2	6.8
28 July	88.4	84.8	33.8	89.6
4 August	127.2	115.4	72.8	87.8
11 August	69.0	55.0	38.8	126.0
18 August	27.0	2.0	5.2	20.8
24 August	6.8	1.2	10.4	.8
1 September	1.4	.6	4.4	1.6
8 September	1.8	.2	.6	.2
15 September	.2	1.6	2.4	0.0
22 September	1.6	1.0	0.0	0.0

[a]Trap plants were exposed to source plants within the field for four-week intervals: 5 May to 2 June, 2 June to 30 June, 30 June to 28 July, 28 July to 25 August, and 25 August to 23 September.

[b]Mean sums of apterous blueberry aphids on five blueberry trap plants touching source plants. There were five source plant replicates per treatment.

[c]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[d]Trap plants and source plants were not enclosed within cages.

Table B-3.--Apterous blueberry aphid populations on blueberry trap plants not touching blueberry shoestring virus-infected blueberry source plants in the field. West Olive, MI, 1981.

Date Sampled[a]	Symptomatic Source Plant[b]		Symptomless Source Plant	
	Cage[c]	No Cage[d]	Cage	No Cage
14 July	15.8	9.0	6.6	5.2
21 July	24.4	45.6	21.6	11.4
28 July	153.8	165.0	105.8	80.6
4 August	97.2	129.4	95.2	72.0
11 August	128.0	86.0	86.8	212.4
18 August	45.4	16.4	19.4	47.2
24 August	7.8	7.4	16.2	3.2
1 September	9.2	1.2	5.8	.2
8 September	2.0	.4	1.6	.2
15 September	.8	0.0	1.8	.2
22 September	.2	.2	4.0	2.8

[a]Trap plants were exposed to source plants within the field for four-week intervals: 5 May to 2 June, 2 June to 30 June, 30 June to 28 July, 28 July to 25 August, and 25 August to 23 September.

[b]Mean sums of apterous blueberry aphids on five blueberry trap plants not touching source plants. There were five source plant replicates per treatment.

[c]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[d]Trap plants and source plants were not enclosed within cages.

Table B-4.--The proportion of viruliferous apterous blueberry aphids on blueberry shoestring virus (BBSSV)-infected blueberry source plants in the field. Aphids were tested for the presence of BBSSV with RIA. West Olive, MI, 1981.

Date Sampled	Symptomatic Source Plant[a]		Symptomless Source Plant	
	Cage[b]	No Cage[c]	Cage	No Cage
14 July	0.00	0.00	0.00	0.00
21 July	0.00	0.00	0.00	0.00
28 July	.10	.06	.04	.04
4 August	.08	.09	.12	.07
11 August	.02	.08	.05	.07
18 August	0.00	.09	0.00	0.00
24 August	.02	0.00	0.00	0.00
1 September	0.00	0.00	.02	0.00
8 September	0.00	0.00	0.00	0.00
15 September	0.00	0.00	0.00	0.00
22 September	0.00	0.00	0.00	.04

[a]Means of five source plant replicates per treatment.

[b]Source plants were individually enclosed within aphid-proof screen cages before budbreak to observe if aphids overwintered within the field.

[c]Source plants were not enclosed within cages.

Table B-5.--The proportion of viruliferous apterous blueberry aphids on blueberry trap plants touching blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. West Olive, MI, 1981.

Date Sampled[a]	Symptomatic Source Plant[b]		Symptomless Source Plant	
	Cage[c]	No Cage[d]	Cage	No Cage
14 July	0.00	0.00	0.00	0.00
21 July	0.00	0.00	0.00	0.00
28 July	.07	.08	.05	.09
4 August	.45	.32	.07	.08
11 August	.08	.03	.01	.06
18 August	.02	.02	0.00	0.00
24 August	.02	0.00	0.00	0.00
1 September	0.00	0.00	0.00	0.00
8 September	0.00	0.00	0.00	0.00
15 September	0.00	0.00	0.00	0.00
22 September	.08	.08	0.00	0.00

[a]Trap plants were exposed to source plants within the field for four-week intervals: 5 May to 2 June, 2 June to 30 June, 30 June to 28 July, 28 July to 25 August, and 25 August to 23 September.

[b]Mean sums of the proportion of viruliferous apterous blueberry aphids on five blueberry trap plants touching source plants. There were five source plant replicates per treatment.

[c]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[d]Trap plants and source plants were not enclosed within cages.

Table B-6.--The proportion of viruliferous apterous blueberry aphids on blueberry trap plants not touching blueberry shoestring virus (BBSSV)-infected source plants in the field. Aphids were tested for the presence of BBSSV with RIA. West Olive, MI, 1981.

Date Sampled[a]	Symptomatic Source Plant[b]		Symptomless Source Plant	
	Cage[c]	No Cage[d]	Cage	No Cage
14 July	0.00	0.00	0.00	0.00
21 July	0.00	0.00	0.00	0.00
28 July	.07	.09	.05	0.00
4 August	.53	.13	.11	0.00
11 August	.08	.08	.04	.02
18 August	.03	.13	0.00	.17
24 August	0.00	0.00	0.00	.06
1 September	0.00	0.00	0.00	0.00
8 September	0.00	0.00	0.00	0.00
15 September	0.00	0.00	0.00	0.00
22 September	.40	.40	.48	.44

[a]Trap plants were exposed to source plants within the field for four-week intervals: 5 May to 2 June, 2 June to 30 June, 30 June to 28 July, 28 July to 25 August, and 25 August to 23 September.

[b]Mean sums of the proportion of viruliferous apterous blueberry aphids on five blueberry trap plants not touching source plants. There were five source plant replicates per treatment.

[c]Groups of five trap plants touching source plants were enclosed with their respective source plants within aphid-proof screen cages.

[d]Trap plants and source plants were not enclosed within cages.

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