The leafhopper vector, *Endria inimica* (Say), of wheat striate mosaic virus is a bivoltine insect which overwinters as second generation eggs in a state of diapause. Under experimental conditions, egg diapause resulted from rearing females under short days (12L:12D) and diapause was averted by rearing females under long days (15L:9D). The effect of rearing females under short days was partially altered by a mean daily temperature of 32.4°C. Short- and long-day females oviposited the majority of their eggs in dead vegetative material on the soil surface. Eggs in diapause were not induced to develop by subjecting them to light and temperature conditions favorable for continuous development. Diapause was terminated more rapidly by exposing the eggs to 0°C than to 8°C.

Sixty-six percent of the short-day females and 52% of the long-day females tested were inoculative after a two-day acquisition feed on durum wheat (cv. Ramsey) plants infected with wheat striate mosaic virus. Frequency of virus transmission was not affected by the photoperiodic regimen under which the females were reared. Short-day females transmitted the virus as frequently as long-day
females. The latent period was 13.21 days in the short-day females and 14.85 days in the long-day females. The mean number of plants infected was 18.46 plants by short-day females and 21.15 plants by long-day females. The number of plants infected per insect day was 0.48 plants by short-day females and 0.50 plants by long-day females.
Environmental Biology of Endria inimica (Say)
(Homoptera: Cicadellidae)

by

Ralph Donald Gustin

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INTRODUCTION

The virus-vector relationship of insect vectors of plant viruses has not been investigated with respect to the response of the vector to different daylengths. It is known that the life cycle of insects must be synchronized with their environment if they are to survive. In temperate latitudes, many insects, at a specific stage in their life cycle, survive the conditions of winter in a state of arrested development or diapause. The state of diapause is not a direct response to the winter conditions, but is initiated prior to the adverse condition. Multivoltine insects with a facultative diapause commonly manifest this response after being exposed to the short days of late summer and autumn. During the summer, the summer generation is exposed to long days and diapause is not manifested. Since the host plant(s) of the vector are typically in a different physiological state or growth stage when the summer and fall generations are present, it is important to know if the virus-vector relationship differs between the generations. As an example, if the fall generation of Endria inimica (Say), vector of wheat striate mosaic virus, is a more efficient vector after being exposed to the short days of autumn, the potential for damage to winter wheat infected at an early growth stage would be greater than if the wheat was infected at an advanced stage of growth by the summer generation. Endo and Brown (1957) reported that barley yellow dwarf virus reduced yield in oats by 90 to 95% when the
plants were infected at the three-leaf stage and 10 to 20% when infected at the boot stage.

Ideally, the investigation of virus-vector relationships requires large numbers of insects of known sex, age, mating condition, and origin. Being able to rear these insects in large numbers for experimental purposes implies that optimum rearing conditions are known. In the past, many virus-vector relationships have been investigated using insects reared under continuous long days, or where rearing has been difficult, insects of unknown environmental histories, age, and mating condition have been field collected and used for experimental purposes. Insects of known sex, age, mating condition, and origin reared under short days which induced the females to oviposit eggs in a state of diapause have not been tested to determine if the virus-vector relationship is different from that of insects reared under daylength conditions favorable for continuous development.

The purposes of this investigation were threefold: (1) characterize the response of the leafhopper, *E. inimica*, to short and long daylengths, (2) determine what environmental factor(s) terminate the state of diapause resulting from exposure to short days, and (3) determine if the virus-vector relationship of females reared under short days (diapause inducing) differed from the relationship of females reared under long days (continuous development).
LITERATURE REVIEW

Definitive studies with respect to egg diapause in leafhoppers (Homoptera: Cicadellidae) are few. Müller (1961) demonstrated that 100% of the eggs oviposited by *Euscelis phebejus* (Fallen) between September 16 and October 6 are in a state of diapause due to short daylength. *Scaphytopius delongi* Young responded to a ten-hour photoperiod by ovipositing eggs which were in a pre-embryonic state of diapause (Swenson, 1971a). Appreciable hatch did not occur until the eggs had experienced a chill treatment of 3°C for at least six weeks. Swenson (1967, p. 276) also found that *Colladonus montanus reductus* (VanD.) oviposited diapause eggs in response to short days.

The geminate leafhopper, *Colladonus geminatus* (VanD.), oviposits overwintering eggs which are not in a state of true diapause, but their development is retarded by cool temperatures (Kaloostian, 1956). *Euscelis lineolatus* Brulle (Müller, 1961) apparently behaves in much the same manner since 10 to 15% of its eggs oviposited in the autumn are in a state of true diapause and the remainder are in a state of quiescence.

Coupe and Schulz (1968) reported that with day, interim, and night temperatures of 80, 75, and 70°F and a 16-hour photoperiod, eggs of *E. inimica* hatched in an average of 13.2 days. Eggs did not hatch in three to four weeks during the winter months if the temperature was not held near 80°F and the natural daylength lengthened with supplementary light. Slykhuis (1963) reported that
eggs hatched in two to six weeks when greenhouse temperatures were held between 24 and 34°C and supplementary light was used to lengthen the natural daylength.

The relationship between photoperiod and temperature has been reviewed by Beck (1968, p. 144) and Lees (1968, p. 76). In long-day insects, elevated temperatures have been shown to shift the critical photoperiod downward. The diapause response may be prevented with elevated temperatures.

Saunders (1971) advanced the hypothesis that the photoperiodic sensitive stage in the life cycle of the insect must undergo a given number of short-day light-dark cycles before the diapause response occurs. Since the rate of development is temperature dependent as long as temperatures are not unfavorable, the sensitive stage may not experience the required number of cycles and diapause does not occur if the stage experiences elevated temperatures. Swenson (1971a), working with *S. delongi*, found that the diapause response was not altered by a mean daily temperature of 24.8°C under a ten-hour photoperiod.

*Endria inimica* is considered to be native to the North American continent (Osborn, 1912). Its geographic distribution includes most of the United States and southern Canada (Nielson, 1968). This leafhopper is commonly found on bluegrass, *Poa pratensis* (L.), and was reported from a wide range of native and introduced grasses (Wilbur, 1954). In Iowa (Osborn, 1912), the overwintering eggs hatched in late April and May and the adults appeared in early July. The second generation adults appeared in late August.
The eggs laid by the second generation adults survived the winter to renew the cycle the following spring. Westdal and Richardson (1966) reported that *E. inimica* did not migrate into Manitoba, although there was evidence that migration occurred from Kansas into both Dakotas. In Manitoba, this insect had two generations per year and overwintered in the egg stage. The first generation developed primarily on grasses and the second generation developed on cereals.

*Endria inimica* and *Elymana virescens* (F.) are the only known vectors of wheat striate mosaic virus (Sinha, 1970). *Elymana virescens* did not transmit the virus as efficiently as *Endria inimica*. After a two-day acquisition feed on the virus source, 81% of *E. inimica* were inoculative as compared to 3% of *Elymana virescens*. The minimum latent period of *E. virescens* was 18 days as compared to four to six days in *Endria inimica*.

Wheat striate mosaic virus was purified and found to be a bacilliform virus particle measuring 200-250X75nm (Lee, 1967). The host range of the virus includes about 20 members of the grass family Gramineae (Sinha and Behki, 1972). The virus is propagative in the insect vector and the initial multiplication site in *E. inimica* was determined to be the alimentary canal. The virus increased 5000-fold between day one and seven after access to the virus (Sinha and Chiykowski, 1969).

The virus was reported as occurring in South Dakota (Slykhuis, 1953), North Dakota and western Minnesota (Timian, 1960), and sporadically in the prairie provinces of Canada (Slykhuis, 1963). The
virus was found to be infecting 25% of the durum wheat plants and 100% of a fall-sown cover crop of hard red spring wheat in 1959 at Fargo, North Dakota (Timian, 1960).

Virus transmission studies have yielded results with a high degree of variability from insect to insect and from one experiment to another. This variability has been most evident in regard to latent period, frequency of transmission, and the number of insects becoming inoculative (Slykhuis, 1953, 1963; Slykhuis and Sherwood, 1964).
GENERAL MATERIALS AND METHODS

The leafhoppers, *E. inimica*, used to start colonies were collected from the bluegrass lawn of the Northern Grain Insects Research Laboratory at Brookings, South Dakota. First-generation nymphs collected in May and June were used to initiate the colonies, since previous experience had indicated that it was extremely difficult to start colonies from insects collected in September.

The host plant used throughout the various experiments was *Triticum durum* Desf. (cv. Ramsey). This durum wheat has been used extensively as an experimental host plant for this leafhopper and as a virus host plant. The host plants were handled in two different ways depending upon their use. For the maintenance of stock insect colonies, 12 to 15 wheat seeds were sown directly into a six-inch clay pot filled with a soil mixture consisting of 12 parts black topsoil, 6 parts rotted cow manure, 2 parts sand, 1.5 parts peat moss, and 1 part sheep manure. Plants which were used for experimental purposes were propagated by placing a large number of seeds on moistened filter paper in a petri dish where they were allowed to germinate. After three days, the seeds which were visually at the same stage of germination were sown one per pot at a uniform depth in a three- or four-inch clay pot containing the previously described soil mixture. It was felt that this method allowed the selection of the most vigorous seed and synchronized the time of emergence from the soil by the seedling. A schedule was followed in planting seeds so that seedlings of a consistent size and age
were available each day. The seedlings were used when the first true leaf was about fully expanded.

A wheat plant infected with wheat striate mosaic virus was obtained from Dr. R. G. Timian, Plant Pathologist, at North Dakota State University in Fargo, North Dakota. The virus was originally isolated by collecting *E. inimica* from a bluegrass lawn at North Dakota State and allowing these to feed on *T. durum* (cv. Mindum). The virus was continuously maintained in this durum wheat.

Two types of cages were used. Stock colonies were caged over the host plants by a cloth-covered cylindrical cage measuring 45 cm tall by 22.5 cm in diameter. This cage was grooved on the bottom to fit over the lip of a six-inch clay pot. The other type of cage was a tubular cellulose butyrate cage measuring 25X5 cm used to confine pairs of insects and individuals over single wheat seedlings. Two opposing rectangular holes were cut in the cage for ventilation and these holes and the top were covered with silk bolting cloth. The cage was held in place by pressing it about 1 cm into the soil surrounding the seedlings.

The experiments were conducted in the facilities of the Northern Grain Insects Research Laboratory, USDA, ARS, located at Brookings, South Dakota. Greenhouse space used for growing wheat seedlings was kept at 21±5°C during the winter months and during the summer the temperature fluctuated with the outside temperature which varied from about 18 to 38°C. During the winter months, supplementary light was provided so that the daylength was 16 hour.
Insects used in the various experiments were held in Scherer-Gillette®, Model CEL 512-37, environmental growth cabinets. These chambers were programmed for the required photoperiod regimens and temperatures.
EXPERIMENTAL RESULTS

A. Diapause Induction

The working hypothesis to determine if *E. inimica* females respond to different photoperiods by ovipositing eggs in diapause or nondiapause was: *E. inimica* has a maternally controlled facultative egg diapause induced by short days. Reports in the literature indicated that when the natural daylength during the winter was extended, the eggs of this insect hatched in about two weeks. When the daylength was not extended, the eggs failed to hatch in two weeks. Knowledge of these reports suggested that females reared under short days responded to short daylengths by ovipositing eggs in a state of diapause.

To investigate the relation between daylength and the period of time it takes the eggs to hatch, two sets of conditions were used which simulated early summer conditions and conditions that prevail in the late summer and autumn months.

Two growth chambers were programmed for those conditions. One had a light regimen of 15L:9D with a temperature of 24 and 18°C respectively, for the light and dark phase. The second chamber was programmed for a regimen of 12L:12D and a temperature of 24 and 18°C respectively. The short daylength was chosen to simulate the daylength at the fall equinox. Hereafter, all insects reared under the 15L:9D regimen will be referred to as long-day insects and those under 12L:12D as short-day insects.
To test the hypothesis of a short-day induced egg diapause, approximately 100 first-stage nymphs were allowed to hatch under long-day conditions. Fifty of these insects were removed from the long-day conditions and were placed under short-day conditions. Both sets of insects were allowed to emerge as adults. From these insects, ten pairs of long-day insects were caged, one pair per cage, over a wheat seedling. Sixteen pairs of short-day insects were handled in the same manner. The 26 pairs were transferred daily to new wheat seedlings. The seedlings from which they were removed were returned to the same environmental conditions to await examination for eggs. This procedure was repeated for 30 days or until the female died. Ten days after the insects had been removed from the plants, the wheat seedlings were examined under a dissecting microscope for the presence of eggs and the embryological condition of the eggs. The criterion used for embryological development was the presence of the distinct red eyespot. Westdal and Richardson (1966) reported that eyespots appeared at about the fifth day and were well developed by the eighth day after oviposition. A sample of 129 long-day eggs was observed and 93 of these were showing an obvious eyespot on the tenth day after oviposition (Table I). Five-hundred sixty eggs oviposited by short-day females were observed and of this number, one had an eyespot on the tenth day after oviposition (Table I). Those short-day eggs not showing eyespots on the tenth day were removed from the plant tissue and placed on moistened filter paper in a sealed petri dish. The eggs were observed for an additional 20 days during which time none developed to the eyespot stage of embryogenesis.
B. Diapause Termination

Preliminary experiments to determine how diapause could be terminated were conducted by collecting additional eggs from short-day females and exposing these to different environmental conditions with results as follows: of 32 short-day eggs, none developed to the eyespot stage of embryogenesis after incubation at 26°C for 50 days; of 51 short-day eggs placed under the long-day conditions, one developed to the eyespot stage of embryogenesis within 50 days; six of ten short-day eggs chilled for 63 days at 4°C and then incubated at a mean temperature of 21.7°C in total darkness developed to the eyespot stage within two weeks; 18 of 31 short-day eggs chilled for 63 days at 4°C and then placed under short-day conditions developed to the eyespot stage within two weeks (Table II).

Westdal and Richardson (1966) and Coupe and Schulz (1968) observed that E. inimica oviposited a large percentage of its eggs in the soil debris or on the soil surface. My investigations verified this observation (Fig. 1). Both long- and short-day insects would oviposit in small pieces of partially decayed vegetative material on the soil surface in preference to ovipositing in the wheat seedlings. Under those conditions, very few eggs could be found in the wheat seedlings. Coupe and Schulz (1968) reported that under summer conditions, most of the eggs were in the leaf blades, but under the conditions of my experiments, this was not true. My observations indicated that the choice of oviposition site is not a behavioral pattern associated with daylength. This
enabled me to collect large numbers of eggs by placing pieces of debris in the cages on the soil surface and then collecting these pieces after several weeks. These pieces of debris were carefully broken apart and the eggs teased free with dissecting needles, forceps, and a camel-hair brush. In this way, about 800 eggs were collected. When eggs were handled in this manner, contamination by mold was minimal as compared to eggs that were dissected from wheat seedlings. Eggs from living plants were almost impossible to clean, whereas, material clinging to the chorion of eggs removed from the debris could be removed, yielding an egg relatively free of all foreign material. These eggs were placed on filter paper which had been boiled in distilled water for ten minutes and partially dried to remove excess water. The eggs and filter paper were placed in sterile plastic petri dishes and closed with parafilm.

Reactivation (Danilevskii, 1965, p. 16) or diapause development (Andrewartha, 1952, p. 53; Lees, 1955, p. 55) of overwintering insects in a state of diapause in temperate regions usually occurs in the 0-12°C temperature range. To test whether *E. imitica* followed this pattern, the petri dishes and eggs were chilled at one of two temperatures, 0 and 8°C, for different lengths of time. Swenson (1971a) found that eggs of *S. delongi* which were in diapause hatched after exposure to 3°C for six weeks. I chose to bracket this time by removing eggs from each temperature treatment after a time lapse of 20, 30, and 67 days. One group of eggs was chilled for 120 days at 0±1°C. After the desired time had elapsed at each temperature, the eggs were placed in an incubator at 26°C and observed daily
to determine when eyespots appeared (Fig. 2) and when hatch occurred (Fig. 3). The number of eggs in each temperature X time experiment is given in Table III.

To determine if elevated temperatures altered the response of females to short days, about 100 first-stage nymphs were caged over wheat seedlings. These insects were reared to the adult stage under a 12L:12D photoperiod regimen at a mean temperature of 32.4°C. About 20 insects in a 1:1 sex ratio were transferred weekly to new host plants, and the eggs from these females were collected and placed on moistened filter paper in a petri dish and sealed. These eggs were then incubated at a constant 26°C for two weeks. A total of 97 eggs was observed and of these, 27 (27%) developed to the eyespot stage within three weeks after oviposition. Eggs laid by females reared in short days at lower temperatures, e.g., 21°C, would not have hatched at all under these conditions.
DISCUSSION

A. Diapause Induction

The data (Table I) show that *E. inimica* females, reared under short days (12L:12D) at a mean temperature of 21.2°C from the time they were first-stage nymphs, responded to short days by ovipositing overwintering eggs. Seventy-two percent of the eggs oviposited by females reared under long-day conditions (15L:9D) at a mean temperature of 21.7°C developed to the eyespot stage within ten days after oviposition while only one of 560 short-day eggs developed to this stage within 30 days. These results confirmed the observations of Coupe and Schulz (1968) and Westdal and Richardson (1966) that this insect will oviposit overwintering eggs if supplementary light is not used to extend the natural daylength during the autumn and winter.

B. Diapause Termination

The data (Table II) show that eggs laid by short-day females were in a state of true diapause in that they did not develop when placed in an environment favorable for continuous development. Only those eggs which were given a chill treatment for 63 days developed. Lees (1968, p.57) cited several examples of insects in which diapause is induced and terminated by daylength. My results with *E. inimica* indicate that diapause is induced by daylength, but termination is not under daylength control. All eggs reported on in Table
were surface sterilized in a 1:130 dilution of Zepharin for 30 seconds and rinsed in distilled water. This treatment with Zepharin may have been detrimental to the eggs since fewer of these eggs developed than did eggs (Table III) which were not surface sterilized.

Using the appearance of the eyespot as the criterion for reactivation or diapause development being completed, 0°C is more effective than is 8°C as is evident from the fact that after a 20-day chill, 57% of the 0°C eggs had developed eyespots within 12 days at 26°C as compared to 34% of the 8°C eggs after 24 days at 26°C. After 30 days at either temperature, 93% of the 0°C eggs had eyespots by the 13th day compared with 93% of the 8°C eggs by the 20th day. After a chill period of 67 days at both temperatures, 94% of the 0°C eggs and 93% of the 8°C eggs had developed eyespots by the 10th and 11th days, respectively. One group of 95 eggs chilled for 120 days at 0°C had a developmental pattern very similar to the pattern exhibited after 67 days at both temperatures. The results shown in Figures 2 and 3 indicate that diapause development or reactivation occurred most rapidly at 0°C, but a longer exposure to 8°C achieved the same results. The maximum termination of diapause occurred after 67 days of chilling. An additional 53 days at 0°C did not synchronize the initiation of embryogenesis and the mean number of days to hatch for the eggs held at 0°C for 67 and 120 days are similar (Table III). The additional exposure to 0°C had no detrimental effect on development.
When comparing development to the eyespot stage (Fig. 2) with hatch (Fig. 3) of the same group of eggs, it is obvious that there are differences between the number of eggs developing to the eyespot stage and the number hatching. This difference is most apparent in the long-day eggs or control where 89% of the eggs developed an eyespot, but only 73% hatched. When examined under the dissecting microscope, the eggs that did not hatch appeared to be normal, but they eventually darkened and were presumed to be dead. A possible explanation for these differences is that there was unequal partitioning of food reserves among the eggs so that some of the last laid eggs may not have possessed sufficient reserves to complete development to hatch (Wellington, 1965).

Murai and Kiritani (1970) found that hatch of green rice leafhopper eggs, *Nephotettix cincticeps* Uhler, decreased with the increase in age of their mothers. The eggs used in my investigations were collected from bits of debris that had been exposed to ovipositing females for several weeks so that the increased age of the females over this time span may also contribute to the difference between those eggs developing to the eyespot stage and those hatching.

The fact that *E. inimica* oviposits in the soil and in bits of organic debris on the soil surface (Fig. 1) was not investigated thoroughly, but it was observed that both short- and long-day females exhibited the same ovipositional behavior. The selection of this oviposition site may have adaptive significance since placement in these types of sites may protect the eggs from weather extremes. It also was observed with approximately 200 long-day eggs, oviposited
over a three-week period and then removed from the debris, that there was no evidence of embryogenesis until the eggs were placed on moist filter paper. The possibility exists that all of the eggs were oviposited on the same day, but this seems improbable. From past experience in rearing this leafhopper, the apparent necessity for the eggs to be exposed to moisture before they will develop may explain why rearing success has been erratic from colony to colony. I have experienced excellent reproduction in some cages and none or very little in others handled in the same way. Pieces of debris lying on the soil surface and in the upper portion of the soil in a caged pot are not normally exposed to moisture when the pots are bottom watered. I have since started to top water the plants used as host plants and the yield of nymphs from cage to cage is much more consistent than previously. This may not be significant from a field standpoint since there is generally dew on the soil surface debris and plant cover each night and into the next morning, but may be an artifact of rearing this insect under artificial conditions.

My results with insects reared under short days at elevated temperatures indicate that the diapause response is altered, but it is not eliminated at a mean daily temperature of 32.4°C. In the flesh-fly, Sarcophaga argyrostoma R.-D., (Saunders, 1971), the entire larval period constitutes the sensitive stage so that increased temperatures compress the developmental period, thereby reducing the number of light-dark cycles this insect experiences during the larval stage. At 26°C, this insect develops to puparia
formation within nine days as opposed to a development period of 22 to 23 days at 16°C. The sensitive stage of *E. inimica* has not been defined, but if it is one or two instars, it may not be possible to reduce the number of light-dark cycles sufficiently to negate diapause in 100% of the insects.
THE working hypothesis for this portion of the study was: leafhoppers reared under short days will differ from insects reared under long days in their ability to transmit wheat striate mosaic virus. The comparative virus transmission study was designed to yield the following information for each daylength: number of females becoming inoculative after a 48-hour acquisition feed on the virus source, mean latent period, mean longevity of transmitting and non-transmitting females, mean number of plants infected, and plants inoculated per insect day (Table IV), and percentage of the inoculative females transmitting the virus each day (Fig. 4). Because of time and space requirements to handle between 9,000 and 10,000 plants, the experiment was done in two parts and the results were combined.

Two colonies of about 125 adult leafhoppers each were set up and partially decayed vegetative debris was scattered on the soil surface to serve as oviposition sites for the females. These two colonies were placed in the growth chamber programmed for long days and kept there for one week. The insects then were removed and the wheat plants upon which they had fed were removed from the soil. Previously germinated wheat seeds were planted in these pots, the pots recaged, and one pot was placed under long-day conditions and the other under short-day conditions. The wheat seedlings were about 5 to 8 cm tall when the eggs began to hatch. The nymphs were observed closely and if the host plants manifested any deterioration
due to heavy feeding, the nymphs were placed on new host plants. The nymphs were allowed to emerge as adults. As they emerged, they were sexed and males and females in about a 1:1 sex ratio were placed on virus-source plants where they were allowed to feed for 48 hours. This procedure was followed daily until enough females had been accumulated for the experiment. A few extra females were used to replace any females which died on the first day after the insects were removed from the virus-source plant and the daily sequential transfers to healthy wheat seedlings had begun. This procedure allowed me to have a full complement of insects for the tests as planned. The virus-source plants had been infected for three weeks and were showing typical symptoms of the disease when the acquisition feed was initiated. The methods used to accumulate insects for the tests enabled me to use females which had emerged as adults within a period of four days. Therefore, the age of the females was relatively uniform. Mating was assumed to have occurred in the two days on the virus-source plant since the insects were held in a 1:1 sex ratio.

Those insects which were surplus to my needs and had not been given access to the virus source were transferred to new host plants. These plants were observed closely for disease symptoms to determine if any of the insects were viruliferous prior to my confining the test insects on the virus source. Both long- and short-day female insects were handled in the same way. Symptoms of the disease were not observed on the plants the surplus insects fed upon and none were observed in my stock colonies.
At the termination of the 48-hour acquisition feed on the virus source, the males and females were removed from the source and the males were discarded. The females were placed individually on healthy wheat seedlings and transferred to a new seedling for 58 consecutive days or until the female died. Both long- and short-day females were kept in the same environment in which they had developed for the 60-day experiment.

The wheat seedlings the females fed upon in the long- and short-day environments for 24 hours were placed in a greenhouse at about 21°C under a 16-hour photoperiod after the females had been removed from them. These plants were held for at least 30 days under these conditions before they were examined for symptoms of the disease.
DISCUSSION OF VIRUS-VECTOR RELATIONSHIP EXPERIMENTS

Virus transmission studies with *E. inimica* have been with insects reared under conditions of supplemental light or with insects collected from the field with unknown environmental histories (Slykhuis, 1953, 1963; Slykhuis and Sherwood, 1964). The sex, age, and mating condition have not been identified and are presumed to have been unknown or not specified. To my knowledge, there is no report where transmission studies have been conducted using insects of specified sex, age, and mating condition reared under a long-day environment versus a short-day environment. The fact that a leafhopper vector such as *E. inimica* where there are two possible developmental pathways determined by the photoperiod to which the females are exposed, seemed to be a reasonable incentive for critical inquiry as to whether these physiologically different females differed in their virus-vector relationship.

Swenson (1971b) stated that transmission experiments should be designed to estimate the number of insects that become inoculative and the frequency of transmission. Table IV summarizes my results when comparing virus transmission of short- and long-day females, mean latent period, mean number of plants infected, and the plants infected per insect day. A total of 52 (66%) of the short-day females and 40 (52%) of the long-day females became inoculative after a two-day acquisition feed on the virus source. The mean latent period for short- and long-day females was 13.21 and 14.85 days, respectively, and would appear to be comparable with the results of Slykhuis
(1963) for temperatures comparable with my temperature conditions. He also reported a decrease in the latent period with an increase in temperature and the shortest period was five days at 27, 30, and 33°C. In my tests at a mean temperature of about 21°C, three (6%), three (6%), and six (11%) of the short-day females transmitted the virus on days three, four, and five. One (2%) of the long-day females transmitted for the first time on day three and the next transmission occurred on day seven when two (5%) females transmitted the virus.

The mean number of plants infected by those insects which were inoculative was 18.46 for the short-day females and 21.15 for the long-day females, but the inoculative long-day females lived a mean number of 56.65 days as compared with 51.31 days for the inoculative short-day females. The ratio of plants infected per insect day was calculated by subtracting the mean latent period from the mean longevity of the transmitters and this difference was divided by the mean number of plants infected (Swenson, 1971b). The short-day females infected slightly fewer (0.484) plants than did the long-day females (0.505) per insect day.

The mean percentage transmission combined in four-day intervals for short- and long-day females was calculated from the daily percent of transmission and is shown in Figure 4. The mean percentage transmission by the inoculative females increased until the 24th day and then increased slightly until the 36th day when both short- and long-day reached peak transmission and transmission then decreased until the conclusion of the experiment on day 60. In making
comparisons between transmission by short- and long-day females, it should be noted that on the 36th day, 39 of the original 40 long-day transmitters were still alive compared with 41 of the original 52 short-day transmitters.

Since the probability of virus transmission increases with the feeding time of the vector, the mean percentage transmission (Fig. 4) would indicate that the differences in feeding activity of short- and long-day females is slight. Chiykowski (1967) and Swenson (1971b) have speculated that female *Macrosteles fascifrons* Stal transmitted more than males because of increased feeding activity by females due to the demands of ovariol development. Coupe and Schulz (1968) stated that overwintering eggs of *E. inimica* are 1.25 times larger than eggs which hatch in several weeks. If the speculation regarding feeding time being related to the demands of ovariol development is true, it would seem reasonable to assume that short-day females feed more than long-day females as judged by the observation that eggs from short-day females are larger. Therefore, they must have greater food reserves. In my experiments, this could explain why 66% of the short-day females and 52% of the long-day females became inoculative after a 48-hour acquisition feed.

It is difficult to make meaningful comparisons with my data and the results obtained by other workers using the same virus-vector combination and different virus and mycoplasma vector relations. My results indicate that short-day females live for a shorter period of time than do long-day females, and in both populations the longevity of nontransmitters was slightly more than that of transmitters.
The greatest difference existed between short- and long-day transmitters where 75% of the long-day transmitters were alive at the end of the test as compared to 63% of the short-day females. The mean longevities are biased in that those insects still alive at the end of the test were killed so that the actual longevity of those insects is not known. Swenson (1971b) working with *M. fascifrons* and aster yellows found that mated female transmitters lived longer than the nontransmitters. Slykhuis (1963) working with *E. inimica* and wheat striate mosaic virus, reported that 16% of his insects were still alive after 60 days, but age at the beginning of the test was not given. In the same tests with a 48-hour acquisition feed, 91% of the insects were inoculative after being transferred at two-day intervals as compared to 66 and 52% of my insects with an identical acquisition feed and a new host plant each 24-hour period.

The vector of curly leaf virus of turnip and beet, *Piesma quadratum* Fieb, overwinters as adults in a state of ovariol diapause induced by short daylength. Völk and Krczal (1957) reported that this insect was a more efficient vector after overwintering than prior to overwintering. Young adults, inoculative prior to overwintering, infected 32.5 and 23% of the young turnip plants they fed upon as compared to 53% of the plants they fed upon after overwintering. This difference in transmission may have been related to diapause. Since the exact conditions of the experiments were not stated, difference may have been related to the separation in time between the experiments, sex, or age of the insects.
My virus transmission studies were not conducted with the stage 6 in the life cycle of *E. inimica* which experienced diapause. Diapause in *E. inimica* is under maternal control and is determined by the daylength under which the mothers are reared. Short-day females which oviposited eggs in a state of diapause were not more efficient vectors than long-day females. Sixty-six percent of the short-day females transmitted the virus at least once during the experiment as compared to 52% of the long-day females, but the mean number of plants infected and the plants infected per insect day were slightly greater for the long-day females.

In applying my results to the situation as it exists in the field, there would appear to be no difference in the virus transmission efficiency of female *E. inimica* maturing under the long days of late spring and early summer compared to females maturing under the short days of late summer and early fall.
TABLE I

NUMBER OF EGGS OVIPOSITED BY ENDRIA INIMICA FEMALES, REARED UNDER 12L:12D AND 15L:9D PHOTOPERIOD REGIMENS AT 24°C DURING THE PHOTOPHASE AND 18°C DURING THE SCOTOPHASE, THAT DEVELOPED TO THE EYESPOT STAGE

<table>
<thead>
<tr>
<th>Photoperiod regimen</th>
<th>15L:9D</th>
<th>12L:12D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number eggs observed</td>
<td>129</td>
<td>560</td>
</tr>
<tr>
<td>Number with eyespots</td>
<td>93</td>
<td>1</td>
</tr>
<tr>
<td>% with eyespots</td>
<td>0.72</td>
<td>0.001</td>
</tr>
</tbody>
</table>
TABLE II

EFFECT OF VARIOUS TREATMENTS ON TERMINATING EGG DIAPAUSE,
INDUCED BY SHORT DAYS (12L:12D) AT A MEAN TEMPERATURE
OF 21.1°C, IN THE LEAFHOPPER ENDRIA INIMICA

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>of 32 diapause eggs without a chill treatment, none developed to the eyespot stage after incubation at 26°C for 50 days</td>
</tr>
<tr>
<td>2</td>
<td>of 51 diapause eggs without a chill treatment placed under conditions favorable for continuous development (15L:9D, 21.7°C), 1 egg developed to the eyespot stage within 50 days</td>
</tr>
<tr>
<td>3</td>
<td>of 10 diapause eggs given a 63-day chill treatment at 4°C and then placed in total darkness at a mean temperature of 21.7°C, 6 eggs developed to the eyespot stage</td>
</tr>
<tr>
<td>4</td>
<td>of 31 diapause eggs chilled for 63 days at 4°C and then placed under diapause inducing conditions (12L:12D, 21.1°C), 18 eggs developed to the eyespot stage</td>
</tr>
<tr>
<td>5</td>
<td>of 31 diapause eggs chilled for 63 days at 4°C and then placed under conditions favorable for continuous development (15L:9D, 21.7°C), 13 eggs developed to the eyespot stage</td>
</tr>
<tr>
<td>Time (days)</td>
<td>Temperature</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>20</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Long-day eggs 56 73% 9.7±1.02 82%

1/ Total percent hatching, mean number days to hatch after chill period, and standard deviation.
2/ Percent of eggs with eyespots hatching.
TABLE IV

COMPARISON OF WHEAT STRIATE MOSAIC VIRUS TRANSMISSION BETWEEN SHORT- AND LONG-DAY MATED ENDRIA INIMICA FEMALES AS RELATED TO NUMBER OF INSECTS INOCULATIVE AFTER A 48-HOUR ACQUISITION FEED, PLANTS INFECTED PER INSECT DAY, LONGEVITY, LATENT PERIOD, AND PLANTS INFECTED

<table>
<thead>
<tr>
<th>Photoperiod regimen and mean temperature</th>
<th>12L:12D 21.1°C</th>
<th>15L:9D 21.7°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number insects</td>
<td>79</td>
<td>77</td>
</tr>
<tr>
<td>Number of inoculative insects</td>
<td>52 (66%)</td>
<td>40 (52%)</td>
</tr>
<tr>
<td>Plants infected/insect day</td>
<td>0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>Longevity transmitters</td>
<td>51.31±14.141/</td>
<td>56.65±8.46</td>
</tr>
<tr>
<td>Longevity nontransmitters</td>
<td>54.92±10.37</td>
<td>57.29±7.86</td>
</tr>
<tr>
<td>Latent period</td>
<td>13.21±6.76</td>
<td>14.85±7.44</td>
</tr>
<tr>
<td>Plants infected</td>
<td>18.46±8.91</td>
<td>21.15±8.27</td>
</tr>
</tbody>
</table>

1/ Mean number days or plants and standard deviation.
Figure 1. Eggs of Endria inimica inserted into partially decayed vegetative material.
Figure 2. Cumulative percent of diapause eggs which reached the eyespot stage of embryogenesis when incubated at 26°C after exposure to 0 and 8°C for different times.
Figure 3. Cumulative percent of diapause eggs which hatched when incubated at 26°C after exposure to 0 and 8°C for different times.
Figure 4. Mean percentage transmission at four-day intervals by short-day females (12L:12D) and long-day females (15L:9D) after a two-day acquisition feed on the virus source and daily sequential transfers.


Wilbur, D. A. 1954. Host plants of the leafhopper Endria inimica (Say) (Homoptera, Cicadellidae). Transactions of the Kansas Academy of Science 57:139-146.