THE TRANSMISSION OF THE BARLEY YELLOW DWARF VIRUS BY THE GREENBUG, TOXOPTERA GRAMINUM (RONDANI)

by

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INTRODUCTION

The barley yellow dwarf virus (BYDV) is quickly becoming a widespread and destructive disease of cereal crops in the United States and throughout the world. Since Oswald and Houston (19) first reported the BYDV in California, it has been reported in several states and countries. Johnston (16), in 1951, reported the "red leaf" disease of oats as still being a mystery in Kansas. In 1954, Sill et al. (31) reported "seeing red leaf" in Kansas but with no severity. A severe outbreak of the BYDV in the United States on oats in 1959 prompted the United States Department of Agriculture to issue a Plant Disease Reporter Supplement, #262 (39). Some Kansas fields suffered estimated losses of 50% or more during this epiphytotic (32).

Oswald and Houston (19, 20) found the virus to be transmitted by 5 species of aphids. Recent studies have shown one more aphid to be a vector of the yellow-dwarf virus (41). Sill et al. (32) reported high aphid populations in Kansas, especially the greenbug, Toxoptera graminum (Rondani), in the fall of 1958 and the spring of 1959. They believed the greenbug was probably the most important vector of the virus during this 1959 BYDV outbreak. Therefore, this research was initiated not only to find techniques to use in studying and transmitting the virus in Kansas but to learn more about its transmission by the greenbug.

REVIEW OF LITERATURE

In 1951, Oswald and Houston (19) reported a widespread and

destructive disease in the California barley crop. The disease was characterized by a brilliant yellowing of leaves accompanied by moderate to severe stunting of the plants. The name yellow-dwarf, which describes the symptoms on barley, was used. As early as 1890 Galloway and Southworth (15) reported a disease of oats that they said was caused by bacteria, but the description of the disease symptoms fits yellow-dwarf of oats. Barrus (5), in 1937, described a red leaf and blast of oats, but he did not know the cause of it. The "red leaf" disease of oats was reported as still being a mystery in Kansas in 1951 (16). Sill et al. (31) reported seeing "red leaf" virus in oats in Kansas in 1954 but with no severity.

The years from 1951 to 1959 brought widespread reports of the disease in the United States (3, 6, 8, 36, 40). Oswald and Tmng (23) found the yellow-dwarf virus on oat and barley plants in The Netherlands. The disease has been reported in Ontario, Northern Europe, and Great Britain (33, 34, 41). The great destructiveness of the disease on oats in 1959, which was reported in the previously mentioned Plant Disease Reporter supplement, resulted in great losses in the North Central United States. Fields of oats that sustained damage severe enough to prevent harvesting were found in Missouri, Kansas, Nebraska, South Dakota, Minnesota, Wisconsin, Iowa, Illinois, and Indiana (18). High aphid populations in Kansas during the fall of 1958 and especially during the spring of 1959 brought about a severe outbreak of the barley yellow-dwarf in spring oats and spring barley (32). Estimates of losses in some Kansas oat fields were 50% or more and in others often 25 to 40%.

Field counts of red leaf plants showed a 50 to 75% infection of individual plants. The percentage of diseased plants was usually higher in late planted fields. (See Figure 1 for loss distribution in Kansas in 1959.)

This disease at first was thought to be due to environmental conditions, but normal plants always found scattered among the yellow-dwarfed plants indicated to Oswald and Houston (19) that this probably was false. No pathogenic fungus was consistently found in the diseased plants. They noticed that the aphid population on grains in California in 1951 was higher than it had been in many years. They, therefore, investigated the possibility of the disease being due to feeding damage caused by aphids, but the disease was severe on barley in areas where aphid populations were not heavy. This forced the conclusion that if aphids were involved, they were probably vectors of a virus disease. Oswald and Houston (19) then proved four species of aphids to be vectors of the yellow-dwarf virus. These were: corn leaf aphid, Rhopalosiphum maidis (Fitch); apple grain aphid, R. prunifoliae (Fitch); English grain aphid, Macrosiphum granarium (Kirby); grass aphid, M. dirhodum (Walker). Recently a European worker (17) reported that R. prunifoliae (Fitch) is used in error and should be R. padi (L). In later work Oswald and Houston (20) found the greenbug, Toxoptera graminum (Rondani), also to be a vector. A more recent study has shown Myzus circumflexus (Buckt.) to also be a BYDV vector (41). Several workers have found the yellow-dwarf virus to be persistent in its vectors from five days to as long as the aphid lives (2, 21, 22, 36). A close relationship between the barley yellow-dwarf virus and the oat red-leaf virus was found by Takeshita

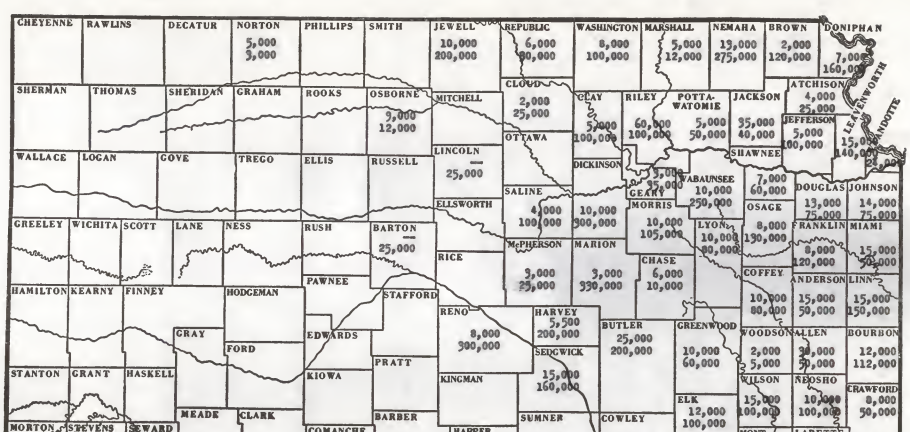

FIGURE 1. Estimated county losses due to barley yellow dwarf in oats and barley in Kansas: 1959 crop. Top number in each county is the bushel loss in barley. Total— 562,000. Bottom number is the bushel loss in oats. Total— 5,923,000. (32)

(36), and he proposed that the two were the same virus. This proposal was based on the similarity of the viruses in regard to symptomatology, incubation period in the host plant, aphid transmission, persistence of the virus in the aphid vector, and failure to transmit either virus by seed, soil, or mechanical means.

Bruehl and Toko (8) found the English grain aphid to be a poor vector of the yellow-dwarf virus collected in Washington; whereas, both the apple grain and English grain aphid were efficient vectors in California (19). This indicated either that strains of the virus might exist, or else vector specificity, possibly both. Allen (1) obtained 43 yellow-dwarf isolates that differed in virulence. He distinguished 16 strains by transmission of the isolates to 4 differential hosts. The hosts were barley varieties Blackhulless, Rojo, and Atlas 46 and the oat variety, Coast Black. Takeshita (37) used a highly virulent isolate and a mildly virulent isolate of the virus in testing very susceptible, intermediate, tolerant, and highly resistant varieties of barley, oats, and wheat previously used by Oswald and Houston (21). The reaction of the highly virulent isolate in most cases coincided with that reported by Oswald and Houston (21). Nearly all host plants manifested either tolerance or high resistance to the mildly virulent isolate. After 129 transfers, Rochow (29) found that the vector specificity of two English grain isolates and two apple grain isolates remained essentially unchanged. However, in all cases, occasional transmission by the "nonvector" aphid occurred. Rochow concluded that the English-grain-aphid-transmitted isolates seemed to represent a strain of the virus common in New York, but different from those found in some other areas of the United States.

Rochow (28) found that vector specificity still prevailed with English grain aphids, but not with apple grain aphids after they had fed on a source leaf infected with both English grain and apple grain virus strains. In cross protection tests, Toko (38) found no interference between the English grain isolate and the apple grain isolate, thus giving proof that they are closely related strains and not distinct viruses. Rochow (30) has demonstrated that greenbugs collected from Florida, Wisconsin, and Illinois varied in their ability to transmit BYDV. The greenbugs from Florida transmitted virus only once in 14 trials while those from Wisconsin and Illinois transmitted 12 of the 14 trials. This is another factor to consider in interpreting any results obtained in transmission experiments.

In their initial work, Oswald and Houston (19) found that the yellow-dwarf virus infected barley, wheat, and oats. Takeshita (36) later found that virus recovered from infected oats produced yellow-dwarf symptoms in rye. There have been several recorded cases of grasses serving as natural hosts to the yellow-dwarf virus (9, 22). Oswald and Houston (22) found that 36 species (representing 8 tribes of the Gramineae) were possible hosts. They found 16 of the 36 species to be symptomless carriers of the virus. The tribes and species that showed BYDV symptoms were:

Tribe Festuceae--Bromus catharticus Vahl, Bromus inermis Leyss., Bromus mollis L., Bromus rigidus Roth., Bromus rubens L., Bromus tectorum L., Cynosurus echinatus L., Festuca myuros L., and Festuca reflexa Buckl.

Tribe Hordeae--Aegilops triuncialis L., Hordeum hystrix Roth.,

Hordeum leporium Link., Hordeum brachyantherum Nevski., and Sitanion hystrix Nutt.

Tribe Aveneae--Avena barbata Brot., and Avena fatua L.

Tribe Agrostideae--Aristida oligantha Michx., and Gastridium ventricosum Gnoan.

Tribe Phalarideae--Phalaris parodoxa L.

Tribe Andropogoneae--Andropogon barbinodis Lag.

The tribes and species that were symptomless carriers of the BYDV were:

Tribe Festuceae--Dactylis glomerata L., Festuca arundinaceae Schref., Poa annua L., and Poa pratensis L.

Tribe Hordeae--Agropyron trachycaulum (Link) Malte., Elymus caputmedusae L., Elymus triticoides Buckl., and Lolium multiflorum Lam.

Tribe Phalarideae--Phalaris tuberosa L., and Anthoxanthum odoratum L.

Tribe Chlorideae--Boutelous curtipendula (Michx.) Torr., Chloris gayana Kunth., and Cynodon dactylon (L.) Pers.

Tribe Andropogoneae--Sorghum sudanense (Piper) Stapf., and Sorghum vulgare Pers.

Bruehl and Toko (9) found that Bromus inermis was immune to a Washington strain of the BYDV, but was susceptible to a California strain. Phleum pratense was susceptible to two Washington strains and was immune to California strains. A Poa species was susceptible to one Washington strain but immune to another. Hence, it also would seem that the virus is extremely variable in respect to host range.

Symptoms produced by the yellow-dwarf virus on cereal crops vary

with the crop and variety. Symptom severity in all hosts depends upon the age of the plant when infected. The following symptoms or similar ones have been described by several investigators (3, 21, 36). Barley infected in the seedling stage is stunted and the leaves become a bright yellow, first at the tip. Later there is a downward progression of yellowing toward the leaf base. Infected oat seedlings develop symptoms similar to those in barley except that a reddening of leaves occurs rather than a yellowing. Complete necrosis of the infected leaves is the usual final stage in symptom expression in both barley and oats. In a highly susceptible variety of oats that has been infected in the seedling stage, heading does not take place. If the plants do head, a blasting of the flower parts occurs. Wheat is the most severely damaged cereal crop when infected in the seedling stage. The wheat seedlings become chlorotic and are severely stunted. Wheat infected at a later stage of growth just shows a bright yellowing of the newly-formed leaves and often is not damaged appreciably.

Shading of inoculated oat, barley and wheat plants increases the incubation period and decreases the severity of the yellow-dwarf virus (12). In temperature studies Endo (12) incubated plants at 65°, 75°, 82°, and 88°F. He found that symptoms were severe at 65° and 75° and progressively less severe at 82° and at 88°F. A highly virulent isolate caused symptoms sooner than a mild one, and it killed oat and barley plants at 65°, 75°, and 82°F. Only moderately severe symptoms develop at 88°F.

Oswald and Houston (21) showed a direct correlation between the time of infection and the effect of yellow-dwarf upon yield. Three barley

varieties, naturally infected at the seedling stage, suffered a 95% yield reduction. Working with a moderately virulent strain of the yellow-dwarf virus, Endo and Brown (13) found plants inoculated in the 3-leaf stage suffered a greater yield reduction than those inoculated in the boot stage. The yield of Clintland oats was reduced 94.4% when inoculated in the 3-leaf stage, and 21.8% when inoculated in the boot stage. Reduction in the yield, when infection occurred in the 3-leaf stage, was due to a reduction in the number of spikelets per panicle and the number of spikelets that produced kernels.

Partial control of the yellow-dwarf virus may be achieved with insecticides for the control of aphids, with breeding for resistance either to control the disease or to prevent feeding of the aphids and with certain cultural practices. Pizarro and Arny (24) found that Systox, a systemic insecticide, protected plants against severe aphid infestation, but did not prevent transmission of the yellow-dwarf virus. Some experimental work has been done with another systemic, Dimethoate, and it has shown good control in preliminary work, but this work has not been confirmed (10). Experiments concerning the use of fertilizers in the control of greenbugs have shown a decrease in infestation when the amount of nitrogenous fertilizer used was increased (4, 7). Probably the best means of control lies with a program of breeding for resistance. Suneson and Ramage (35) found a single recessive gene difference between Rojo and California Mariout barley varieties, and after four backcrosses, it was homozygous in plant predominantly like California Mariout. Later work demonstrated the existence of the same type of gene for resistance to yellow-dwarf in each of four barley varieties: C.I. 1227, C.I. 1237,

C.I. 2376, and Abate (25). The future use of these varieties will be important in breeding for resistance to yellow-dwarf. Oswald and Houston (21) found that the late-planted barley fields suffered the greatest losses due to the BYDV. Sill et al. (32) reported the greatest losses in late planted oat fields in the 1959 BYDV outbreak. The late planted fields suffered great losses because the severity of symptoms is wholly dependent on plant age when infected and viruliferous aphid populations were high when the late plants were in the seedling, or most susceptible, stage.

MATERIALS AND METHODS

Virus Source

The original source of the barley yellow-dwarf virus used in this investigation was supplied by W. F. Rochow of Cornell University. The virus was received in the form of infected California Red oat plants. A later shipment was received as infected leaves between moist blotter papers. Rochow (27) referred to this BYDV isolate as Ag-I and found that it was regularly transmitted by the apple grain aphid but only occasionally by the English grain aphid. A later letter from Rochow[1] indicated that it was also transmitted by the greenbug. Early in the study, plants showing supposed symptoms of the virus were collected from

[1]Personal correspondence from W. F. Rochow of Cornell University to W. H. Sill, Jr., of Kansas State University.

Kansas fields, but possibly due to inadequate techniques no BYDV isolates were obtained from them. Later, using improved techniques an isolate of the virus was recovered from plants collected in Kansas, but it has not been used thus far in transmission trials.

Rearing of Aphid Colonies

Due to its importance in Kansas cereal crops, the greenbug, Toxoptera graminum (Rondani) was used in all investigations. The aphids were originally obtained from the Entomology Department at Kansas State University. Stock colonies of the aphid were maintained on virus-free barley plants in an isolated laboratory to prevent outside contemination by other aphids or viruses. Reno barley was thickly planted in 6-inch pots. The pots were placed on window sills of the laboratory and the plants were infested with non-viruliferous aphids while still in the seedling stage. Reno seed was planted at regular intervals in order to always have a supply of young infested plants. Leaves from plants previously infested with aphids were pulled and placed among new plants in other pots. The aphids moved to the new plants at will which provided a quick method of transfer without injury.

Plants Used in Transmission Studies

Clintland and Andrew oat and Lee and Baart 46 wheat varieties were used as test plants in this study. The plants were grown in 6-inch

clay pots and were thinned to 4 plants per pot. A mixture of top soil, peat moss, sand and sheep manure was used for potting soil. Seed was planted on a regular schedule in order to always have young plants available for all studies. Plants were regularly fertilized with a liquid fertilizer (Hyponex). Virus-infected Clintland oat plants were kept in the greenhouse and used as a continuing virus source.

Types of Cages

Humidified plastic boxes were used as containers for detached barley-yellow-dwarf-virus-infected leaves where aphid vectors were given an acquisition feeding period on the leaves (Plate I). High humidity was maintained by lining the boxes with a seed germination pad moistened with distilled water. This system was adapted from Rochow (26). During the 3-day inoculation feeding period, the aphids were caged on seedlings by means of 100 ml plastic test tube cages (Plate II). Air holes were cut in the tubes and then covered with nylon fabric. These cages were developed by H. W. Somsen, entomologist with the U. S. Department of Agriculture stationed at Kansas State University. Other cages used were 9-inches square and 18 inches tall. They were wooden frames covered on 3 sides by nylon fabric and on the front by a sheet transparent plastic. These cages were sealed to 6-inch pots with Armstrong's caulking compound. An opening in the top of the cage provided access to the plants. Del Rosario and Sill (11) reported and illustrated this cage. These wooden frame cages were used in early transmission studies, but proved unsuccessful at that time. Large wooden framed glass cages measuring 33 x 31 x 23 inches

EXPLANATION OF PLATE I.

Plastic boxes used to confine aphids on detached, diseased leaves for acquistion feeding. The boxes were lined with a moist germination pad.

PLATE I.

EXPLANATION OF PLATE II.

Plastic test tube cages used to cage aphids on seedlings during the inoculation feeding period.

PLATE II.

were also used (Plate III). The base and opening door of these cages was wood. Air was supplied to each large cage by a Dayton blower with a capacity of 150 cubic feet per minute. This cage was developed by Fellows and Connin (14).

Working Areas

All work of this investigation was performed in sections of the mosaic greenhouse at Kansas State University. Fumigation with Plantfume 103 smoke generators (active ingredient tetraethyl dithiophrophosphate) manufactured by the Plant Products Corporation was followed on a regular schedule to prevent contamination by insects coming from outside the greenhouse. In winter the greenhouse temperature averaged about 70°F, but the summer temperatures varied greatly. Thermostatically controlled steam heat with a blower system was used in the greenhouse during the winter. During the summer, work was carried on in a greenhouse section cooled by a Floral Breeze Greenhouse Cooling System, an evaporative cooler, built by the Acme Manufacturing Company of Muskogee, Oklahoma.

Techniques Used in Virus Transmission and Greenbug Injury Experiments

In all studies the aphid, Toxoptera graminum (Rondani), was used. Non-viruliferous aphid colonies were maintained in a laboratory on Reno barley plants as stated previously. The aphids were transferred from one pot to another by pulling a heavily infested plant and placing it

EXPLANATION OF PLATE III.

Large wooden-framed glass cage used in transmission studies. The left side of the cage opens allowing access to the plants.

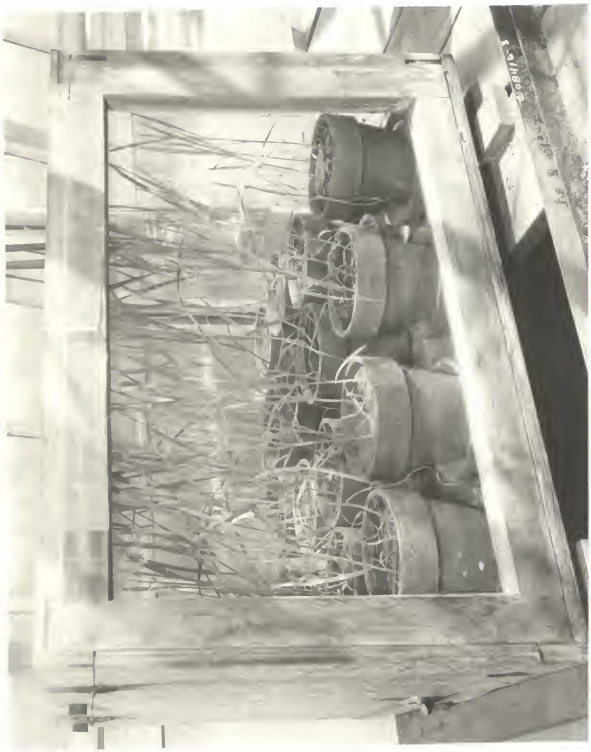

PLATE III.

among the non-infested plants, allowing the aphids to move to new plants at will.

Aphids were placed on detached, diseased leaves by gently brushing a heavily infested plant with the hand as it was held over the open plastic boxes that contained the leaves. The aphids then fell on the diseased leaves. After a 3-day acquisition feeding period the viruliferous aphids were transferred by means of a camel's hair brush to the test plants. One aphid per plant was caged on the test plants which were then covered with the previously mentioned 100 ml test tube cages, and allowed a 3-day inoculation feeding period. Following the inoculation feeding period, the plants were uncaged and the aphids killed with nicotine sulfate spray. After an incubation period of 10 to 15 days, the symptoms of the virus appeared and the percentage of transmission was calculated. One hundred plants were used for each trial and thus the percentage was easily calculated. Control plants of the same age infested with non-viruliferous aphids were used with each trial. Also a series of healthy control plants was grown with each trial.

Viruliferous aphids were caged on plants during a sufficient number of trials to determine that they were eventually lethal to the plants. The aphids were allowed to feed and reproduce at will; thus the population built up and gradually destroyed the plants. Non-viruliferous aphids were handled in the same way with similar results, indicating that the damage caused by large aphid populations in the greenhouse where no predators occur might be primarily aphid damage.

A study was made to compare the amount of greenbug-feeding-damage

with that due to barley yellow-dwarf virus. Viruliferous and non-viruliferous greenbugs were allowed to build up on plants in separate cages. Observations of these were made to find the amount of damage caused when the greenbugs were allowed to feed and reproduce at will. Healthy plants and virus-infected plants kept free of all insects were observed at the same time, allowing a comparison to be made.

EXPERIMENTAL RESULTS

Rearing of Aphids

Originally aphid colonies to be used for transmission studies were grown in the pot cage previously described. The greenbug population built up such large numbers that the plants were killed within 10 days. This resulted in much time being spent in caging young plants and infesting them with aphids. The caged plants also were occupying space in the greenhouse which could have been used for other work. When the aphids were uncaged and placed in the windows of an isolated laboratory far from the greenhouse, the winged forms escaped, helping to prevent a large population build-up and sudden destruction of the host plants. Therefore, this technique of growing uncaged plants in an isolated laboratory was used routinely for growing non-viruliferous source aphids.

Transferring Aphids

The technique of moving aphids individually with a camel's hair brush proved excessively slow when preparing for the 3-day acquisition feeding period. The aphids were moved most efficiently by gently brushing the heavily infested plants with the hand. The aphids then fell into the open plastic boxes placed on a table below. Since one aphid was used on each plant for the inoculation feeding, the transfer by a small camel's hair brush, even though slow, proved most satisfactory.

Cages Used

Petri dishes lined with moist filter paper were used first as acquisition feeding chambers but these allowed the aphids to escape and the detached leaves suffered from desiccation. Later the plastic boxes, previously mentioned, lined with moist seed germination pads proved to be successful (Plate I). The aphids survived in the closed plastic boxes for the 3 day acquisition period and gave successful transmission results. The 100 ml plastic test tube inoculation cages allowed single aphids to be confined to just one plant and hence were more satisfactory than the large pot cages which allowed the aphids to move from plant to plant and created the possibility of inaccurate transmission percentage counts (Plate II). The best results were obtained in transmission studies when all plants could be left uncaged without aphids after the inoculation feeding period. The large, wooden-framed glass cages provided a favorable environment for a comparison of greenbug

feeding damage with that due to the BYDV (Plate III). Smaller cages seemed to give an inaccurate picture of the results because the aphids built up at an extremely rapid rate and plant growth was not normal even in control plants.

Virus Transmission and Greenbug Injury Experiments

The detached leaf method, previously described, proved most efficient in obtaining viruliferous aphids for transmission experiments. At the beginning of the study, non-viruliferous aphids were caged on BYDV-infected plants and allowed to feed for an extended period of time. This method proved more time consuming and less accurate than the detached leaf method. Also individual aphids were more easily removed from a small plastic box than from a cage. There was also more danger of viruliferous aphids escaping from a cage and contaminating the greenhouse.

During 5 trials involving 500 oat plants, the greenbugs collected here in Kansas proved to be efficient vectors to oats of the strain of the BYDV used. The percentage of transmission varied from 23 to 47% (Table 1). Since these percentages were obtained by using only one aphid per plant, it would seem that a large mobile aphid population could cause a severe outbreak of the BYDV in oats. Three transmission trials using wheat plants proved unsuccessful. In a fourth trial some mild symptoms were expressed as the plants were maturing. The transmission percentage in wheat was not recorded. Difficulties encountered may

Table 1. Percent of successful transmission of the BYDV during five transmission trials using the greenbug, Toxoptera graminum (Rondani).

Trial	Variety (Oats)	No. of plants per trial	No. of aphids placed per plant	No. of plants showing symptoms	% of successful transmission
1	Clintland	100	1	28	28
2	Clintland	100	1	36	36
3	Clintland	100	1	47	47
4	Andrew	100	1	23	23
5	Clintland	100	1	32	32
Total		500		166	Ave. 33.2%

indicate that the greenbug used in this study does not readily transmit the virus to wheat.

Symptoms expressed in oats began with a discoloration of the leaf tips of older leaves. The tips usually became reddish-brown and later blotches of yellow to orange appeared in the lower portion of the leaves. A reddening of the infected leaves was followed by the leaf finally becoming necrotic beginning at the tip (Plate IV). Some stunting of infected oat plants was observed as compared to healthy plants of the same age (Plate V). Symptoms observed on maturing wheat started with the appearance of chlorotic striping on the older leaves. This was followed by complete chlorosis and necrosis of the leaves.

Three cages, previously described, were used in the comparison of the greenbug feeding damage with that caused by the BYDV. One cage con-

EXPLANATION OF PLATE IV.

Clintland oat leaves showing varying degrees of BYDV symptoms. Leaf on left is from healthy control plant.

PLATE IV.

EXPLANATION OF PLATE V.

Fig. 1. Healthy Clintland oat plants.

Fig. 2. Barley yellow-dwarf virus-infected Clintland oat plants. Notice stunting and delayed maturation.

PLATE V.

Fig. 1 Fig. 2

tained 64 plants infested with non-viruliferous greenbugs, another cage contained 64 plants infested with viruliferous greenbugs and the third cage contained 32 BYDV-infected plants without aphids and 32 healthy control plants. After one trial the technique used was successful enough to recommend its use in future trials; however, one trial did not give sufficient data to record. More greenhouse trials followed by field trials will need to be done to complete this study.

DISCUSSION AND SUMMARY

Rochow (26) reported growing aphid colonies in cages, but in this study that method proved less satisfactory and very time consuming. Maintaining greenbug colonies in a laboratory on uncaged Reno barley plants proved much more successful than using cages. The laboratory provided an isolated location where the colonies were kept free from contamination by viruses and other aphids. Transferring aphids from the infested plants to detached diseased leaves for an acquisition feeding period was best accomplished by brushing them from the plants with the hand so that they dropped into the plastic boxes containing diseased leaves. The viruliferous aphids were transferred most satisfactorily to test plants from the detached diseased leaves by means of a camel's hair brush as reported by Rochow (26).

Plastic boxes used in the acquisition feeding proved more effective than petri dishes as used by Rochow (26). The plastic boxes prevented the escape of aphids and retained moisture more effectively. Oswald and Houston (19) reported caging pots, each containing 5 plants, during a

3-day inoculation feeding period. The 100 ml plastic test-tube cages used in this study allowed each individual plant to be caged instead of caging 4 or 5 plants together. The caging of individual plants gave better control of the viruliferous aphids used for transmission than the technique used by Oswald and Houston (19). Endo (12) found that the BYDV symptoms were less severe and the incubation period was increased when the inoculated plants were shaded. This seemed to support not using any cages that might shade inoculated plants.

The detached-leaf method, adapted from Rochow (26) for this study, produced viruliferous aphids with a minimum of time and work. This method allows a leaf to be split, with one species of aphid feeding on half of the leaf and another species on the other half. In this way, the vector specificity of a virus strain may be easily determined.

The greenbugs collected in Kansas were efficient vectors of the BYDV strain used even when only one viruliferous aphid per plant was used. Oswald and Houston (19, 20) have reported a greater percentage of transmission of the virus, but they were using several viruliferous aphids per plant. It would seem that a large greenbug population in the field, transmitting the BYDV 33.2% of the time, as found in this study could easily cause a severe outbreak of the disease similar to that which occurred in 1959 (39). However, future studies should involve strains of the BYDV found in Kansas as well as all known greenbug biotypes. The literature does not reveal any transmission studies of the BYDV to wheat using the greenbug. Therefore, findings in this study cannot be compared with previous work, but would indicate that the greenbug may not be an efficient vector to wheat. In future trials with wheat, virus

strains found in Kansas should be used to find how efficiently the greenbug will transmit them.

The virus symptoms observed are similar to those previously described by several workers (3, 21, 36).

A complete evaluation of the comparative damage caused by the BYDV alone, by the viruliferous greenbug plus the virus, and by the non-viruliferous greenbug alone would be of great importance to a better understanding of the disease. A preliminary trial was made during this work, but no final conclusions could be drawn. However, it is clear that severe damage to the plants may be due to a combination of the virus and greenbug together or by either one separately. In the greenhouse it would appear that the greenbug often may be more damaging than the virus, but whether this actually is true in the field where aphid predators abound needs to be determined.

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THE TRANSMISSION OF THE BARLEY YELLOW DWARF VIRUS BY THE GREENBUG, TOXOPTERA GRAMINUM (RONDANI)

by

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Since the first reports of the barley yellow-dwarf virus (BYDV) in 1951, it has become a widespread and destructive disease of cereal crops in the United States and in other parts of the world. In 1959 the Kansas oat crop suffered severe and widespread losses because of the disease. Five common species of aphids have been found to be vectors of the virus. The greenbug, Toxoptera graminum (Rondani), is perhaps the most common vector in Kansas. This research was initiated to find techniques to use in studying and transmitting the virus in Kansas and to learn more about its transmission by the greenbug.

A method of rearing non-viruliferous aphid colonies without cages in a laboratory was developed. Caging the colonies allowed the aphid population to become so large that the plants were destroyed within 10 days. When they were uncaged, the winged aphids escaped and populations did not increase so rapidly. Removing aphids from caged plants also proved to be a more difficult and time-consuming task than shifting them from uncaged plants.

Preparation for a 3-day acquisition feeding period involved placing detached, diseased leaves in small plastic boxes lined with a moist germination pad and allowing non-viruliferous aphids to feed on the leaves. Petri dishes lined with moist filter paper were first used, but aphids escaped from them and the leaves suffered from desiccation. The aphids were gently brushed from the host plants and allowed to fall into the plastic boxes containing the diseased leaves. Moving one aphid at a time had previously proven unsatisfactory. After the acquisition feeding period, individual viruliferous aphids were moved from the boxes to test

plants by means of a camel's hair brush. Individual plant cages made from 100 ml plastic test tubes were used to confine one of these aphids on each of 4 plants in a 6-inch pot. The aphids were allowed a 3-day inoculation feeding period and then were killed with nicotine sulfate spray. The test tube cages provided a quick method of caging one or more aphids on individual plants. After the inoculation feeding period the best results were obtained when all plants were left uncaged. Large wooden framed glass cages measuring 33 x 31 x 23 inches, provided a favorable environment under which greenbug feeding damage could be compared with damage caused by the BYDV. Smaller cages measuring 9-inches square by 18 inches tall seemed to give inaccurate results since the aphid population built up at an extremely rapid rate and the cages produced excessive shade.

Five transmission trials involving 500 Clintland and Andrew oat plants proved that greenbugs collected in Kansas were efficient vectors of the strain of BYDV used. The percentage of transmission varied from 23 to 47%. Since these percentages were obtained by using only one aphid per plant, it would seem that a large mobile greenbug population could cause a severe outbreak of the BYDV in oats and probably did in the spring epiphytotic of 1959. Three transmission trials using wheat plants proved unsuccessful. Some mild symptoms developed in a fourth trial as the plants were maturing. These symptoms were so diffuse that the transmission percentage could not be recorded. No one has reported greenbug transmission to wheat and difficulties encountered in this study may indicate that the greenbug does not readily transmit the virus to wheat.

A complete evaluation of the comparative damage caused by the BYDV alone, by the viruliferous greenbug plus the virus, and by the non-viruliferous greenbug alone would be of great importance in understanding the disease. In the greenhouse the virus alone appears to cause the least amount of damage to oat plants. Damage caused by the viruliferous greenbug plus the virus or by the non-viruliferous greenbug alone seems to be more severe. Field trials will be necessary to complete this study, however, since aphid predators abound in the field and are absent in the greenhouse.