VIRUS - HOST AND VIRUS - VECTOR STUDIES WITH MANITOBA ISOLATES OF BARLEY YELLOW DWARF VIRUS

A Thesis

Submitted to

the Department of Graduate Studies and Research

The University of Manitoba

In partial fulfillment

of the requirements for the degree

Master of Science

by

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October 1970

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ABSTRACT

by

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Virus-host and virus-vector studies were carried out with several vectors and isolates of barley yellow dwarf virus (BYDV) from Manitoba. Interference between certain isolates of BYDV was observed in the plant and, possibly, also in the vector. Serial transfers on test plants of aphids infected with certain isolates of BYDV revealed differences in the pattern of transmission by nymphs and adults.

Interference studies were carried out in the plant with a Macrosiphum avenae-specific, a Rhopalosiphum padi-specific,and a non-specific isolate of BYDV. There was reciprocal interference between the M. avenae-specific and the non-specific isolates, both when the isolates were inoculated simultaneously into the plant and when there was a time interval between inoculations.

Although no cross protection was evident, the fact that interference occurred between these two isolates in the plant suggested that they were related. There was a lack of interference in the plant between either of these isolates and an R. padi-specific isolate. The R. padi-specific isolate was therefore considered to be unrelated to the M. avenae-specific and the non-specific isolates. Two Schizaphis graminum-specific isolates were considered to be unrelated to each other because of a lack of interference between these two in the host plants.

In a similar study, interference between one of the S. graminum-specific isolates and the M. avenae-specific isolate suggested that they were possibly related. This interference also occurred between these isolates at 15° and 30°C., although results at 30°C. were not as clear-cut because of the adverse effect of this temperature on the test plants.

In a limited study with a number of different BYDV isolates, three types of effect were observed in the aphid vectors when they were allowed access in succession to a given pair of the isolates. The most common was an addition effect. A second effect was termed a suppression effect and a third a synergistic effect.

In single transfers of aphids, more individuals of *R. padi*, *M. avenae*, and *S. graminum* transmitted an *R. padi*-specific isolate or an *M. avenae*-specific isolate if the vectors were held on healthy plant material for a period of three days between the acquisition and inoculation feeding periods, than if there was no delay between the acquisition and inoculation feeding periods.

In similar tests with the same vectors and a non-specific and a so-called *S. graminum*-specific isolate of BYDV, an opposite effect was observed. Fewer vectors transmitted virus when held for three days on healthy plant material between the acquisition and inoculation feeding periods, than if there was no delay between these two feeding periods.

A higher proportion of nymphs than adults of *S. graminum*-specific isolates of BYDV. Nymphs also transmitted virus to a higher percentage of plants than adults when moved serially on the test plants. Nymphs of the same clone of *S. graminum* also transmitted a non-specific isolate of BYDV more efficiently than adults.

With both of the *S. graminum*-specific isolates, aphids that acquired virus as nymphs usually lost their ability to transmit after the final moult. With one of these isolates, adults that had access to the virus as nymphs, were less efficient vectors than aphids which had access to the virus as adults.

ACKNOWLEDGMENTS

This study was undertaken at the suggestion of Dr. C. C. Gill, Research Scientist, Canada Department of Agriculture, Research Station, Winnipeg, Manitoba. Grateful appreciation is extended to Dr. Gill for his guidance and helpful criticism of my research work and to Dr. A. E. Hannah, Director of the Research Station, for laboratory space and facilities.

Grateful acknowledgment is made to Dr. C. C. Bernier of the Faculty of Agriculture, University of Manitoba, and Dr. B. R. Irvine, of the Department of Botany, University of Manitoba, for their helpful criticisms. Thanks are extended to Dr. R. J. Baker, Research Scientist, Canada Department of Agriculture Research Station, Winnipeg, Manitoba, for suggestions regarding the statistical analysis.

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CHAPTER I

INTRODUCTION

Review of Literature

Although barley yellow dwarf virus (BYDV) was not recognized as a virus until 1951 when it was discovered by Oswald and Houston (1951), several workers prior to 1951 had worked with diseases, similar to, and in some cases, diseases which are now believed to have been BYDV (Barrus, 1937; Manns, 1909; Sprague, 1950). Oswald and Houston found that BYDV could not be mechanically transmitted to plants, that it was a yellow type of disease, and that the virus had a circulative relationship with its aphid vectors (Oswald and Houston 1951, 1952).

Since this virus causes a disease of considerable importance in small grains and grasses, much work has been done on control, epidemiology and related practical problems. The virus also had been the subject of many studies on virus-vector-host relationships. A major development in the knowledge of BYDV has been the elucidation of several kinds of variability among virus isolates, with particular regard to host range, symptomatology, vector specificity, and physiological specialization among vectors.

Watson and Mulligan in England (1960 a) and Allen in the United States (1957) found that individual isolates of the virus differed widely in their host range. However, data on host range is of little value in establishing relationships among isolates. Hollings (1959) found widely different viruses to have similar host ranges and even cause similar symptoms on several test plants. Similarly three distinct cereal viruses, Agropyron mosaic, wheat streak and Hordeum mosaic virus, were found to differ only slightly from each other in host range and symptom expression (Slykhuis and Bell, 1966).

Symptomatology and variation in virulence are two other criteria used to characterize strain relationships of BYDV. Allen (1957) differentiated 16 strains of BYDV on the basis of their ability to discolor each of three barley and one oat varieties. However, many isolates of a given virus resemble each other in terms of symptoms produced, but are quite different in terms of transmission, host range and stability (Hollings 1959; Slykhuis and Bell 1966). By itself, virulence is of little value, but in combination with other factors such as vector specificity, differences in virulence have been helpful in determining kinds of

variation occurring among isolates of BYDV (Bruehl 1961; Gill 1969; Rochow 1968; Rochow et al 1965).

Vector specificity, and the relative efficiency with which an aphid species will transmit a virus isolate have become the most important criteria for differentiating BYDV isolates. Oswald and Houston (1952) originally reported Rhopalosiphum padi (Linnaeus) and Macrosiphum avenae (Fabricius) to be the most efficient vectors of BYDV. Rochow (1958) found in a later study, that efficiency of transmission of BYDV depended on the isolate of the virus. Since then many non-specific isolates (Gill 1969; Rochow 1965; Timian and Jensen 1964; Toko and Bruehl 1959; Watson and Mulligan 1960 b) have been discovered throughout the world.

Based on vector relationships, isolates of BYDV fit into one of the following five main categories:

1. non-specific isolates
2. Rhopalosiphum padi-specific isolates
3. Macrosiphum avenae-specific isolates
4. Rhopalosiphum maidis-specific isolates
5. Schizaphis graminum-specific isolates

These categories of isolates have been characterized by their transmissibility with four aphid species, R. padi, R. maidis, M. avenae, and S. grmainum.

There is great variation among the non-specific isolates of BYDV (Gill 1967, 1969; Rochow 1967). One

group of these isolates has been reported from several areas (Gill 1967; Jedlinski and Brown 1959; Rochow 1965 b; Smith 1963 a; Tetrault et al 1963; Timian and Jensen 1964). This group is transmitted most efficiently by R. padi (av. 58%) and least efficiently by R. maidis (av. 0%)(Gill 1967; Tetrault et al 1963). What appears to be a separate group of non-specific isolates was reported from Kansas (Saksena et al 1964 b). These isolates were transmitted more efficiently than the isolates described by Gill (1967), and Tetrault et al.(1963) (R. padi, av. 93%; R. maidis, av. 37%). A third group, transmitted most efficiently by R. maidis (av. 52%) and least efficiently by R. padi, (av. 28%) (Gill 1967), has been reported from Canada (Gill 1967; Smith 1961).

The R. padi-sepcific isolates are transmitted most efficiently by R. padi, are rarely transmitted by S. graminum and R. maidis, and never by M. avenae. This is the only BYDV strain present in Israel(Harpaz and Klein, 1965) and has been found by several authors in Canada, the United States and Britain (Gill 1969; Rochow 1959 a; Toko and Bruehl 1959; Watson and Mulligan 1960 b).

Specificity in the third category, which includes isolates transmitted specifically by M. avenae, is very marked. Transmission by R. padi, S. graminum and R. maidis

is very rare. In several localities these isolates have been relatively common in many years (Rochow 1965; Gill 1967).

Isolates of the R. maidis-specific type have been isolated from field samples in some locations (Gill 1967, Gill 1969; James, Gill and Halstead 1969; Rochow 1965 b). S. graminum-specific isolates have only been described in Manitoba (Gill 1967, Gill 1969).

A complicating factor in the use of virus-vector relationships as a criterion for classification is the variation within a vector species. Aphids are known to vary in transmission efficiency depending on which clone, developmental stage or form of a species is used (Rochow 1960; 1963 a). Although vector specificity will continue to be important in relating viruses at the strain and species level, it does not tell much about the relatedness of the viruses. More precise criteria are needed to compare isolates of BYDV and in particular, to evaluate the importance of variations among isolates.

Serological relationships between viruses or isolates of viruses are usually a reliable indication of virus affinity, as serologically related viruses share many other properties. Rochow and Bell (1967) and Aapola (1968) have obtained evidence for serological differences among isolates of BYDV using the infectivity neutralization test

and the Ouchterlony agar double diffusion technique, respectively.

McKinney in 1929 discovered that a plant systemically infected by a virus was immune to establishment of a closely related strain of the same virus. This phenomenon, called cross protection, has been widely used to demonstrate strain relationships of many viruses, including studies with BYDV isolates. Holmes devised a thesis with regard to interaction of viruses in the plant (1956). He pointed out that closely related strains do not induce a more severe disease in mixed infections than either virus alone, but that unrelated viruses usually do. Early studies showed no evidence of cross protection between strains of BYDV in the plant (Allen, 1957; Rochow 1958; Toko and Bruehl 1959; Watson 1960 a). Recently however, cross protection between M. avenae-specific isolates and non-specific isolates has been shown to occur in the plant (Aapola 1968; Jedlinski and Brown 1965; Smith 1963). Two authors have reported that no interaction occurs in the aphid between the isolates tested (Allen 1957; Watson and Mulligan 1960 a), while Smith (1963) reports cross protection in the aphid between M. avenae-specific and R. padi-specific isolates of BYDV.

PURPOSE OF THESIS

There is considerable heterogeneity and variability among isolates of BYDV, even isolates from the same locality. Various criteria, including host range, symptomatology, physical properties, and serology, have been used in the comparison of BYDV isolates. As BYDV is an aphid-transmitted disease of plants, both virus-host and virus-vector studies are possible. This thesis was undertaken to find relationships, if any, between some of the BYDV isolates found in Manitoba, using both virus-host and virus-vector studies.

In the virus-host studies, possible relatedness between two isolates was indicated if interference occurred between the two isolates inoculated into a common test plant. In this work interference was deemed to have occurred if the presence of two isolates of BYDV in the host, caused an effect less than the additive effects of the isolates present singly in the host. Evidence for interference was derived from observation of symptoms and measurements of height, seed yield, and dry weight of the inoculated plants. Evidence for possible protection or exclusion, which may be regarded as an extreme case of interference, was sought by determining whether only one isolate was present in the doubly-inoculated plant. A study was also done, to compare the effect of temperature

on the interference between two isolates of BYDV.

Virus-vector studies were also carried out with several BYDV isolates. In the first series of these experiments, attempts were made to find evidence for interference between pairs of BYDV isolates in both aphid vectors and non vectors when the aphids were allowed to feed on two isolates in succession.

A second series of experiments was done to study vector relationships, particularly transmission of the virus by nymphs compared with transmission by adults. In separate experiments both nymphs and adults were given an acquisition feed on the isolates, then transferred serially to test plants, to compare transmission patterns and transmission efficiency of nymphs as compared with that of adults, and to ascertain whether nymphs lost any ability to transmit the virus when they moulted to adults.

CHAPTER II

GENERAL MATERIALS AND METHODS

Aphid Vectors

The aphid species used were as follows:

1. *Macrosiphum avenae* (Fabricius)
   the English grain aphid
2. *Rhopalosiphum padi* (Linnaeus)
   the cherry oat aphid
3. *Schizaphis graminum* (Rondani)
   the greenbug
4. *Rhopalosiphum maidis* (Fitch)
   the corn leaf aphid
5. *Acyrthosiphon dirhodum* (Walker) = *Metopolophium dirhodum* (Walker)
   the rose grass aphid

All clones of the five aphid species were supplied by Dr. C. C. Gill, Canada Department of Agriculture, Winnipeg. These clones of virus-free aphids were maintained on caged barley (*Hordeum vulgare* L.) plants in an isolated growth chamber.

Life Stages

Adult non-winged aphids from the aphid colonies were used in all interference studies in the plant and in the aphids. In serial transfer experiments, 12-hour old nymphs and 10-day old adults were used in the comparison of nymphs

versus adult transmission of the BYDV isolates.

Barley Yellow Dwarf Virus Isolates

Barley yellow dwarf virus isolates were as follows:

1. 6508, transmitted non-selectively by M. avenae and R. padi obtained in 1965 in Manitoba (Gill 1967).

2. 6515, transmitted as above and obtained in 1965 in Manitoba (Ibid).

3. 6801, transmitted as above and obtained in 1968 in Manitoba (characterized in this work).

4. 6407, transmitted specifically by M. avenae and obtained in 1964 in Manitoba (Gill 1967).

5. 6524, transmitted specifically by R. padi and obtained in 1965 in Manitoba (Ibid).

6. 6711, transmitted specifically by S. graminum and rarely by R. padi and M. avenae and obtained in 1967 in Manitoba (Gill 1969).

7. 6718, transmitted specifically by S. graminum and obtained in 1967 in Manitoba (Ibid).

Stocks of these virus isolates were maintained in oats, Avena byzantina K. Koch "Coast Black", and were transmitted to new plants every 4-6 weeks using the most efficient vector.

Acquisition and Inoculation

The aphids in all experiments were either allowed an acquisition feed on detached leaves in a petri dish or on a caged leaf of a plant. When a detached leaf was used it was

cut into three-inch lengths and one was placed in moistened sand in the petri dish. Each leaf was cut into a number of pieces equal to the number of aphid species used. The petri dishes were then placed in a chamber maintained at 15°C.

When the aphids were allowed to acquire virus from an intact, infected plant, an eight inch long plastic tube one inch in diameter was fitter over a leaf, and split urethane foam plastic plugs were placed at each end of the tube. This type of tube was used primarily for acquisition feedings for serial transfer experiments. The plants were maintained in a growth chamber at 15°C. with a 16 hour photoperiod for the length of the acquisition feed. All inoculations were made in the greenhouse, unless controlled temperature conditions were warranted by the experiment. Most inoculations were made on plants about 10 days old, which were in the $1\frac{1}{4}$ to $1\frac{1}{2}$ leaf stage. In the interaction studies in the plant, when the inoculation was made at a late stage of growth, cages were used large enough to fit over the entire plant. In experiments requiring serial transfers, each aphid was caged on a seedling with a ventilated plastic tube eight inches tall and one inch in diameter. After transfer of the aphid to the next plant in the series, the plants were sprayed with tetraethylpyrophosphate (TEPP) insecticide.

Experimental Design and Calculation of Statistics

In interference studies in the plant, plants were grown 30-40 to a flat measuring 16 inches by 24 inches. Usually treatments were completely randomized among the plants. I n some experiments, treatments were randomized between flats of plants. No attempts were made in either the serial work, or in the interaction studies in the aphid, to randomize the test seedlings. All F tests were calculated with an Olivetti Underwood "programma 101" desk top computer.

CHAPTER III

VIRUS - HOST STUDIES

Effect on Oats Doubly-Inoculated with Paired Combinations of Five Barley Yellow Dwarf Virus Isolates

Introduction

Early studies showed no evidence of cross protection between strains of barley yellow dwarf virus (BYDV) in the plant (Allen 1957; Rochow 1958; Toko and Bruehl 1959; Watson and Mulligan 1960 a). Recently it has been shown that complete protection can occur between Macrosiphum avenae-specific isolates and non-specific isolates of BYDV both in Clintland oats (Avena sativa L.) (Jedlinski and Brown 1965; Smith 1963), and in Coast Black oats (Avena byzantina K. Koch) (Aapola, 1968). Aapola, who also included a Rhopalosiphum padi-specific isolate in his studies, found that no protection occurred between this isolate and either the non-specific or the M. avenae-specific isolates.

This paper reports the results of interference studies in Clintland oats doubly-inoculated with isolates of BYDV found in Manitoba. In the first group of experiments, a non-specific, an M. avenae-specific, and an R. padi-specific isolate were tested in paired combinations. Two other isolates, both transmitted most efficiently by Schizaphis graminum

(Rondani), were tested in a separate experiment. Isolates of the latter type were first described from Manitoba (Gill 1967, 1969) and were termed S. graminum-specific, though some of them were also transmitted by R. padi and rarely by M. avenae.

Materials and Methods

The species of aphids used were the English grain aphid, Macrosiphum avenae (Fabricius), the cherry oat aphid, Rhopalosiphum padi (Linnaeus), the greenbug, Schizaphis graminum (Rondani), the corn leaf aphid, R. maidis (Fitch), and the rose grass aphid, Acyrthosiphon dirhodum (walker) = Metopolophium dirhodum (walker). Methods for rearing the clones were the same as those previously described (Gill 1967).

The five BYDV isolates were 6407 (M. avenae-specific), 6524 (R. padi-specific (Gill 1967) 6711 and 6718 (S. graminum-specific) (Gill 1969) and 6801 (non-specific). Isolate 6718 was transmitted only by S. graminum, while 6711 was also transmitted by R. padi, but with low efficiency. Isolate 6801 was obtained from naturally infected oats in southern Manitoba in 1968. The ability of five species of aphids to transmit this isolate was determined by the method already described (Gill 1967).

The plants used for the interference tests were spring oats, Avena sativa L. var Clintland, grown in flats of soil in greenhouses at 17-23°C. Stocks of the virus isolates were maintained in Coast Black oats (Avena byzantina K. Koch). These isolates were transmitted to new stock plants every 4-6 weeks using the most efficient vector.

In experiments where plants were simultaneously doubly-inoculated, different batches of plants were treated as follows:

1. infested with seven aphids for each isolate
2. infested with seven aphids carrying one of the isolates and with seven virus-free aphids
3. as for #2 but using the other isolate

In experiments where there was a delay between the introduction of the individual isolates into the doubly-inoculated plants, 10 aphids were used per treatment instead of seven. Treatments for singly-inoculated plants were arranged so that inoculation with relevant isolate corresponded with that in the doubly-inoculated plants. An infestation with non-viruliferous aphids on the singly-inoculated plants was also made to correspond with the inoculation of the other isolate in the doubly-inoculated plants.

To determine the possible effect of aphid feeding on plant weight and seed yield, batches of plants in each experiment were also infested with virus-free aphids only,

or were merely caged without aphids. Treatments were randomized among the plants. In experiments with isolates 6801, 6407, and 6524, there were five plants per treatment, and with isolates 6711 and 6718 there were 10 plants per treatment. Aphid vectors used to inoculate the plants were R. padi for isolates 6801 and 6524, S. graminum for 6718 and 6711, and M. avenae for 6407. Plants were inoculated by allowing virus-free aphids to feed for two days at 15° C. on leaves detached from the virus stock plants and maintained in petri dishes with moist sand. These aphids were then transferred to the individually caged test plants. After two days, the aphids were sprayed with tetraethylpyrophosphate (TEPP) insecticide.

Inoculations were made when the plants were at the $1\frac{1}{4}$ to $1\frac{1}{2}$ leaf stage. Where there was an interval between inoculations, the second inoculation was made 16 days after the first inoculation.

Attempts to transmit the individual isolates from the singly-or doubly-inoculated plants were made 2-3 weeks after the simultaneous or the second inoculations. The detached, split-leaf method was used (Gill 1969). R. padi and M. avenae were used as vectors, with Clintland oats as test plants, for isolates 6801, 6524 and 6407; and R. padi and S. graminum, with Coast Black oats, for

isolates 6711 and 6718. Because isolate 6801 was transmissible by R. padi and M. avenae, virus transferred from plants doubly-inoculated with this isolate and isolate 6407 or 6524 was transferred to additional test plants one or more times for proof of identity, with the two aphid species as potential vectors.

Results

Characterization of Isolate 6801

The percentage of individual aphids that transmitted isolate 6801 from oats to oats was 41% for R. padi, 29% for M. avenae, 17% for A. dirhodum, 15% for S. graminum and 2% for R. maidis. These values were averages for a total of 240 individuals of each species tested in 24 trials. Symptoms of this isolate on oats were severe. These results indicate that this isolate belongs to the non-specific strain of BYDV (Gill 1969; Rochow 1969).

Simultaneous Inoculation of Plants with Paired Combinations of Isolates 6801, 6407, and 6524

All inoculated plants developed symptoms in 12-14 days. No symptoms appeared on plants infested with non-viruliferous aphids.

Symptoms on control plants inoculated with one isolate only were progressively more severe for 6524, 6407 and 6801.

This was reflected in plant weight, seed weight, and plant height (Table 1).

Plants doubly inoculated with isolates 6801 and 6524, or with 6524 and 6407, were more severely affected in terms of plant weight, seed weight and plant height than were the relative control plants inoculated with only one isolate (Table 1). The isolates in these paired combinations appeared to act synergistically.

On the other hand, when plants were doubly inoculated with isolates 6801 and 6407, height and weight of plants, and seed weights were intermediate between those for the control plants singly inoculated with isolates 6801 and 6407 (Table 1). These results appear to indicate that interference occurred between the two isolates.

In these, and subsequent experiments, there were no significant differences in the heights, dry weights, or seed weights of plants singly-or doubly-infested with non-viruliferous aphids, or of plants not infested with aphids.

Inoculation of Plants with Paired Combinations of Isolates 6801, 6407, 6524, the Two Inoculations Separated by an-Interval of Time

Symptom expression and incubation periods of these isolates in singly-inoculated control plants were similar

Table 1. Effect on Clintland Oats of Single or Simultaneous Double Inoculations With Paired Combinations of Barley Yellow Dwarf Virus Isolates 6801,6407, and 6524.

Treatment	BYDV - Isolate Combination								
	6801 / 6407			6801 / 6524			6524 / 6407		
	Mean dry[a] weight per plant g.	Mean Seed wt. per plant g.	Mean[b] ht. per plant cm.	Mean dry weight per plant g.	Mean Seed wt. per plant g.	Mean ht. per plant cm.	Mean dry weight per plant g.	Mean Seed wt. per plant g.	Mean ht. per plant cm.
Isolate 1[c]	1.08*[d]	0.10**	58.0**	1.28**	0.30[N.S.]	76.8**	6.28**	0.55*	94.4**
Isolate 2	2.96**	0.93**	91.2**	5.44**	0.52[N.S.]	83.0**	4.82**	0.28[N.S.]	89.6**
Isolate 1 &2	1.66	0.45	74.8	0.52	0.00	35.2	1.84	0.26	74.6

a - mean values for 5 plants

b - measured at maturity

c - isolate 1 is listed first in each combination

d - N.S.= F value not significantly different at the 5% level from the corresponding double inoculation

* = F value significant at the 5% level from the corresponding double inocuation

** = F value significant at the 1% level from the corresponding double inoculations

to those described in the preceding section, though plants were less affected by the later of the two inoculations.

Symptoms in plants doubly inoculated with isolates 6801 and 6524, or with 6524 and 6407, were more severe than in singly-inoculated control plants. The weight of the doubly-inoculated plants was consistently less than the weights for either of the singly-inoculated plants. (Table 2). This was true independently of the order in which the isolates were introduced into the plant. In most cases the differences were statistically significant. With one exception, results for seed weights in these combinations supported the results for plant weights (Table 2).

On the other hand, neither plant weight nor seed weight of plants doubly-inoculated with isolates 6801 and 6407 were significantly different from the relative weights for singly-inoculated control plants (Table 2). Plant and seed weights for doubly-inoculated plants were greater than those of either of the singly-inoculated controls when the milder isolate (6407) was used for the first inoculation, but intermediate between the controls when the more severe isolate (6801) was used first (Table 2). Symptoms in doubly- and singly-inoculated plants were of similar severity.

Table 2. Effect on Clintland Oats of Single or Double Inoculations With Paired Combinations of Barley Yellow Dwarf Virus Isolates 6801,6407, and 6524 When the Paired Inoculations were Separated by an Interval of Time.

Treatment[a]	BYDV - Isolate Combination					
	6801 / 6407		6801 / 6524		6524 / 6407	
	Mean dry weight per plant[b] g.	Mean Seed wt. per plant g.	Mean dry weight per plant g.	Mean Seed wt. per plant g.	Mean dry weight per plant g.	Mean dry weight per plant g.
Isolate 1[c] early	2.7[N.S.d.]	0.35[N.S.]	2.5**	0.15[N.S.]	9.9**	3.35**
Isolate 2 late	6.0[N.S.]	1.43**	8.4**	1.06*	7.7**	1.69**
Isolate 1 & 2	4.3	0.39	1.4	0.16	4.1	1.08
Isolate 2 early	7.1[N.S.]	1.23[N.S.]	4.7**	1.01**	5.8[N.S.]	2.40[N.S.]
Isolate 1[c] late	7.0[N.S.]	1.04[N.S.]	4.6**	0.66**	7.8**	3.06**
Isolate 1 & 2	8.2	1.38	1.7	0.15	4.4	1.65

a - late inoculation - 16 days after early inoculation

b - mean value for 5 plants

c - isolate 1 is listed first in each combination

d - N.S. = F value not sig. different at the 5% level from the corresponding double inoculation

- * = F value sig. at the 5% level from the corresponding double inoculation

- ** = F " " " " 1% " " " " " "

Double Inoculation of Plants with Isolate 6711 and 6718

Symptoms were mild in oats inoculated early with isolate 6711. Isolate 6718 caused leaf discoloration 3-4 days later on oats and stunted the plants to a greater degree than isolate 6711. Both isolates produced relatively mild symptoms in the late-inoculated control plants.

Plant weights and seed weights for doubly-inoculated plants were significantly less than the relative weights for either of the singly-inoculated controls (Table 3). This was true regardless of the order in which the isolates were used. Heights of doubly-inoculated plants were also less than those of singly-inoculated plants, though the differences were not significant.

Attempted Transmission of the Virus Isolates from Inoculated Plants

In the above experiment, BYDV was successfully transmitted to test oat seedlings from all singly- and doubly-inoculated plants that were so tested. Recharacterization tests showed that the viruses isolated were identical with those originally used. Therefore, in doubly-inoculated plants, there was no evidence of protection by one isolate against another for any combination.

Table 3. Effect on Clintland oats of single or double inoculations with barley yellow dwarf virus isolates 6718 and 6711 when the paired inoculations were separated by an interval of time.

Treatment [a]	Mean dry weight per plant[b] g.	Mean seed weight per plant g.	Mean height per plant cm.
Isolate 6718 early	5.6**[c]	1.56**	70.9[NS]
Isolate 6711 late	7.1**	1.18**	73.0[NS]
Isolate 6718 early & 6711 late	3.6	0.66	66.1
Isolate 6711 early	4.2**	0.63**	76.8[NS]
Isolate 6718 late	8.8**	2.33**	78.9[NS]
Isolate 6711 early & 6718 late	3.3	0.39	74.2

[a] the late inoculation was made 16 days after the early inoculation

[b] mean values for 10 plants

[c] NS = F value not significantly different at the 5% level from the corresponding double inoculation.
** = F value significant at the 1% level from the corresponding double inoculation.

R. padi and M. avenae always transmitted virus from plants doubly-inoculated with isolates 6407 and 6524. Further subtransfers of the virus isolated by R. padi, using R. padi and M. avenae as differential vectors, indicated the presence of the R. padi-specific isolate, 6524. Similar subtransfers of the virus isolated by M. avenae, indicated the presence of the M. avenae-specific isolate, 6407. There was no evidence for a loss of vector specificity.

R. padi and M. avenae always transmitted virus from plants doubly-inoculated with isolates 6801 and 6524. The isolate transmitted by M. avenae was 6801. Further subtransfers of the virus isolated by R. padi, using R. padi and M. avenae as differential vectors, indicated the presence of the R. padi-specific isolate 6524, though occasionally there was evidence that R. padi had transferred both isolates from the doubly-inoculated plants.

Similarly, both R. padi and M. avenae transmitted virus from plants doubly-inoculated with isolates 6801 and 6407. Again, the less selective vector, in this case M. avenae, transmitted its specific isolate 6407, more frequently than the non-specific isolate 6801.

Discussion

As a general rule, Holmes (1956) states that the occurrence of additive effects from mixed inoculation with two causal agents of disease implies that the pathogens under consideration are not closely related, and that the occurrence of intermediate effects from mixed inoculations implies that the causal agents of disease are closely related. Holmes used this "simultaneous infection test" as a basis for this general thesis on viral interrelatedness.

In the present study, when simultaneous inoculations were made with a non-specific isolate and an M. avenae-specific isolate of BYDV the disease effect in the doubly-inoculated plants was intermediate in terms of symptom severity and plant data, between that of the singly-inoculated controls. According to Holmes thesis, the above results infer relatedness between the two isolates. With similar isolates from New York, Aapola (1968) found an additive effect in Coast Black oats, doubly-inoculated with the two isolates, and a synergistic response when an R. padi-specific isolate was simultaneously inoculated into the test plants with an M. avenae or a non-specific isolate. In my tests, plants simultaneously inoculated with the R. padi-specific isolate and either the M. avenae-specific or the non-specific isolates, were also more severely affected

than plants inoculated with only one of these isolates (Table 1). Aapola (1968) suggested that there was a lack of relatedness between his R. padi-specific isolates and both an M. avenae-specific isolate and a non-specific isolate.

In the present study, the results from experiments with a delayed second inoculation generally supported the results for simultaneous inoculations. The results for plant data are similar to those obtained by Aapola (1968) in his tests with a delayed second inoculation. However, Aapola also found evidence for protection between his M. avenae and non-specific isolates. There was no evidence for protection in my study. This discrepancy could be caused by the use of different isolates. Also, Aapola used Coast Black oats as his test plants, and Clintland oats were used in my study. These factors may have had some bearing on the results of the recovery data.

Loss of vector specificity has been reported in cases where oats were doubly-inoculated with an M. avenae-specific and an R. padi-specific isolate of BYDV (Aapola 1968; Rochow 1965). This loss of vector specificity is believed to be caused by a phenotypic mixing (Rochow 1970). Toko and Bruehl (1959) found no evidence for a loss of vector specificity when an M. avenae-sepcific and an R. padi-specific isolate

of BYDV from Washington were present simultaneously in the same plant. There was no evidence of a loss of vector specificity in the present study. This phenomenon, therefore, may not occur in all cases where a plant is doubly-inoculated with an M. avenae-specific and an R. padi-specific isolate of BYDV.

There was no evidence for interference or cross-protection between the S. graminum-specific isolates used in this study. Except in the case of plant heights, the data for the doubly-inoculated treatments is significantly lower than that for the singly-inoculated controls. Both isolates were recovered from all doubly-inoculated plants tested. This may indicate that the isolates are multiplying independently in the plant and are not related (Aapola 1968; Holmes 1956; Kassanis 1963; Rochow 1965 a). Unpublished work with regard to studies on double-inoculation with an M. avenae-specific isolate (6407) and an S. graminum-specific isolate (6711) in the plant, give some evidence that these two isolates may be related. The plant data for isolates 6407 and 6711 was similar in many ways to that obtained in this study when isolate 6407 (M. avenae-specific) and 6801 (non-specific) were doubly-inoculated into the plant. As isolate 6711 is also transmitted by M. avenae and R. padi it might be termed an S. graminum-non-specific isolate, and

isolates, such as 6801, R. padi-non-specific.

Effect on Oats Doubly-Inoculated with an Additional Combination of Two Barley Yellow Dwarf Virus Isolates

Introduction

Cross-protection between a non-specific isolate and a Macrosiphum avenae-specific isolate of barley yellow dwarf virus (BYDV) had been demonstrated by several authors (Smith 1963 b; Jedlinski and Brown 1965; Aapola 1968). In the previous section of this thesis interference was demonstrated between a non-specific isolate of BYDV (6801) and an M. avenae-specific isolate (6407). Isolate 6711 is similar to isolate 6801 in that it is transmitted by Rhopalosiphum padi, M. avenae, and S. graminum, but it differs from 6801 in that S. graminum is clearly the most efficient vector (Gill 1969). Therefore, an experiment was performed in the greenhouse, similar to those in the previous section of this report, to see if any interference occurred between isolates 6407 and 6711.

Dizon (1968) found that temperature caused differences in the efficiency of transmission of BYDV by four aphid vectors. Temperature also influences the development and severity of symptoms in cereals. Thus the most pronounced symptom expression in barley occurred at low temperatures

while symptoms were mild or masked at high temperatures (Gill and Westdal 1966; Jensen 1968). To see if temperature influenced the degree or type of interference between isolates 6407 and6711 in the plant, the test was repeated in growth cabinets at 15° and 30°C.

Materials and Methods

A similar method to that used in the interference studies with isolates 6801, 6407 and 6524, was used in studies on isolates 6407 and 6711. Schizaphis graminum was used for the inoculation of isolate 6711, and Macrosiphum avenae for the inoculation of isolate 6407. Clintland oats was used as the test plant.

The first experiment was run in the greenhouse. There was a 10-day interval between the inoculations with the individual isolates and 10 plants were used per treatment. No attempts were made to isolate the virus from the inoculated plants. The weight and number of seeds per plant were recorded at maturity.

A second experiment was run at 15° and 30°C. in growth cabinets. There was a 20-day interval between the inoculations with the individual isolates, and 5 plants were used per treatment. Four weeks after the second inoculation, attempts were made to isolate the virus isolates

from one plant each of the singly-inoculated treatment, and two plants each of the doubly-inoculated treatments. The height, dry weight and seed weight per plant was recorded at maturity.

Results

Inoculation of Plants with BYDV Isolates 6407 and 6711 under Greenhouse Conditions, the Two Inoculations Separated by an Interval of Time

In the singly-inoculated control plants, symptoms of isolate 6711 appeared 3-4 days later than symptoms of isolate 6407. Although obvious symptoms for isolate 6711 were not as strong as those for isolate 6407, the effect on yield was greater for isolate 6711 than for isolate 6407 (Table 4). In the plants doubly-inoculated with isolates 6711 early and 6407 late, the symptoms in the doubly-inoculated plants were more severe than those for isolate 6711 inoculated early in the singly-inoculated controls. When isolate 6407 was inoculated early in the doubly-inoculated plants, the symptoms on these plants were no more severe than the symptoms on the plants singly-inoculated with isolate 6407 early.

Table 4 gives the mean seed number and mean seed weight per plant for the singly- and doubly-inoculated treatments for the tests performed in the greenhouse.

Table 4. Effect on Clintland Oats under Greenhouse Conditions of Single or Double Inoculations of Barley Yellow Dwarf Virus Isolates 6711 and 6407 When the Paired Inoculations Were Separated by an Interval of Time.

Treatment[a]	Plant Data[b] Mean Number of Seeds per plant	Mean Seed weight per plant
Isolate 6711 early	37.5**[c]	1.04**
Isolate 6407 late	64.6**	1.24**
Isolate 6711 & 6407	20.2	0.50
Isolate 6407 early	25.3^{NS}	0.74^{NS}
Isolate 6711 late	49.0^{NS}	0.92^{NS}
Isolate 6407 & 6711	33.9	0.70

a - late inoculation was 10 days after early inoculation

b - mean values for 10 plants

c - NS= F value at 5% not sig. from corresponding double inoculation data

\- **= F value at 1% sig. from corresponding " " "

When isolate 6711 was inoculated early and isolate 6407 inoculated late, the seed numbers and seed weights of doubly-inoculated plants were significantly lower than those of the singly-inoculated controls (Table 4). When the isolates were inoculated in the reverse order, ie., isolate 6407 early, isolate 6711 late, there were no significant differences between the seed weight and seed number of the singly-inoculated controls and the doubly-inoculated test plants.

Inoculation of Plants with BYDV Isolates 6407 and 6711 in Growth Cabinets at 15° and 30°C., the Two Inoculations Separated by an Interval of Time

Symptoms at 15°C. were more severe than symptoms at 30°C. and appeared 3-4 days later. At both temperatures, plants inoculated with isolate 6711 developed symptoms while isolate 6407 only showed visible symptoms at 15°C. At 15°C., plants inoculated with isolate 6711 developed symptoms 4-6 days later than plants inoculated with isolate 6407. At maturity, symptoms in plants doubly-inoculated with isolate 6711 early and 6407 late were no more severe than the symptoms in plants singly-inoculated early by isolate 6711.

Table 5 gives the results of attempted reisolation of virus from plants singly- and doubly-inoculated with isolates 6407 and 6711. The low transmission by M. avenae

Table 5. Tests for recovery by Macrosiphum avenae and Schizaphis graminum of two isolates of Barley Yellow Dwarf Virus inoculated singly or successively into Clintland oats.

Treatment[a]	Plant No.	Transmission of virus from plants inoculated with isolate indicated			
		15°C		30°C	
		Macrosiphum avenae	Schizaphis graminum	Macrosiphum avenae	Schizaphis graminum
Isolate 6711 early	1	3/5[b]	5/5	1/5	3/5
Isolate 6407 late	1	5/5	0/5	5/5	0/5
Isolates 6711 early and 6407 late	1	1/5	4/5	4/5	4/5
	2	3/5	3/5	3/5	4/5
Isolate 6407 early	1	5/5	0/5	0/5	0/5
Isolate 6711 late	1	0/5	0/5	0/5	4/5
Isolates 6407 early and 6711 late	1	5/5	0/5	1/5	0/5
	2	5/5	0/5	4/5	3/5

[a] late inoculation was 20 days after the early inoculation.

[b] number of plants infected of the number infested with 10 aphids each. Acquisition and inoculation feeding periods were 2 and 2 days respectively.

from the plants doubly-inoculated with isolates 6711 early and 6407 late at 15°C. appears to indicate that isolate 6407 has not been recovered. This is uncertain as further subtransfers to isolate the individual virus isolates were not attempted. In the same virus isolate combination at 30°C., it appears as if both isolates were recovered, as both M. avenae and S. graminum transmitted efficiently from the doubly-inoculated plants.

Interpretation of the results of the isolates 6407 early, 6711 late combination, was complicated by the failure to recover isolate 6711 from the singly-inoculated late control at 15°C. or isolate 6407 singly-inoculated early at 30°C. However, as symptoms of isolate 6711 were observed at 30°C. when it was inoculated late in both the singly - and doubly-inoculated plants, isolate 6407 could not have protected against isolate 6711 at 30°C. In this experiment further subtransfers were not carried out to prove identity of the isolates recovered from the doubly-inoculated plants.

At maturity, symptoms in plants doubly-inoculated with isolates 6407 early and 6711 late were more severe than the symptoms of the singly-inoculated control plants. Except for plant heights at 15°C., the plant data for the doubly-inoculated plants was consistently less than that for

the singly-inoculated plants (Table 6). In the case of plants doubly-inoculated with isolates 6711 early and 6407 late, all of the plant data except for plant heights at 30°C. was intermediate between the values for the singly-inoculated controls. Independently of the order in which the isolates were introduced into the plants, the data from the doubly-inoculated plants was not significantly different from the plant data for the corresponding early singly-inoculated controls.

Discussion

In this study, there appeared to be interference occurring between the two isolates, especially so at 15°C. Complete protection may have occurred, although incomplete recovery data makes this difficult to discern. The overall results of this study, are very similar to those obtained by Smith (1963), Jedlinski and Brown (1965), Aapola (1968) and previous results obtained in this thesis, with regard to interactions between an M. avenae-sepcific isolate, and a non-specific isolate of BYDV. The plant data from the doubly-inoculated plants was not significantly different from that of the early singly-inoculated control. The term interference, as previously described, can be applied to the results of the experiment in the greenhouse when isolate 6407 was inoculated early and isolate 6711 late and

Table 6. Effect on Clintland Oats of Single or Double Inoculations of Barley Yellow Dwarf Virus Isolates 6711 and 6407 at 15 and 30°C. When the Paired Inoculations Were Separated by an Interval of Time.

Treatment[a]	Plant Data					
	Mean dry weight[b] per plant g.		Mean Seed weight[b] per plant g.		Mean height[b] per plant cm.	
	15°	30°	15°	30°	15°	30°
6711 early	7.0[NSc]	1.5[NS]	0.7[NS]	0.0[NS]	89.5[NS]	68.0[NS]
6407 late	16.3**	2.4[NS]	3.6**	0.0[NS]	100.0[NS]	69.3*
6711 & 6407	8.8	1.9	1.7	0.0	95.0	60.7
6407 early	9.1[NS]	3.2[NS]	2.4[NS]	0.0[NS]	101.6[NS]	67.2[NS]
6711 late	15.1**	3.5[NS]	4.0*	0.3[NS]	102.0[NS]	74.8*
6407 & 6711	7.7	2.7	2.2	0.0	105.4	62.6

a - late inoculation was 20 days after early

b - mean values for 5 plants

c - NS= F value at 5% not sig. from corresponding double inoculation data

*= " " " " significant from " " " "

**= " " " 1% " " " " " "

reciprocally when these isolates were inoculated into test plants grown at 15° and 30°C. Although isolate 6711 has S. graminum as its major vector, and isolate 6801 has R. padi as its major vector, the two BYDV isolates, 6711 used in the previous section, and isolate 6801, used in the previous study, are both actually non-specific in a broad sense. Isolate 6801 could possibly be termed an R. padi-non-specific isolate and 6711 a S. graminum-non-specific isolate.

The degree of interference depends on such conditions as: time interval between inoculations, age of plant, site of inoculation, identity of isolates, plant species, and dosage of second isolate (Kassanis, 1963). Protection is, therefore, not always absolute, and partial protection is often the case (Vlinkowski and Schmelzer 1957; Munro 1955; Schmelzer et al,1960).

The virus isolates, in terms of effect on plant data, appeared to react similarly at 15°C. and 30°C., although the severity of the conditions at 30°C. affected morphology, even in the control plants. Gill and Westdal (1966) found that symptoms at 27°C. were very mild and at 32°C. were non-existent, using an M. avenae-specific isolate of BYDV on barley. In my work, the M. avenae-specific isolate (6407) did not develop symptoms on the plants at 30°C., while isolate 6711 developed distinct symptoms. However, temperature did not appear to influence possible interference in the doubly-inoculated plants.

CHAPTER IV

VIRUS - VECTOR STUDIES

Effect on Transmission of Barley Yellow Dwarf Virus by Aphids Allowed Access to Two Isolates in Succession

Introduction

Watson and Mulligan (1960 a) and Allen (1957) were not able to find any evidnece for interaction in the aphid between the BYDV isolates that they tested. Smith (1963) however, gave some evidence for what he termed cross-protection between an M. avenae-specific isolate and an R. padi-specific isolate of BYDV in the aphid vector M. avenae.

Several experiments in the present work were done with pairs of various isolates in an attempt to find evidence of interference. Experiments were performed with aphid species that did not normally transmit the isolate as well as with those that did transmit the isolate. Smith (1963) reported that he found no cross-protection in non-vectors. However, Rochow and Pang (1961) have shown that aphids can acquire strains of BYDV that they do not transmit. Since the S. graminum-specific isolates were discovered only recently (Gill 1969), several experiments were also performed with these isolates to determine if they showed any evidence of

interference in the vector with previously described isolates.

In early attempts by Watson and Mulligan (1960 a) and Allen (1957) to obtain cross-protection in the aphid, about 10 of these aphids were put on each plant for the inoculation feed after the double acquisition feeding. Unless complete protection occurred in all aphids, the resulting effects o n plant height, or whatever criterion was used in the comparison, would be highly variable. When a single plant is inoculated by several aphids feeding on two isolates in succession, some of the aphids may transmit one of the isolates. This could also result in interaction in the plant,,accounting for the variability that both Watson and Mulligan (1960 a) and Allen (1957) found. Smith (1963) modified the above procedure somewhat by giving the aphids access to both isolates in succession, then allowing them to feed singly on healthy test plants. He obtained evidence of complete protection, using symptom severity on the test plants as his criterion.

In the present study, percent transmission was used as a criterion for differentiating types of interference in the vector, since different vectors transmit different isolates of BYDV with varying efficiency (Allen 1957; Gill

1967, 1969; Rochow 1958, 1959 a).

Materials and Methods

The species of aphids used were the English grain aphid, Macrosiphum avenae (Fabricius), the cherry oat aphid, Rhopalosiphum padi (Linnaeus), and the greenbug, Schizaphis graminum (Rondani). Methods for rearing the clones were the same as those previously described (Gill 1967). Aphid controls were run periodically from these clones and they were found to be virus-free at all times.

The five barley yellow dwarf virus (BYDV) isolates used were 6407 (M. avenae-specific), 6508 (non-specific) 6524 (R. padi-specific) (Gill 1967), 6711 and 6718 (S. graminum-specific) (Gill 1969). Stocks of the virus isolates were maintained in oats, Avena byzantina K. Koch "Coast Black" in a greenhouse at 17-23°C. The isolates were transmitted to new stock plants every 4-6 weeks using the most efficient vector.

Aphids were allowed to acquire virus from leaves detached from the virus source plants and placed with one end in moist sand in a petri dish at 15°C. A split-leaf technique was used in all the experiments. For a given isolate, one half of the leaf was used for aphids that would be feeding on a total of two isolates in succession.

and the other half for aphids that would be feeding only on this isolate as a control. In all of the treatments, the acquisition feeding period was six days and consisted of two three-day feeding periods. Aphids allowed acquisition on both virus isolates fed on these in succession. Aphids allowed access to only one isolate were maintained on detached healthy leaves of Coast Black oats in petri dishes, for either the first or the second three-day feeding period. After the six-day feeding period, the aphids were moved singly to individually caged Clintland oat seedlings. After a two-day inoculation period, the aphids were killed with tetraethylpyrophosphate (TEPP) insecticide. Final readings for the number of infected test plants were made four weeks after their inoculation.

Results

Because of the number of isolates and vectors studied, only one experiment was carried out for each vector and specific combination of isolates. All interpretation of results is subject to this limitation. The results from some of the experiments are included in table 7. In the series of experiments, three types of results were observed. One of these was an addition effect, one a suppression effect, and a third, a synergistic effect.

Table 7. Effect on Transmission When Aphids Were Allowed to Feed on Paired Combinations of Five Barley Yellow Dwarf Virus Isolates.

	Transmission of BYDV Isolates by Indicated Aphid									
Treatment	6407/6508[a] Macrosiphum avenae	6407/6524 Macrosiphum avenae	6407/6524 Schizaphis graminum	6407/6711 Macrosiphum avenae	6407/6711 Schizaphis graminum	6407/6711 Rhopalosiphum padi	6508/6711 Schizaphis graminum	6524/6711 Schizaphis graminum	6524/6718 Schizaphis graminum	6524/6718 Rhopalosiphum padi
1) Isolate 1 early	10[b]	26	2	24	0	0	1	22	8	19
2) Isolate 2 late	3	0	3	2	9	4	14	4	0	0
Total of 1) and 2)	13	26	5	26	9	4	15	26	8	19
Isolates 1 early and[c] 2 late	15	30	2	27	11	2	18	21	5	12
1) Isolate 2 early	4	6	2	0	4	0	23	0	2	0
2) Isolate 1 late	13	21	0	23	0	0	0	24	1	9
Total of 1) and 2)	17	27	2	23	4	0	23	24	3	9
Isolates 2 early and 1 late	16	25	12	29	12	0	23	25	3	7

[a] - isolate 1) is listed first in each combination.

[b] - number of plants infected of 30 infested, each with a single aphid (except for the totals).

[c] - aphids were allowed to feed on the two isolates in succession.

The results were termed additive when the addition of the values for transmission of virus by aphids given an early or a late single acquisition on the virus source, was about equal to the values for transmission by aphids given an acquisition on the same two isolates in succession. Often the results were additive no matter which isolate the aphid vector was given access to first. This was the case when M. avenae was given access to isolates 6407 and 6508, and also when S. graminum was given access to isolates 6508 and 6711 (Table 7). In other cases the results were only additive when the vectors fed on the virus sources in a specific sequence. This sequence was generally the same regardless which vector fed on the isolates. For example, when both M. avenae and S. graminum were given access to isolates 6407 and 6711, additive effects were only observed when the vectors were given access to isolate 6407 first in the sequence. Similar results were observed in the vectors S. graminum and R. padi, when the aphids were given access to isolates 6718 and 6524 in that order. This so-called additive effect was the most common effect observed.

Another type of result, termed a suppression effect was observed in some of the virus isolate combinations. In this case, the addition of the values for the transmission of virus by aphids given an early or a late single

acquisition on the virus source, was greater than the values for the transmission by aphids given a n acquisition on the same two isolates in succession. This phenomenon was observed in both vectors S. graminum and R. padi, when given access to isolates 6524 and 6718 in that order (Table 7). This suppression effect was also observed when S. graminum was given access to isolates 6524 and 6711 in that order.

A third response, called a synergistic effect, was noted for certain vectors and combinations of isolates. When synergism was involved, the number of transmissions by the aphids given access to the two isolates in succession was greater than the addition of the number of transmissions by aphids in both the early and the late single acquisition controls. Synergism occurred when M. avenae was given access to isolates 6407 and 6524 in that order and when S. graminum was given access to isolates 6524 and 6407 in that order. Synergism also occurred with both S. graminum and M. avenae when they were given access to isolates 6711 and 6407 in that order.

In the total of experiments performed in attempts to find interference between BYDV isolates in the vector, a difference was noted in the ability of the aphids to transmit certain isolates when the aphids were given a

three-day holding period between the acquisition on the test plants for the inoculation feed after the acquisition feed. For isolate 6407, M. avenae, and in one case, S. graminum, transmitted virus to the test plants. These vectors transmitted virus to a greater number of plants when the aphids were held for three days on healthy plants than when they were placed on the test plants directly after the acquisition feeding (80 of 110 vs. 69 of 110). For isolate 6524, R. padi, S. graminum and M. avenae transmitted to a greater number of test plants after a three-day holding period than similar aphids placed on test seedlings with no holding period (total 72 of 200 vs. 45 of 200).

A different effect was noted for transmission of isolates 6711 and 6508. The aphids which were held for three days on healthy plants between the acquisition and inoculation feeding consistently transmitted virus to less plants than those aphids given access to the virus and then placed directly onto the test plants (71 of 325 vs. 83 of 325 for isolate 6711 and 14 of 115 vs. 20 of 115 for isolate 6508).

Discussion

The present work and that of Smith (1963) are the only experiments ofa type where single aphids were used in

studies after these aphids were given access to two isolates of BYDV in succession. Smith found no evidence of mixture or recombinations between any of the isolates tested in both vectors R. padi and M. avenae. It is possible that single aphids will not simultaneously transmit two isolates of BYDV. In the present study aphids transmitting both viruses to the test plants would obscure the actual transmission values. In spite of this factor, both synergistic and suppression effects were observed in this series of experiments as well as an addition effect.

The addition effect occurred either unilaterally or reciprocally when the aphids were allowed to feed on an M. avenae-specific isolate (6407), with either a non-specific isolate (6508), and R. padi-specific isolate (6524), or an S. graminum-specific isolate (6711) (Table 7). Smith using a similar M. avenae-specific and a non-specific isolate, has shown that protection can occur between these two isolates. The use of different isolates could account for the lack of protection observed in the present study.

The suppression effect rarely occurred in this study. Suppression indicative of cross-protection, ie. where the transmission by aphids given access to both isolates in succession was about equal to the transmission by aphids

given the corresponding early acquisition control feeding, only occurred twice, and then only unilaterally. In S. graminum, isolate 6407 appeared to protect against isolate 6524, and isolate 6524 appeared to protect against isolate 6711. A different type of suppression may have occurred between isolates 6524 and 6718. The transmission by aphids given access to both isolates was below that of the early single acquisition control. This may actually be a type of antagonism, and only noticeable because isolate 6718 causes little or no visible symptoms on Clintland oats (Gill 1969). However, why this so-called antagonism occurred only when isolate 6718 was acquired late is not known.

Another phenomenon observed in this series of experiments was the synergistic effect. This could be analogous to the effect produced in plants when an assisting virus is necessary for the multiplication of a second virus in the plant (Kassanis 1963). This effect was only observed in S. graminum (not M. avenae) when the aphids were given access to isolates 6524 and 6407 in that order. In one case, M. avenae is a non-vector for isolate 6711, and in the other case S. graminum is a non-vector for isolate 6407.

Further in-depth studies are necessary in the areas of transmission by vectors after having access to two virus

isolates in succession. This study has shown possible evidence for both a type of suppression and synergism in the vector.

The comparison of the controls in this series of experiments showed the differing ability of aphids to transmit the various isolates when placed directly on the test plants after having access to the virus source, and when held for three days between the acquisition and inoculation feeding. Rochow (1963 b) has shown that there is a latent period in the most efficient vectors of both an R. padi-specific and an M. avenae-sepcific isolate of BYDV. He found that aphids allowed acquisition periods of 12 hours or less, generally require about five days to attain maximum transmission ability. The results reported in my work with isolates 6407 (M. avenae-specific) and 6524 (R. padi-specific), support the findings of Rochow. A latent period could account for the fact that aphids transmitted less often when given access to the virus source directly before placing them on the test plants, than when they were held for three days between the acquisition and inoculation feeding periods.

Totally different results were observed in the vectors M. avenae, R. padi, S. graminum with a non-specific isolate (6508) and an S. graminum-specific isolate (6711). Both

of these isolates were transmitted by a higher percentage of aphids when these aphids were placed directly on the test plants after the acquisition feeding period, than when the inoculation feeding was delayed for three days after the acquisition feed. These results appear to indicate that the vectors of both of these isolates lose their ability to transmit these isolates very readily. Subsequent tests using serial transfers of aphids given access to isolate 6711 confirmed this observation (see next section in thesis). In these tests, virus transmission by S. graminum fell off rapidly. When the virus was acquired by nymphs, there were no transmissions by the aphids after 10 days. When virus was acquired by adults, the virus appeared to be lost even more rapidly (see Table 9). It appears that this isolate is not multiplying in the vector.

Transmission of Barley Yellow Dwarf Virus by Different Stages of the Greenbug

Introduction

Very little work has been done comparing the life stages of aphids in the transmission of isolates of barley yellow dwarf virus (BYDV). Previously Toko and Bruehl (1959) found no difference between the ability of nymphs and adults to transmit two isolates of BYDV. One

of these isolates was Macrosiphum avenae-specific, the other Rhopalosiphum padi-specific. Dizon (1968) found no difference in virus transmission efficiency by three life stages of the aphid species, Macrosiphum avenae (Fabricius), Rhopalosiphum padi (Linnaeus), Rhopalosiphum maidis (Fitch), and Schizaphis graminum (Rondani), using a non-specific isolate of BYDV. Watson and Mulligan (1960) obtained similar results using an isolate transmitted efficiently by R. padi. Sana and Shultz (1962) reported nymphs of R. fitchii (sand) to be less efficient than adults in the transmission of a "North Dakota" strain of BYDV. Gill, (1970) working with an R. maidis-specific isolate of BYDV, found that the proprotion of nymphs transmitting this isolate was consistently greater than the proportion of adults that transmitted. In the same work, Gill also found that S. graminum nymphs were more efficient than adults in transmitting an R. maidis-specific isolate.

Few authors have compared the efficiency of transmission by aphids before and after moulting. Sylvester (1949) found that the infective ability of nymphs carrying beet yellow-net virus was reduced when they moulted. Watson and Mulligan (1960 b) reported that when nymphs of R. padi passed through the final moult they lost some of their ability to transmit a BYDV isolate.

The present work reports the results of experiments with S. graminum, and a non-specific and two S. graminum-specific isolates of BYDV, in which the ability of nymphs and adults to transmit the virus by single or serial transfers was compared.

Materials and Methods

The species of aphids used were the cherry oat aphid, Rhopalosiphum padi (Linnaeus), and the greenbug, Schizaphis graminum (Rondani). Methods for rearing the clones were the same as those previously described (Gill 1967). Aphids from these colonies were tested regularly to insure that they were free from virus.

The three barley yellow dwarf virus (BYDV) isolates used were 6515 (non-specific) (Gill 1967), 6711 and 6718 (S. graminum-specific) (Gill 1969). Stocks of the virus isolates were maintained in oats, Avena byzantina K. Koch "Coast Black" in a greenhouse at 17-23°C. The isolates were transmitted to new stock plants every 4-6 weeks using the most efficient vector.

In one test with isolate 6515, virus-free nymphs and adults of S. graminum and R. padi were selected from regular aphid colonies and caged together on a single leaf of the virus source plant. After an acquisition feeding period

of three days, the stages of S. graminum and R. padi were caged separately in groups of three aphids per plant on Clintland oat seedlings grown in wooden flats of soil in the greenhouse. After three days the aphids were sprayed with tetraethylpyrophosphate (TEPP) insecticide.

In all of the experiments involving daily transfers of individuals of R. padi or S. graminum on test plants, Coast Black oats, grown two seedlings to a 5-inch pot, and inoculated at the $1\frac{1}{4}$ to $1\frac{1}{2}$ leaf stage, were used as the virus source plants 10-15 days after the inoculation. Aphid nymphs with an average age of 12 hours were derived by placing virus-free apterous adults on healthy detached oat leaves in plastic dishes at 15°C. for 24 hours. Ten-day old aphids were derived by placing 12-hour old nymphs on healthy caged Herta barley in a growth cabinet at 15°C. The mature forms were removed after 10 days. For the acquisition feeding 12-hour old nymphs and 10-day old adults were caged together on a single leaf of the virus source plant in a growth cabinet at 15°C. Aphids were removed from this leaf and caged individually on the first batch of test seedlings at the 1-2 leaf stage. After 24 hours, each aphid was transferred to a second caged test seedling at the 1-2 leaf stage, the transfer continuing until the aphid died or the experiment was concluded.

Results

Transmission of Virus Isolate 6515 by Apterous Adults of Schizaphis graminum and Rhopalosiphum padi When Transferred Daily to Successive Test Seedlings

In this trial, the serial transfer was only carried out until the 10th day. The object was to determine the pattern of transmission by S. graminum for the non-specific isolate 6515. R. padi, the most efficient vector for this isolate, was also included. All 10 adults of R. padi transmitted the isolate. Seventy-two percent of the 89 seedlings inoculated became infected. No decrease in the infection rate was apparent by the 10th day. Only 2 of 10 S. graminum adults transmitted virus. Each aphid transmitted only once, one on day 1 and the other on day 4. These results confirm the findings of Rochow (1959 b) and Dizon (1968) that R. padi adults are efficient vectors of non-specific BYDV isolates, and of Dizon (1969) that only a small proportion of S. graminum adults transmit, and then only rarely.

Transmission of Virus Isolate 6515 by Nymphs and Adults of Rhopalosiphum padi and Schizaphis graminum

The discovery by Gill (1970) that nymphs of R. maidis and S. graminum transmitted an R. maidis-specific isolate more efficiently than adults, initiated an investigation

of the comparative ability of nymphs and adults of R. padi and S. graminum to transmit the non-specific isolate 6515, tested above. In two trials with R. padi, all of 8 plants infested with nymphs and all of 9 plants infested with adults became infected. In one trial with S. graminum 12 out of 18 plants infested with nymphs and only one out of 19 plants infested with adults became infected. In the other trial, 13 out of 20 plants infested with nymphs and 5 out of 20 plants infested with adults became infected.

Transmission of Virus Isolate 6718 by Apterous Adults or Nymphs When Transferred Daily to Successive Test Seedlings

These studies were then extended to isolates for which S. graminum was the most efficient, or the sole vector. The object was to compare transmission patterns of nymphs and adults when the aphids were transferred serially on the test seedlings, and to compare the rate of transmission of adults that had fed on the plants when 10 days old, to that for adults which had fed on the source plants as young nymphs. A 4-day acquisition feed was used because a very low transmission by S. graminum adults resulted from a 2-day acquisition feed. In one trial only 1 of 10 and in another trial only 2 of 12 adults of S. graminum transmitted this isolate following a 2-day acquisition feed.

Table 8 shows the transmission pattern obtained when 12 hour-old nymphs and 10-day old apterae were allowed to feed on the source plants. Four of 15 adults and 8 of 15 nymphs transmitted virus. The four adults transmitted to 5% of the 80 seedlings inoculated, while the eight aphids that fed on the virus source as 12-hour old nymphs transmitted to 13% of the 160 plants inoculated. Four of the eight aphids matured as apterous adults and four matured as alatae. For aphids that acquired virus as nymphs, 26% of 34 plants inoculated by the nymphal stages and 11% of 116 plants inoculated by the adult stages became infected. The percent transmission for nymphs and adults of all aphids that fed on the virus source as 12-hour old nymphs including those that did not transmit, was 10%, while the percent transmission for all the aphids that fed on the virus source as 10-day old adults was 1.5%.

A second trial was run with 10 daily transfers per aphid, using ten 12-hour old nymphs and ten 10-day old adults. Five of 10 aphids that fed on the virus source as 12-hour old nymphs transmitted the virus to 18% of the 50 plants inoculated, while 2 of 10 aphids that fed on the virus source as 10-day old adults, transmitted virus to 10% of the 20 plants inoculated. Of the aphids that acquired virus

Table 8. Transmission of Barley Yellow Dwarf Virus Isolate 6718 by Individuals of Schizaphis graminum When Nymphs or Apterous Adults were Allowed to Feed on the Virus Source for 4 days and were then Transferred Daily to Successive Seedlings of Coast Black Oats.

Aphid Stage	Aphid No.	Days of transfer[1] 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Adult	1	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-
(10	2	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
day	3	P	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-
old)	4	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-
Nymph	1	-	-	-	-	x̲x	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-
(12-hr.	2	-	P	P	-	-	x̲x	-	P	-	-	-	-	-	-	-	-	-	-	-	-
old)	3	-	P	-	-	x̲	-	P	-	-	-	P	P	-	-	P	-	-	-	-	-
	4	P	P	-	P	-	x̲x	P	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	x̲x	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-
	6	-	P	-	-	x̲	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	P	P	-	x̲	P	P	-	-	-	-	P	-	-	-	-	-	-	-	-
	8	-	-	-	-	x̲	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-

1 P= plant infected
-= plant not infected
x= aphid matured as an aptera on this plant
xx= aphid matured as an alata on this plant

as nymphs, 24% of 35 plants inoculated by the nymphal stages and 10% of 10 plants inoculated by the adult stages became infected. The percent transmission for nymphs and adults of all aphids that fed on the virus source as 12-hour old nymphs was nine percent, while the percent transmission for all the aphids that fed on the virus source as 10-day old adults was two percent.

Transmission of Virus Isolate 6711 by Apterous Adults or Nymphs, When Transferred Daily to Successive Test Seedlings

The object of the three trials run with this isolate was similar to that of the tests on isolate 6718 above. Partial results from two of the trials are included in Table 9, and a summary of the results of the three trials in Table 10.

In trial 1, 5 out of 15 adults and 3 of 4 nymphs transmitted virus to at least one plant in the serial transfer. In trial 2, 2 of 8 adults and 9 of 12 nymphs transmitted the virus, while in trial 3, 3 of 10 adults and 8 of 10 nymphs transmitted the virus. Therefore, more aphids that acquired virus as nymphs transmitted (77%) than did aphids that acquired virus as adults (30%). Furthermore, aphids transmitted more frequently during serial transfers as nymphs than as adults (Table 10). Although all of the 59 aphids tested survived 20 serial transfers, none transmitted beyond the 10th day.

Table 9. Transmission of Barley Yellow Dwarf Virus 6711 by Individuals of Schizaphis graminum When Nymphs or Apterous Adults were Allowed to feed on the Virus Source for 2 days and were then Transferred Daily to Successive Seedlings of Coast Black Oats.

Trial	Aphid Stage	Aphid No.	Day of Transfer[1] 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Adult (10 - day old)	1	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		3	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		4	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		5	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Adult (10-day old)	1	-	P	-	-	P	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-
		2	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Nymph (12-hr. old)	1	-	P	P	-	-	-	x	-	-	P	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	P	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	x	P	-	-	-	-	-	-	-	-	-	-	-
		4	-	P	-	-	P	x	-	-	-	P	-	-	-	-	-	-	-	-	-	-
		5	P	P	-	P	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
		6	-	P	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
		7	-	P	P	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
		8	-	P	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
		9	-	-	-	-	P	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-

[1] P= plant infected

-= plant not infected

x= aphid matured as an aptera on this plant

xx= aphid matured as an alata on this plant

Table 10. Percent transmission of barley yellow dwarf virus isolate 6711 by nymphs and adults of Schizaphis graminum in three serial transfer experiments.

Life stage allowed aquisition to virus	Number of aphids that transmitted of number tested	Percent plants infected of those infested by Aphids that transmitted virus				All Aphids[a]
		Adults	Nymphs			
			Nymphal Stages	Adult Stages	Both Stages	
10-day old adults	10/33	8	--	--	--	3
12-hr old nymphs	20/26	--	25	4	11	8

[a] includes aphids that transmitted and those that did not transmit.

Discussion

Nymphs transmitted virus more frequently than adults when they were moved serially on test plants (Tables 8, 9, and 10). For both of the S. graminum-specific isolates tested a greater proportion of nymphs than adults of S. graminum transmitted virus. Gill (1970) obtained similar results with nymphs and adults of the aphid vectors R. maidis and S. graminum and an R. maidis-specific isolate of BYDV.

Previous to Gill's work (1970), several workers found no evidence for a difference in percent transmission between nymphs and adults of several aphid species with BYDV (Dizon 1968; Sana and Shultz 1962; Toko and Bruehl 1959). Dizon (1968) found that there was no significant difference between the ability of nymphs and adults of S. graminum, R. padi, R. maidis, and M. avenae to transmit a non-specific isolate of BYDV from New York. However, in the present study with a non-specific isolate (6515) similar to that used by Dizon, nymphs of S. graminum transmitted more efficiently than adults. Rochow (1960) found that different collections of S. graminum adults varied greatly in their ability to transmit several isolates of BYDV. It is, therefore, possible that two collections of S. graminum could vary in the relative

ability of nymphs and adults to transmit the isolates. The fact that Dizon used a different isolate of BYDV than used in the present study may also account for the difference between the results.

In the present study, there was a marked drop in percent transmission when nymphs moulted to adults for both isolates 6711 and 6718 (Tables 8 and 9). This is believed to be the first report of a profound decrease in transmission of a BYDV isolate when the nymphs moulted to adults. Similar results were recorded by Sylvester (1949) using beet yellow-net virus and the green peach aphid Myzus persicae (Sulzer). Watson and Mulligan (1960 b) reported that adults of R. padi, derived from nymphs feeding on the BYDV isolate were less efficient than adults that had fed on the same source. However, they did not compare the transmission of the nymphs before and after the moult as was done in my study. Gill (1970) compared percent transmission by nymphs of R. maidis with the transmission by adults after the moult. He found a slight increase in percent transmission from nymphs to adults (42% vs. 45%).

In my work with isolate 6718, S. graminum adults, which required virus as nymphs, were equal in efficiency to, or were more efficient than aphids which acquired virus as adults. However, with BYDV isolate 6711, adults which

acquired virus as nymphs were always less efficient vectors than aphids which acquired virus as adults. This is similar to what Watson and Mulligan (1960 a) found in a limited test with the vector R. padi.

CHAPTER V

SUMMARY AND CONCLUSIONS

Virus-host and virus-vector studies were carried out with several vectors and isolates of barley yellow dwarf virus (BYDV). Interference between certain isolates of barley yellow dwarf virus was observed in the plant and, possibly, also in the vector. Serial transfers on test plants, of aphids infected with certain isolates of BYDV, revealed differences in the pattern of transmission by nymphs and adults.

Interference studies were carried out in the plant with an M. avenae-specific (6407), an R. padi-specific (6524), and a non-sepcific (6801) isolate of BYDV from Manitoba. There was reciprocal interference between the M. avenae-specific and the non-specific isolates with both simultaneous and delayed inoculations. Although no protection was evident, the fact that interference occurred between these two isolates in the plant, suggested that they were related. There was a lack of interference in the plant between either of these isolates and an R. padi-specific isolate. The R. padi-specific was, therefore, considered to be unrelated to the M. avenae-specific and the non-specific isolates. S. graminum-specific isolates (6711 and 6718) were considered to be unrelated to each other because of a lack of interference between these two in the host plants.

In a similar study, interference between one of the S. graminum-specific isolates (6711) and the M. avenae-specific isolate (6407) suggested that they were possibly related. This interference also occurred between these isolates at 15° and 30°C., though results at 30°C. were not as clear-cut, because of the adverse effect of this temperature on the test plants.

Three types of effect were observed in the aphid vectors when they were given access to two isolates of BYDV in succession. The most common was an addition effect which possibly indicated that there was no interference between the isolates in the aphid vector. A second effect was termed a suppression effect. In this case, the number of transmissions of virus by aphids given access to the two isolates in succession was less than that obtained by adding the number of transmission from both of the corresponding controls, where aphids were given access to only one of the isolates. A third effect was termed a synergistic effect. In this case, there was a marked increase in transmission of virus by aphids given access to the two isolates in succession compared to the transmision by aphids given a single acquisition feeding period.

In single aphid transfers, an R. padi-specific isolate (6524) and an M. avenae-specific isolate (6407) were tested

with the vectors R. padi, M. avenae, and S. graminum. These isolates were transmitted to a greater number of plants if the vectors were held on healthy plant material for a period of three days between the acquisition and inoculation feeding periods, than if there was no delay between the acquisition and inoculation feeding periods. A long latent period in the vector between acquisition of the virus and ability to transmit the virus, would account for transmission being lower when the aphids were placed directly on the test plants after the acquisition feeding period on the virus source. It is also possible that the virus is multiplying in the vector.

In similar tests with the same vectors, and a non-specific (6508) and a so-called S. graminum-specific isolate (6711), an opposite effect was observed. The vectors appeared to lose virus when held for three days on healthy plant material between the acquisition and inoculation feeding periods. This was possibly due to a lack of multiplication of the virus in the vector.

A higher proportion of nymphs than adults of S. graminum transmitted two S. graminum-specific isolates (6711 and 6718) of BYDV. Nymphs also transmitted virus to a higher percentage of plants than adults when moved serially on the test plants. Nymphs of the same clone of

S. graminum also transmitted a non-specific isolate (6515) of BYDV more efficiently than adults.

With both of the S. graminum-specific isolates, aphids that required virus as nymphs usually lost their ability to transmit after the final moult. With one of these isolates (6711) adults derived from nymphs having access to the virus, were less efficient vectors than aphids which had access to the virus as adults. This confirmed an earlier finding that this isolate is rapidly lost by the vector.

SUGGESTIONS FOR FUTURE RESEARCH

Although advances have been made in recent years with regard to characterization of strains and isolates of BYDV, the mechanisms involved in multiplication in the plant, and whether or not multiplication occurs in the vector, have received little attention. As important as the study on multiplication in the vector and in the host, is the study of interactions causing different types of interference.

In conjunction with the mechanisms of the interference phenomenon and the multiplication and movement of virus in the plant and the vector, the following areas are suggested for future research:

1. Effect of varying the length of the time interval between the

inoculation of the two BYDV isolates in the plant on the degree of interference.

2. Effect of the stage of development of the leaf inoculated by the challenging virus, and degree of interference in different leaves of the same plant.

3. Further studies for relatedness between the R. padi-non-sepcific isolate (6801) and the S. graminum-non-specific isolate (6711).

4. Effect of different temperatures on interference between isolates of BYDV in the aphid vectors. Different temperatures could be used both in the acquisition and inoculation feeding period.

5. Reisolation of virus from all plants which became infected in a study on interference in the vector where either addition, suppression or synergism effects are involved. It is still not clear which isolate is being transmitted by the vector.

6. Effect of different time intervals between acquisition and inoculation feeding periods on the possible interference phenomenon in the aphid vector.

7. Compare the transmission of nymphs and adults of several aphid clones with several isolates of BYDV. These clones and isolates could come from many different areas of the world.

CHAPTER VI

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