Comparisons of Beet Yellows Virus Isolates from Arizona

E. G. RUPPEL[1]

Received for publication October 13, 1969

Beet yellows, incited by the beet yellows virus (BYV), was discovered in sugarbeet seed fields in the Salt River Valley of Arizona in 1955 (1)[2]. Monthly assays of yellowed beets, grown for sucrose production during the seasons beginning in September 1965, 1966, and 1967, indicated that the virus first appeared in the field late in the beet campaign, namely, April or May (6). At least, the disease incidence was too low to be detected earlier by the chosen method of sampling.

Since early infection with BYV induces greater reductions in yield of seed and roots (2,3), the tardy occurrence of the disease in central Arizona may restrict the crop losses due to this virus. However, severe strains of BYV possibly could induce important losses even late in the season (2). Thus, the present study was conducted to determine if strains of BYV with different degrees of virulence exist in Arizona.

Materials and Methods

Five BYV isolates, arbitrarily named A, B, C, D and E, were recovered from yellowed sugarbeets in central Arizona. The isolates were identified as BYV by symptoms in indicator hosts and by their virus-vector relationships. Isolates A and E were recovered in April 1967 and May 1968, respectively, from beets growing in experimental plots at the University of Arizona Agricultural Experiment Station in Mesa, Arizona. Isolates B and C were recovered in May 1966 from beets growing in two widely separated commercial sugarbeet fields. Isolate D was recovered from a diseased beet in a commercial field in June 1967. As determined by host reaction, all isolates have been relatively stable throughout their retention in the greenhouse; however, it is not definitely known that they represent single viruses.

Aviruliferous green peach aphids [*Myzus persicae* (Sulz.)] were allowed to feed on the leaves of diseased beets for 12 hours and then transferred to seedlings of *Chenopodium capitatum* (L.) Asch. for an inoculation feeding of 24 hours. When typical beet yellows symptoms were evident, aviruliferous aphids again were

[1] Research Plant Pathologist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Mesa, Arizona (Present address: Crops Research Laboratory, Colorado State University, Fort Collins, Colorado 80521). In cooperation with the Arizona Agricultural Experiment Station, Department of Plant Pathology, University of Arizona, Tucson; Journal article No. 1542.

[2] Numbers in parentheses refer to literature cited.

used to transfer the isolates to healthy sugarbeets where they were maintained. *C. capitatum* was used to eliminate the beet western yellows virus from possible mixed infections with BYV (5). At the beginning of the present study, the entire screening process through *C. capitatum* was repeated. The resultant infected beets, all inoculated on the same day, served as virus source plants.

Green peach aphids were reared on caged sugarbeets which were tested frequently for the presence of contaminating viruses. In routine transmissions of the BYV isolates, aphids were given a 12-hour acquisition feeding on the source plants and a 24-hour inoculation feeding on the test plants. All transmissions were carried out in insect-proof cages.

Eleven hosts of BYV were selected to test their reaction to infection with the five isolates. The hosts included *Amaranthus caudatus* L., *Beta vulgaris* L. 'Asgrow Wonder' (red table beet), *B. vulgaris* (sugarbeet hybrid F58-554 H1, supplied by R. J. Hecker), *B. vulgaris* 'S301-H' (sugarbeet, supplied by Spreckels' Sugar Company), *Celosia argentea* var. *cristata* Kuntze, *Chenopodium amaranticolor* Coste & Reyn., *C. capitatum, C. murale* L., *C. quinoa* Willd., *Nicotiana clevelandii* Gray, and *Spinacia oleracea* L. Seedlings were inoculated in groups of five with aphids that had fed on the virus source plants; 10 aphids were used per test plant. Thirty days after inoculation, each seedling was assessed an arbitrary disease index of 0 to 5 inclusive, in ascending order of severity. Presence of BYV then was ascertained by back-inoculations to *C. capitatum*.

The effect of the five BYV isolates on yield of sugarbeet was determined by inoculating seedlings ('S301-H') with aphids that had fed on the virus source plants. Seeds were planted in 6-inch pots and the seedlings were thinned to one plant per pot in the cotyledon stage. Ten replications were made in a randomized complete block design in each of two tests. In the first test, the beets were inoculated 8 weeks after planting when they had 10 to 12 leaves. In the second test, 3-week-old seedlings in the 2-leaf stage were inoculated. In both tests, the beets were harvested 60 days after inoculation and dry weights of tops and roots were determined.

Bennett (1) differentiated strain 5 of BYV from strains 2 and 4 by symptom severity in beet and by the apparent ease with which strain 5 could be transmitted by individual green peach aphids. Transmission of the Arizona isolates of BYV and their retention by individual aphids were compared by two methods. Aphids were placed on the virus source plants for a 24-hour acquisition feeding before being transferred in mass to radish

(*Raphanus sativus* L.) which is immune to BYV (1). The aphids then were transferred singly to seedlings of *C. capitatum* at feeding intervals of 0, 24, 48, 72, and 96 hours on radish. Five replications were made for each interval for each virus isolate; two trials were conducted. In the second method, aphids were given a 24-hour acquisition feeding on the source plants and then transferred singly to seedlings of *C. capitatum*. These aphids then were used singly in daily serial transfers to healthy *C. capitatum* seedlings. Five replications were made at intervals of 0, 24, 48, 72, and 96 hours after the acquisition feeding for each virus source.

The more virulent strains of BYV apparently are easier to transmit by mechanical inoculation than are the mild strains, and they also induce more local lesions in beet and *C. capitatum* (1,4). Essentially the same techniques used by Costa and Bennett (4) and Bennett (1) were employed to determine the ease of mechanical transmission of the Arizona isolates of BYV and the relative number of local lesions produced by the isolates. Briefly, *C. capitatum* plants in the 6- to 8-leaf stage were placed in subdued light in the laboratory 48 hours before inoculation. The plants then were dusted with 600-mesh silicon carbide and the inocula applied with cheesecloth pads. Inocula were prepared from infected *C. murale* plants by triturating 0.5 g of leaf tissue in 2.0 ml of 0.1 M phosphate buffer, pH 7.0, with a mortar and pestle. After a 24-hour post-inoculation period in subdued light, the plants were placed in the greenhouse. Five test plants were inoculated with each isolate. A second test was conducted in which inocula for each isolate was prepared from infected plants of *C. argentea* var. *cristata, C. murale,* and sugarbeet. Three *C. capitatum* seedlings were inoculated with each of the 15 inocula.

Striking differences in symptom severity were noted in *C. capitatum* plants inoculated with the five isolates. Therefore, tests were conducted to determine if an isolate that induced milder symptoms in this host would protect against infection by the more virulent isolates. Seedlings of *C. capitatum* were divided into six groups of five plants each. Four groups were inoculated by aphids that had had access to isolate E (mild); the remaining two groups were inoculated with isolates A and D, respectively. In 10 days, when typical yellows symptoms were evident, one group of plants infected with E was inoculated with A; another group infected with E was inoculated with D; and a third group infected with E was reinoculated with E. Additional healthy plants were inoculated with each isolate to check the infectivity of the aphids from the source plants. Two trials were made.

Plants maintained for periods of more than 1 month were given a N/10 Hoaglund's solution three times a week.

Results

Host Reactions

All isolates induced similar symptoms in the test species, and only in a few hosts were there obvious differences in disease severity (Table 1). In *C. capitatum,* isolate A and D were most severe while isolates B, C, and E induced milder symptoms. All plants inoculated with A and D were dead at the conclusion of the test. Isolate D also induced more severe symptoms in *A. caudatus* and *B. vulgaris* 'S301-H' than the other isolates; isolate A induced very mild symptoms in these hosts.

Table 1.—Reactions of 11 selected hosts to five isolates of beet yellows virus from Arizona.

Host	Severity of symptoms induced by indicated strain[1]				
	A	B	C	D	E
Amaranthus caudatus	1.3	2.5	2.5	2.7	2.0
Beta vulgaris 'Asgrow Wonder'	3.0	3.3	2.7	2.5	2.7
B. vulgaris hybrid	1.7	1.7	2.0	2.0	2.3
B. vulgaris 'S301-H'	1.3	1.3	1.7	2.7	2.3
Celosia argentea var. *cristata*	3.3	3.3	3.5	3.5	3.0
Chenapodium amaranticolor	2.0	2.0	2.0	2.0	2.0
C. capitatum	5.0	3.3	2.3	5.0	3.0
C. murale	2.0	2.0	2.0	2.0	2.0
C. quinoa	2.0	2.0	2.0	2.0	2.0
Nicotiana clevelandii	2.3	2.5	1.7	2.0	2.0
Spinacia oleracea	2.0	2.3	2.0	2.0	2.0

[1] Based on numerical system with a range from 0 to 5 inclusive, in ascending order of severity; 0 = no infection and 5 = plants dead. Means of three replications.

Effect on Yield

In Experiments 1 and 2 (Table 2), there were no significant differences between isolates of BYV as measured by dry weight of roots. The non-inoculated checks, however, significantly outyielded the inoculated beets regardless of isolate. Analysis of the data on dry weight of tops in Experiment 1 indicated that the difference in yield between the non-inoculated beets and those inoculated with isolate C was not significant; however, the checks again significantly outyielded beets inoculated with isolates A, B, D, and E. In Experiment 2, differences in dry weight of tops between the non-inoculated checks and plants inoculated with A, B, or C were not significant; however, dry weights of plants in these treatments were significantly greater than those inoculated with D or E. In both experiments, losses in dry weight of tops, based on percentage yield of the non-inoculated beets, ranged from 16 to 38%, whereas losses in roots were greater and ranged from 37 to 65%.

Table 2.—Dry weight of sugarbeets infected with five isolates of beet yellows virus; beets harvested 60 days after inoculation.[1]

Isolate	Experiment 1		Experiment 2	
	Tops g	Roots g	Tops g	Roots g
A	175.2a	38.7a	308.5a	173.6a
B	179.3a	46.7a	310.6a	149.4a
C	228.4b	63.9a	306.6a	168.4a
D	209.0a	60.8a	253.7b	102.2a
E	201.8a	52.1a	265.8b	114.3a
Check	284.2b	111.6b	366.3a	276.1b

[1] Means of ten replications; means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Virus Retention in the Aphid

Of the 75 aphids transferred directly from diseased beets to *C. capitatum* in the two retention experiments, only 32 (43%) were able to transmit the virus. The ability of single aphids to pick up and transmit the isolates varied considerably between experiments; however, 33, 0, 95, 55, and 47% of the aphids transmitted isolates A, B, C, D, and E, respectively, in the two experiments. Only one of 50 aphids was able to transmit isolate A after a post-acquisition feeding of 24 hours on an immune plant. Similarly, only one of 25 aphids retained isolate E for at least 24 hours while feeding on a host of BYV. All other attempts to transmit the isolates after various durations on radish or *C. capitatum* were negative.

Mechanical Transmission

In the first test, with infected *C. murale* as the virus source, isolate A and D induced minute local lesions in the leaves of *C. capitatum*. No quantitative differences in lesion numbers were evident. Only isolate E induced lesions in *C. capitatum* in the second test, and only when *C. murale* served as the virus source. The results of all other transmission attempts were negative, and no systemic infection occurred in any inoculated plant.

Protection Tests

Plants of *C. capitatum*, infected with the relatively milder isolate E, were not protected from the effects of infection with the more severe isolates A and D. When A and D were used as the challenging isolates after symptoms induced by E were evident, all infected plants died within 40 days. Plants only infected with E were alive at the conclusion of the tests.

Discussion

Bennett (1) divided six strains of BYV into two main groups. Three strains were placed in group 1 because: they did not induce vein-clearing on sugarbeet, they were not lethal to plants

of *C. capitatum,* and they could not be transmitted by juice inoculation. Strains placed in group 2, conversely, did produce vein-clearing, were lethal to *C. capitatum,* were more readily transmitted mechanically, and were somewhat more readily transmissible by individual aphids. All Arizona isolates of BYV used in this study produced vein-clearing in sugarbeet; however, only isolates A and D were lethal in *C. capitatum.* Thus, it appears that we must add to Bennett's system a third group for isolates that do induce vein-clearing in beet but are not lethal in *C. capitatum.* Group 3 then would incorporate Arizona isolates B, C, and E reported herein. Isolates A and D, presumably, would be relegated to group 2.

Further classification of BYV isolates from Arizona would be more difficult, because characteristics usually used to denote greater or lesser virulence followed no consistent pattern. For example, isolate A produced a lethal reaction in *C. capitatum* but very mild symptoms in *A. caudatus* and 'S301-H' sugarbeets. Isolate C was most readily transmitted by individual aphids, but induced relatively mild symptoms in most hosts. The effect of the isolates on yield also was inconsistent with other results. Although A and D were considered the most virulent isolates as determined by symptom severity in most hosts, yields of roots from beets infected with these isolates were not significantly different from those of beets inoculated with the other isolates.

Under the conditions of this study, virus retention in the vector, yield trials in the greenhouse, and juice inoculation, proved too unreliable for differentiating strains of BYV. The failure of a less virulent isolate of BYV to protect against infection by more virulent isolates agreed with results of tests conducted by Bennett (1) with California strains.

This study demonstrated that all of the BYV isolates reported herein fall into the category of the more virulent strains as defined by Bennett (1); however, differences were noted between the Arizona isolates and the California strains of BYV. Studies that include Arizona and California isolates would have to be made under the same experimental conditions to determine if such differences are real.

Although differences between isolates were mostly subtle, variant strains of the virus apparently do exist in Arizona.

Summary

Five isolates (A, B, C, D, and E) of beet yellows virus (BYV) from Arizona induced similar symptoms in 11 selected hosts of the virus. Only isolates A and D, however, induced a lethal reaction in *Chenopodium capitatum.* All isolates induced vein-clearing in sugarbeet.