Reaction of Sweet Corn to Inoculation with Barley Yellow Dwarf Virus RMV-IL

Ronald L. C. Itnyre, Cleora J. D'Arcy, and W. L. Pedersen, Department of Crop Sciences, University of Illinois, Urbana 61801; and Laura E. Sweets, Department of Plant Pathology, University of Missouri, Columbia 65211

ABSTRACT

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The reaction of five sweet corn hybrids to barley yellow dwarf virus (BYDV-RMV-IL) was determined in 1992 and 1993. In 1992, symptoms were observed in three of the five hybrids planted 20 May and four of the five hybrids planted 20 June. No symptoms were observed in hybrids planted June or July 1993. The mean virus incidences of RMV-IL determined by enzyme-linked immunosorbent assay (ELISA) in all plots for the May and June 1992 planting dates were 3.5 and 21%, respectively. The mean virus incidence for the inoculated plots for the June 1992 planting date was significantly higher than incidence for the control plots (29 versus 13%). Ear weights were significantly lower for inoculated plots than for the control plots (1.2 kg versus 1.4 kg/10 ears). The mean virus incidences of RMV-IL in all plots for the June and July 1993 planting dates were 31 and 23%, respectively. The mean virus incidence for the inoculated plots for the June 1993 planting date was significantly higher than incidence for the control plots (49 versus 14%). Plots inoculated in June 1993 also had a significantly lower ear weight than the control plots (1.8 kg versus 2.0 kg/10 ears). A range of symptoms, infection levels, and yield responses of sweet corn hybrids to BYD-RMV-IL was demonstrated in this study. Hybrid susceptibility to this strain of BYDV should be considered when planting sweet corn from middle to late June in order to limit potential yield losses.

Additional keyword: luteovirus, *Zea mays*

Barley yellow dwarf viruses (BYDVs) are members of the family *Luteoviridae* group and infect members of the Poaceae family (4). A majority of the research on BYDVs has been done on wheat, barley, and oats, with little work on the effects of BYDVs on corn (*Zea mays* L.) (8). BYDVs have been shown to infect both sweet and dent corn (1,3,11-13). Decreases in dry matter of 50%, as well as reductions in height and in digestible crude protein, have been reported in sweet corn infected by BYDVs (12). Yield losses in BYDV-infected dent corn in Europe ranged from 25 to 57% (11).

Typical symptoms of barley yellow dwarf (BYD) in corn are reddish purple or yellow leaves, fine chlorotic irregular spots, and stunting (3,6,11,13). Symptom expression depends on the cultivar or hybrid grown, the strain of BYDV, and the environment (4,8). Enzyme-linked immunosorbent assay (ELISA) is more reliable than symptoms for detecting viral infection (9,10).

BYD disease development in corn is influenced by temperature. Eweida et al. (6) reported that the corn hybrid Jubilee was most susceptible to an MAV-like strain above 20°C; conversely, the cultivar Edo was not susceptible in this temperature range. The hybrid Sundance was most susceptible to an RPV-like strain between 20 and 22°C.

Sweet corn in the U.S. Midwest is planted from early May until early July to maintain a steady supply of corn for fresh markets and processing. As a result, a particular cultivar or hybrid may be grown under a wide range of environmental conditions. The goal of our research was to determine the effect of one strain of BYDV (RMV-IL) on infection, symptomatology, and yield of five sweet corn hybrids planted early or late in the growing season.

MATERIALS AND METHODS

Five sweet corn hybrids supplied by Pillsbury/Green Giant (designated H3, H5, H10, H50, and H99) were planted in a randomized complete block with four replicates on 20 May and 20 June 1992. Plots were 2.3 m wide and 5.2 m long and consisted of three rows: an inoculated row and a noninoculated control row, separated by a single border row. Twenty plants in each inoculated row were infested on 1 July (early planting) or 15 July (late planting) with aphids (*Rhopalosiphum padi* L.) from colonies reared on barley (*Hordeum vulgare* L.) infected with barley yellow dwarf virus strain RMV-IL. Individual plants

were inoculated with approximately 18 aphids using a modified bug bazooka (2,14). Plots were sprayed 2 weeks after infestation with the insecticide dimethoate (Cygon 2E, Rigo, a.i. 23.4%) at the rate of 1.2 liters of dimethoate with 94.5 liters of water per hectare. Samples 8 to 10 cm in length were collected from leaf tips. Samples from the top leaf of 10 plants in each row were collected 31 July from the early-planted plots. Samples from the top leaf, the bottom leaf, and a middle leaf from 10 plants in each plot were collected on 4 September from the late-planted plots. Several plots had leaves collected from fewer than 10 plants due to senescence or other plant damage. Leaf samples were stored at -80°C, and each leaf was individually analyzed for RMV-IL by ELISA as described in the next paragraph. The uppermost ear from 10 plants was collected from each plot and weighed on 18 August for the early planting date and on 10 September for the late planting date. For plots with insect damage, only undamaged uppermost ears were weighed, and 10-ear values were calculated.

Individual leaf tissue collected from control and RMV-IL inoculated plots was tested for RMV using a modification of double antibody sandwich ELISA as reported by D'Arcy et al. (5). Immulon U microtiter plates were coated with 3 µg of RMV immunoglobulins per ml (Agdia, Elkhart, IN). Leaf samples were ground in 1.2 ml of phosphate-buffered saline (PBS) with a leaf sap extraction press (Erich Pollahne, Germany). Alkaline phosphatase conjugate (Agdia, Elkhart, IN) was diluted 1:400 in PBS with 0.1% nonfat dry milk. Plates were read at 405 nm with an ELISA plate reader (Dynatech MR700, Dynatech Laboratories Inc., McClean, VA). Inoculated plants were considered infected when the absorbance was greater than twice the mean absorbency of the control plants collected from the field. If a control sample was suspected of being infected with virus, it was excluded from the calculation. Data were analyzed by analysis of variance with SAS (SAS Institute, Cary, NC).

In 1993, the experimental design was modified to a split-plot factorial, with the main plots consisting of the RMV-IL and the noninoculated controls. Two rows of border were placed between the two main plots in an effort to reduce aphid spread to the noninoculated plots. Main plots were subdivided into five subplots, one for each hybrid. Subplots consisted of a single row 5.2 m long. The hybrids were planted on 7

Corresponding author: Cleora J. D'Arcy
E-mail: c-darcy@uiuc.edu

Current address of R. L. C. Itnyre: FFR Cooperative, 4112 East State Road 225, W. Lafayette, IN 47906.

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June and 7 July. Ten to 15 plants in each subplot of the RMV main plot were infested with viruliferous aphids as described previously on 9 July (early planting) and 30 July (late planting). Plots were inspected for aphids 2 weeks after infestation and treated with dimethoate as described previously. Samples from the top leaf, the bottom leaf, and a middle leaf from 10 plants in each plot were collected on 30 August for the early planting and 24 September for the late planting. Several plots had leaves collected from fewer than 10 plants due to senescence or other plant damage. The uppermost ear from 10 plants, where available, was collected from each plot and weighed on 25 August for the early planting date. A heavy corn earworm infestation damaged a large number of ears in the late-planted plots, so ear weight data were not taken.

In 1993, the three leaves collected from each plant were ground together, and a composite sample was tested to determine if the plant was infected. The ELISA procedure was the same as described above. Data were analyzed by analysis of variance with SAS (SAS Institute, Cary, NC).

RESULTS

1992. Neither hybrid nor inoculation with RMV-IL had a significant effect on either virus incidence or ear weight for the May planting. Red-purple symptoms at the leaf margins were present in three (H5, H10, and H50) of the five sweet corn hybrids in both artificially infested and naturally infected plots. Symptoms were most obvious on tips and margins of leaves located above the ear and, over time, expanded inward. The mean virus incidence for all plots as determined by ELISA was 3.5%, with a range from 0 to 40%. The mean weight of 10 ears was 1.9 kg, with a range from 1.5 to 2.5 kg. There were no hybrid $\times$ inoculation interactions for either virus incidence or yield.

In the June (late) planting, there were no significant differences among hybrids for virus incidence or yield, but there was a significant effect of the inoculation. Red-purple leaf symptoms were present in plants from four (H3, H5, H10, and H99) of the five sweet corn hybrids in both artificially infested and naturally infected plots. The mean virus incidence for virus-inoculated plots was 29%, with a range from 0 to 60%. The mean virus incidence of the noninoculated plots was significantly lower (13%), with a range from 0 to 30%. The mean weight of 10 ears for the plots inoculated with RMV-IL was 1.2 kg, with a range from 0.8 to 1.7 kg. The mean weight of 10 ears for the noninoculated plots was significantly higher at 1.4 kg, with a range from 0.8 to 2.0 kg. There were no hybrid $\times$ inoculation interactions for either virus incidence or yield.

RMV-IL was detected by ELISA in top, middle, and bottom leaves of all five hy-

brids in the June planting date (Table 1). However, no leaf position was consistently positive for virus infection, and the leaf position with the highest number of positive samples varied from hybrid to hybrid. There was a highly significant negative correlation ($R = -0.39$) between virus detection in the top and bottom leaves. The overall incidence of virus infection for each hybrid was consistently higher than the incidence at any single leaf position (Table 1).

1993. Red-purple leaf symptoms were not observed on any of the hybrids for either planting date. The mean and standard deviation of virus incidence as determined by ELISA for plots in the June planting inoculated with RMV-IL were 49 and 30%, with a range from 0 to 100%. The mean virus incidence of the non-inoculated plots was significantly lower at 14%, with a range from 0 to 50%. The mean weight of 10 ears for the virus-inoculated plots was 1.8 kg, with a range from 0.7 to 2.4 kg. The mean weight of 10 ears for the noninoculated plots was significantly higher at 2.0 kg, with a range from 0.9 to 2.9 kg. There were no hybrid $\times$ inoculation interactions for either virus incidence or yield. H5 had a significantly higher virus incidence than the other hybrids (Table 2). H3 had a significantly lower ear weight than the other hybrids (Table 2).

There were no significant main effects for either hybrid or inoculation with RMV-IL for virus incidence for the July planting. Red-purple leaf symptoms were not observed in any of the sweet corn hybrids tested. The mean virus incidence for all plots was 23%, with a range from 0 to 87%. There were no hybrid $\times$ inoculation

interactions for virus incidence. Data were not collected for ear weight due to a heavy corn earworm infestation.

DISCUSSION

The five sweet corn hybrids tested in this study exhibited a range of responses to RMV-IL in their infection level, yield response, and symptomatology. Symptom development varied from year to year and was an unreliable predictor of virus infection. Yield loss occurred and virus was detected by ELISA in symptomless plants.

In 1992, symptom development may have been influenced by temperatures in August (42 to 63 days after planting). One of the authors (L. E. Sweets) previously noted a relationship between cool temperatures in early August and development of red-purple leaves in sweet corn fields. Minimum temperatures in Urbana in August 1992, when symptoms were observed, averaged 14.5°C, 2.3°C below normal. The following year, minimum August temperatures (18.3°C) were above average, and no symptoms were seen in plots inoculated with RMV-IL.

Virus was detected twice as often and yield was 14% lower in plots inoculated with RMV-IL than in control plots of the 1992 June planting. The results for the 1993 June planting date were similar, with virus detected three and a half times as often in the RMV-IL inoculated plots and a 10% greater yield loss than in the control plots. These results were consistent across the five hybrids in this study and indicate that infection with RMV-IL reduces yields in sweet corn hybrids. This is the first report of yield loss in corn due to an RMV

Table 1. Detection of barley yellow dwarf virus RMV-IL by enzyme-linked immunosorbent assay in the top, middle, and bottom leaves from the June 1992 planting of five inoculated sweet corn hybrids at Urbana, Illinois

Hybrid	Virus incidence (%) ^a			
	Top leaf	Middle leaf	Bottom leaf	Plant ^b
H3	38	43	45	68
H5	28	13	43	70
H10	23	23	8	35
H50	48	53	38	68
H99	13	28	23	53

^a A total of 40 plants was tested, 10 from each of four replicates.

^b Percentage of plants positive for RMV-IL in at least one of the three leaves tested.

Table 2. Means and standard deviations for strain RMV-IL of barley yellow dwarf virus detected by enzyme-linked immunosorbent assay and weights of 10 ears for the June 1993 planting of five sweet corn hybrids at Urbana, Illinois

Hybrid	Virus incidence (%) ^a			Ear weight (kg)		
	Mean	SD ^b	Range	Mean	SD	Range
H3	30	33	0-100	1.2	0.3	0.7-2.0
H5	47	36	0-100	2.1	0.2	1.6-2.4
H10	31	27	0-78	2.0	0.3	1.3-2.6
H50	27	29	0-80	2.0	0.4	1.4-2.9
H99	22	18	0-50	2.2	0.2	1.8-2.6
LSD (0.05)	14			0.2		

^a A total of 40 plants was tested, 10 from each of four replicates.

^b SD = standard deviation.

strain of BYDV. Previously, an RPV-like strain was reported to cause yield loss, as well as reduced height and digestible protein in both dent and sweet corn (11,12).

BYDV-RMV-IL caused yield loss in one sweet corn hybrid (H3) when no symptoms were evident on the plants. The fact that virus incidence in H3 was no higher than in several other lines indicates that some sweet corn lines are highly susceptible to BYDV-RMV-IL. Conversely, the fact that sweet corn line H5 had a significantly higher incidence of virus but no greater yield loss than several other lines indicates that some sweet corn lines have some level of tolerance to this disease. Further work is needed to identify sweet corn lines with tolerance or resistance to BYDVs.

For all of the planting dates, there was "natural" infection of the control plots ranging from 4 to 23% incidence. A possible source of inoculum was the inoculated plots. We did attempt to minimize spread by using border rows between the inoculated and control plots and an aphicide on the inoculated plots. Another possible explanation is that both RMV-IL and its vectors arrived in the control plots naturally from alternative hosts.

Planting date greatly influenced RMV-IL infection in sweet corn and the resulting yield. The 1992 and 1993 June planting dates had higher levels of infection and yield losses. The 1992 May planting date had the lowest percent infection at 4%. Cooler night temperatures (9.4°C average minimum in May 1992 versus 16.1°C historical average May minimum) may have affected aphid populations, hybrid susceptibility, and/or virus replication. Eweida et al. (6) reported some normally resistant cultivars were susceptible to an RPV-like strain when the plants were grown at higher temperatures (18 to 22°C). Although the average temperature was high (24.4°C) for July 1993, the level of virus detection (23%) was lower than in 1992. The high level in the control plots may indicate increased aphid movement; the lower detection level may indicate decreased virus replication.

It is difficult to determine the incidence of BYDVs in corn. Symptom expression was inconsistent from 1992 to 1993 and therefore is not a reliable indicator of either infection or potential yield loss. Virus distribution has been reported to be uneven in the corn plant (7). RMV-IL was not consistently detected in any particular leaf (top, middle, or bottom); thus no individual leaf could be considered representative of the whole plant. For example, in the hybrid H3, virus was detected in 15 top leaves, 17 middle leaves, and 18 bottom leaves from the 40 plants tested. Overall, virus was detected in 27 of the 40 plants. In this example, testing of any one leaf would result in an estimation of incidence 23 to 30% lower than the incidence estimated by testing three leaves per plant. Results were similar for the other hybrids. Collecting samples from different portions of the plant helped to reduce this problem, but most likely did not eliminate it.

Since testing three leaves individually for each plant would be both time and cost prohibitive, a composite mix of the three leaves collected was tested in 1993. Previous studies in dent corn inbreds showed that a composite sample could be used to detect virus, even if only a single leaf was infected (R. L. C. Itynre, *unpublished*). This method increases the chance of detecting virus infection and is quicker and more economical.

The results of this study indicate there is the potential for yield losses in sweet corn hybrids planted from middle to late June in Illinois when infected by RMV-IL. Infection can be confirmed by ELISA, but not by symptoms. Sweet corn hybrid susceptibility to strains of BYDV needs to be studied further in order to determine which hybrids can be grown successfully where these viruses are endemic.

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