THE IMPACT OF PLANT AGE AND GENETICS ON CURLY TOP DISEASE DEVELOPMENT IN MODERN SUGARBEET VARIETIES

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ABSTRACT
Performance of current California adapted sugarbeet varieties, which have little resistance to curly top disease, caused by *Beet curly top virus* (BCTV), were compared with some of the most tolerant (Inter-mountain West adapted) and susceptible varieties available for effect of infection on disease severity and plant weight. Field studies conducted in the 1970s demonstrated that sugarbeet plants were more susceptible and losses more severe when seedlings were infected by BCTV, but less severe when plants were larger at the time of infection (Duffus and Skoyen, 1977). To evaluate more precisely the relationship between age at infection and yield loss in modern varieties which were not bred for curly top reesistance, individual sugarbeet plants were inoculated with 20 viruliferous beet leafhoppers (*Circulifer tenellus*) each, when plants had either 2, 4 or 6 true leaves, and maintained in a greenhouse for 6 weeks. When plants were inoculated at the 2 leaf stage, all varieties became severely stunted with high disease ratings and similar rates of symptom development, regardless of tolerance or susceptibility of the variety. Plants inoculated at 4 and 6 leaf stages exhibited increasing separation between tolerant and susceptible phenotypes, with highly tolerant varieties performing well with low disease ratings and slower symptom development relative to susceptible varieties. California varieties performed only slightly better than the susceptible control line, Seedex Monohikari. At the conclusion of experiments, soil was carefully removed from beet roots by washing, and total plant biomass was determined. All varieties were severely stunted when inoculated at the two leaf stage, as indicated by individual plant weight. As plants achieved larger size prior to infection, the effect of curly top on total weight was diminished. Results from greenhouse trials matched those from field trials conducted under heavy curly top pressure.

INTRODUCTION
Beet curly top virus (BCTV), and related viruses transmitted by the beet leafhopper *C. tenellus* (Baker) have caused significant problems to irrigated agriculture in the western US since 1899 (Carsner and Stahl, 1924). BCTV is known to infect a broad range of crop and weed hosts in many plant families (Bennett, 1971). The leafhopper vector also feeds and breeds on an extensive range of plants from different families (Cook, 1967), although sugarbeet is clearly its favorite crop host. *C. tenellus* transmits curtoviruses most efficiently after a 48-hour acquisition-access feed on an infected source plant, but shorter feeding times (2-20 min.) also result in a low frequency of transmission. Curtovirus transmission by the vector requires a 4 hour latent period following ingestion, and leafhoppers can inoculate healthy plants by feeding for as little as a 1 min inoculation access period. Symptoms develop in plants in a minimum of 5 days. Leafhopper vectors retain the ability to transmit curly top for days to weeks and probably for their lifetime (Bennett, 1971).

BCTV is a monopartite geminivirus and the type member of the genus *Curtovirus* within the family *Geminiviridae.* Many strains (up to 14) of BCTV were initially distinguished on the basis of differential symptomatology in sugarbeet (reviewed in Klein, 1992). Molecular characterization of BCTV in beet, demonstrated that the virus primarily existed as three strains, CFH, Worland, or California/Logan, and genotypic variants of these strains (Stenger and McMahon, 1997). Based on sequence similarity and severity on sugarbeet, the three strains are now designated as separate species with the names *Beet severe curly top virus* (BSCTV, formerly CFH), *Beet mild curly top virus* (BMCTV, formerly Worland), and *Beet curly top virus* (BCTV, formerly California/Logan) (Stenger, 1998). Studies conducted over the past two years have examined epidemiology of curtoviruses to determine if individual curtovirus species are specifically associated with certain weed hosts, as well as whether localized areas may serve as reservoirs for the more severe curtovirus species. These studies have suggested some clustering of infection centers in California, but it is not yet clear whether such pockets contribute to prevalence of specific virus species (Wintermantel et al., 2005).

The wide host range of curly top and abundance of the leafhopper vector has made managing the virus difficult. The present management strategy focuses on the use of curly top-resistant or tolerant varieties when available for a specific crop and large scale spraying of insecticides to control the leafhopper vector in its overwintering grounds (Bennett, 1971; Clark, 1995). The spraying of overwintering ground is believed to be effective in some years, but effectiveness varies considerably from year to year (Morrison, 1969). In addition, crop applied insecticides are recommended to delay infection, and early planting is recommended to allow significant growth prior to infection (Kaffka et al., 2002). Curly top-tolerant sugarbeet varieties (These are often referred to as resistant, but BCTV and other curtoviruses can replicate well in these varieties; Wintermantel unpublished) have been grown with some success in California, however, tolerant varieties do not yield as well as non-curly top tolerant varieties in the absence of curly top. The tolerance is a multigenic trait with low heritability that is very difficult to move between varieties. Field applied insecticides can delay infection when applied appropriately (Kaffka et al., 2002), however, since the vector needs only a brief feeding interval in which to introduce the virus into a healthy plant, insecticides will not block virus transmission.

OBJECTIVES

Previous studies have shown that plants infected very young are more susceptible and losses will be more severe. Losses decrease the larger the plant at the time of infection. The present study compares performance of current varieties, which have little resistance to curly top, with some of the most resistant varieties available. Although most of the resistant varieties used in this study are not adapted for growth in California, they offer a direct comparison as to how performance might be influenced by the availability of resistance, whether traditional or through genetic engineering. Through this study we were able to:

- Determine variety performance relative to known resistant and susceptible varieties under equivalent conditions, including number of virus-laden leafhoppers and plant age in a 2 month period.

- Demonstrate that greenhouse testing yields equivalent results with those obtained through field testing, while eliminating variability in inoculation as occurs in the field.
- Determine performance of current varieties grown in California with what might be possible if more resistant germplasm were available.

MATERIALS AND METHODS

Nine sugarbeet varieties were compared for curly top severity and effect of disease on plant weight in greenhouse experiments conducted over a 7 week period. Seedex Monohikari was used as the susceptible control variety, since this is one of the most susceptible varieties available. Resistant controls consisted of the standard California resistant variety USH11, as well as three curly top resistant varieties adapted for the Inter-mountain West production area, including HM Owyhee, HM PM21, and Beta 8118. These varieties were compared with the current varieties grown in California, Beta 4430, HH Phoenix, HH 142, and HH Alpine. Plants were inoculated by attaching leaf cages containing 20 virus-containing leafhoppers each, when plants had either 2, 4 or 6 true leaves. Scoring was conducted weekly using the standard curly top disease severity scale (1=earliest symptoms, 9=dead plant). At the conclusion of the experiment, 7 weeks after inoculation, plants were harvested and total plant biomass determined as an estimation of yield. Each experiment was repeated 3 times.

A large scale resistance trial was conducted during a severe curly top outbreak at the University of California West Side Research and Education Center (WSREC) in Fresno, Co. in 2001. This trial also addressed varietal performance in California. This experiment also used Seedex Monohikari as the susceptible and USH11 and HM Owyhee as resistant controls. 40 individual varieties were included in the trial. Plants were scored for disease severity 4 times over the summer using the standard curly top disease severity scale.

RESULTS & DISCUSSION

Disease Severity

Overall, resistant varieties had much lower disease severity scores than Monohikari, as well as current varieties B4430 and Phoenix. Owyhee performed the best with a mild median score of 4 when infected at the 2 leaf stage, when plants in this experiment were at their most susceptible stage. USH11, Beta 8118 and HM PM21 exhibited only slightly increased severity compared to Owyhee. When plants were inoculated at 4 and 6 leaf stages, these same varieties continued to perform well (Table 1). In contrast, the California varieties were all highly susceptible at the 2 leaf stage. With later inoculation, however, two of the newer varieties, HH 142 and Alpine performed slightly better with regard to disease severity scores than the standard California varieties (Table 1). Severity results in the greenhouse trials closely resemble those of a field trial conducted in 2001, when curly top incidence was at its highest level in over a decade (Fig. 1). In the field study, all California varieties were below the average of 40 test varieties, and some were almost identical in severity to the susceptible control.

Estimated Yield Potential by Age at Infection

At the conclusion of experiments (6 weeks after inoculation), soil was carefully removed from beet roots by washing, and total plant weight was determined. Total weight is a better estimation of yield potential under greenhouse conditions than root weight, since it is more difficult in the greenhouse to establish fertilizer regimes that enhance root rather than top growth. Figure 2 lists a comparison of total weights among varieties for plants inoculated at the 2 leaf growth stage. Clearly the greatest difference between healthy and infected beets occurs when plants are very young at the time of inoculation. At this stage, the effect of BCTV infection on sugarbeet plants is so severe that even growth of resistant varieties is severely impacted by BCTV infection, as determined by plant weight (Fig. 2). As plants achieved larger size prior to infection, the effect of curly top on weight in greenhouse tests was diminished, although the trends observed with infection at the 2 and 4 leaf stages were maintained (Fig. 2). Plants were only maintained for 7 weeks after inoculation, since prior studies have indicated that plant weights begin to be impacted by pot size after approximately two months, preventing accurate weight comparisons in plants maintained over long periods of time in pots.

Table 1: Median curly top severity scores by variety in sugarbeet plants inoculated when plants had 2, 4 and 6 true leaves[1].

Variety	Res./Susc.[2]	2 Leaf	4 Leaf	6 Leaf
SX Monohikari	VS	8[3]	7	7
USH11	R	5	5	4
HM Owyhee	VR	4	4	3
HM PM21	VR	5	3	3
B 8118	R	5	4	5
B 4430	Test	7	6	6
HH Phoenix	Test	7	6	6
HH 142	Test	7	6	5
HH Alpine	Test	7	5	4

1. Plants were inoculated using 20 virus-containing leafhoppers per leaf cage, and one leaf cage per plant.
2. Level of resistance/susceptibility of each variety. R, resistant; S, susceptible; VR, very resistant; VS, very susceptible. Test varieties are those being tested to determine level of resistance or susceptibility. Resistance of control varieties based on performance against curly top in Amalgamated Sugar/University of Idaho comparative variety trials http://www.uidaho.edu/sugarbeet/av02/02vttext1.htm. Seedex Monohikari has long been the industry standard for susceptibility. USH11 has been used for many years as a curly top severity indexing variety.
3. Score shown is the cumulative median score from two replications of the experiment (rounded to the nearest whole number) of all infected plants for each variety at 7 weeks post-inoculation.

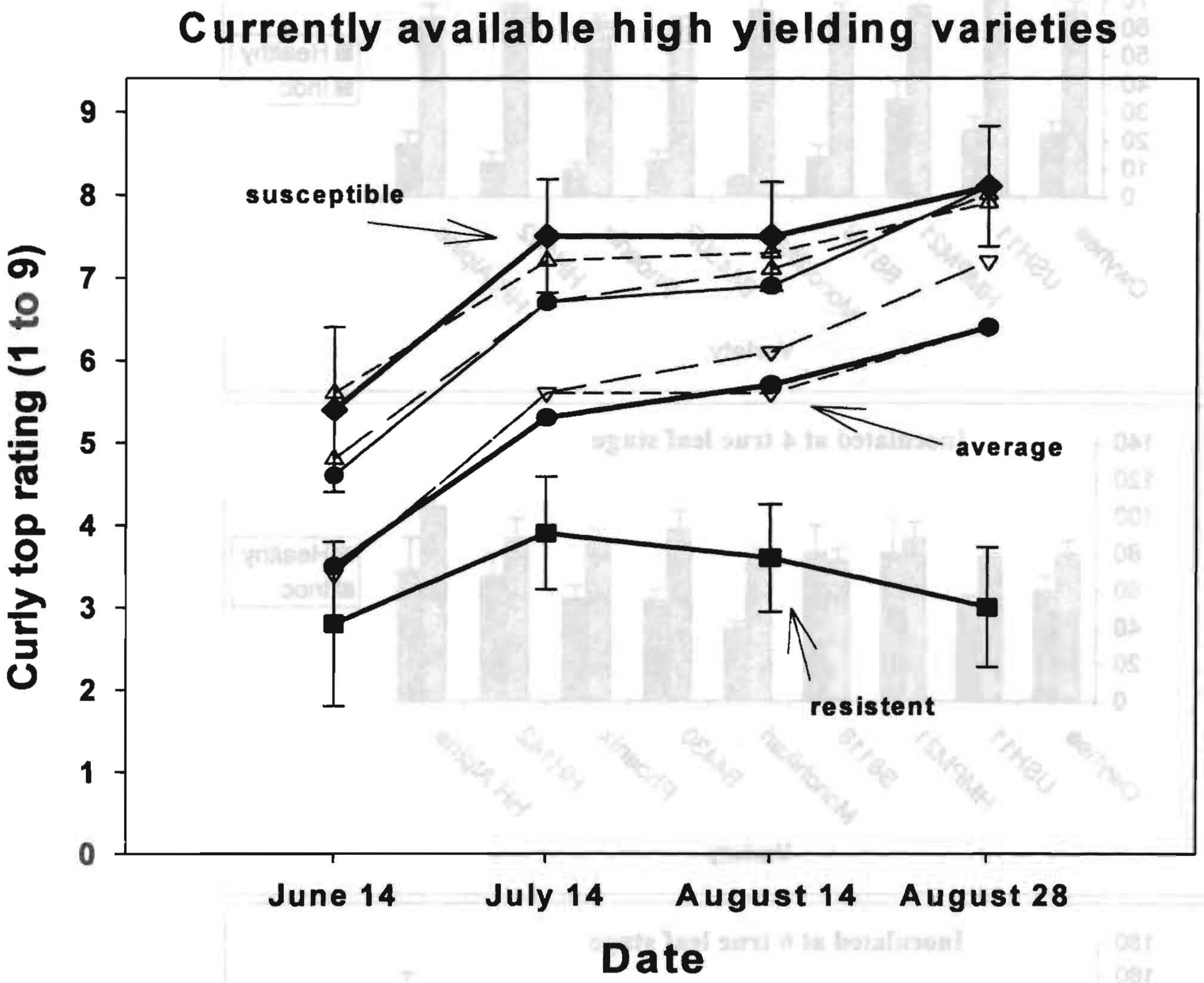

Figure 1. Disease severity comparison of current varieties of sugarbeet grown in California with industry standard susceptible and resistant varieties. Field evaluations were conducted at the WSREC in Fresno, Co, CA in 2001, under severe natural curly top pressure. Numbers on the Y (vertical) axis represent scores based on a disease severity scale. Scale: rating of 1 (earliest symptoms detected) to 9 (plant dead as a result of curly top). Variety Code: Susceptible control = Seedex Monohikari, Resistant controls = HM Owyhee and USH11 (pooled means), Average = means of 40 varieties included in trial. Dashed lines represent current varieties grown in California. Variety names are intentionally omitted from the graph.

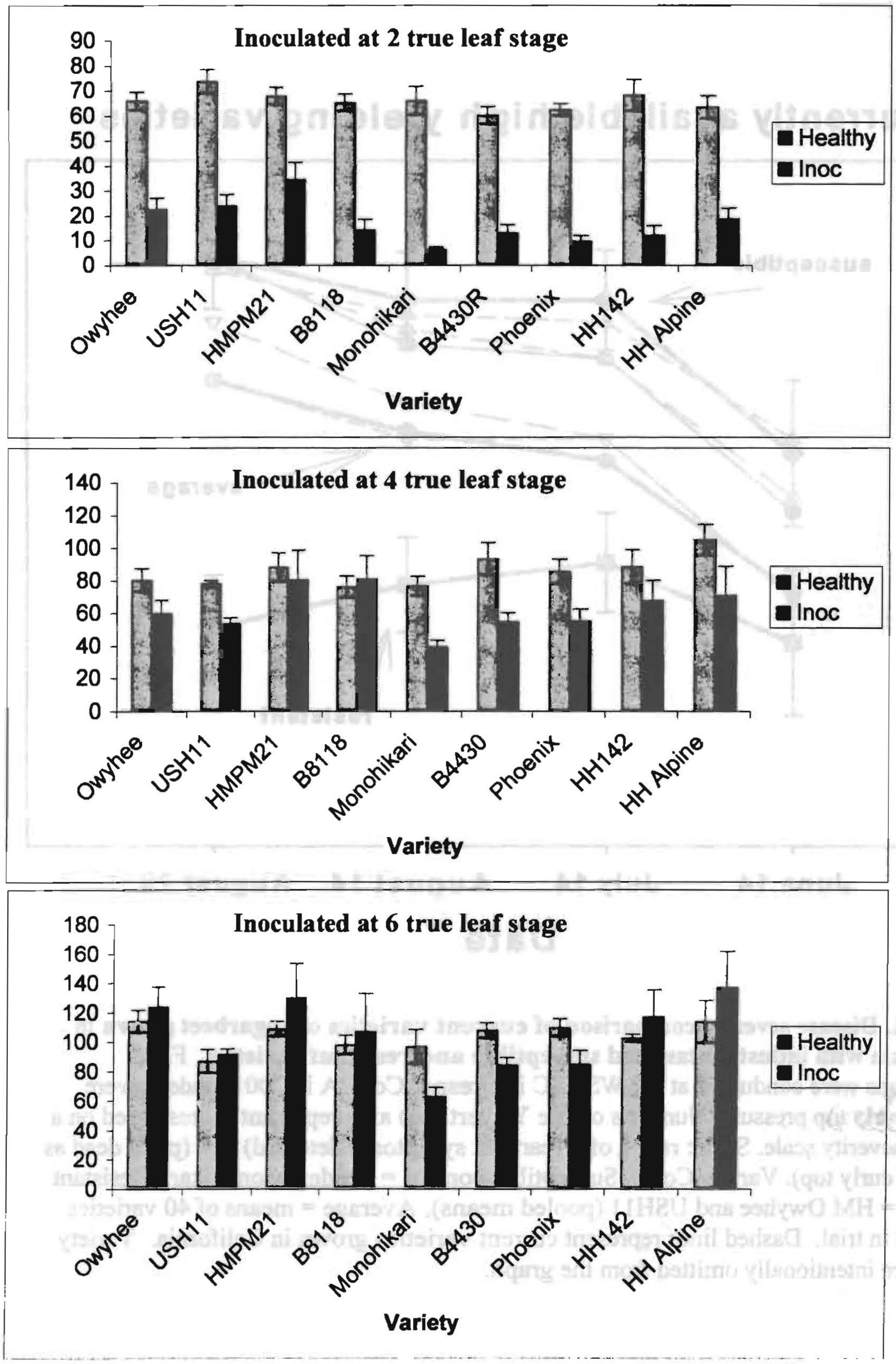

Figure 2. Mean fresh weight of sugar beet plants 7 weeks after inoculation when inoculated at either 2, 4 or 6 true leaf growth stage.

ACKNOWLEDGEMENTS
This project was supported by funds provided by the California Beet Growers Association Industry Research Committee.

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