Evaluation of *Beta corolliflora* for Resistance to Curly Top in Idaho

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ABSTRACT

Curly top of sugarbeet is caused by *Beet severe curly top virus* (BSCTV) or closely related curtovirus species which are vectored by the beet leafhopper (*Circulifer tenellus*). *Beta corolliflora*, shown in 1969 to impart a very high level of curly top resistance to sugarbeet into the BC_2 generation, is a wild relative of cultivated sugarbeet that has not been utilized in breeding programs. The nature of curly top resistance from *B. corolliflora* seems to be reduced symptoms and resistance to viral accumulation. Field screening of 14 *B. corolliflora* accessions for resistance to curly top followed by PCR detection of BSCTV did not identify any accessions with phenotypic symptoms of curly top and 9 accessions did not have detectable virus. Clip cage inoculations followed by PCR detection of BSCTV and of related species, *Beet mild curly top* and *Beet curly top viruses*, were difficult to interpret due to small sample size but indicated that accessions BETA 408, BETA 414, BETA 528, BETA 690, and BETA 805, from Genebank Gatersleben, Foundation Liebniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany had no visible curly top symptoms or evidence of virus accumulation. Results of a preference test showed that beet leafhoppers did not

have a strong aversion to *B. corolliflora* and likely would have at least sampled the plants in the field. Therefore, field screening for resistance to curly top, at least in the early generations of an introgression program, should be successful.

Additional key words: sugarbeet, curtovirus, interspecific crosses, disease resistance, beet leafhopper, *Circulifer tenellus*, insect resistance, introgressive hybridization.

The sugarbeet crop in the western United States has been afflicted with curly top (caused by *Beet severe curly top virus* (BSCTV) or closely related curtovirus species (Stenger, 1998; Strausbaugh et al., 2008)) since severe losses first were noted in Utah and California in the late 1890s (Bennett, 1971). Initially, this disease threatened to destroy the sugarbeet industry in the west. Suitable levels of resistance were identified in sugarbeet, *Beta vulgaris* ssp. *vulgaris* L., and incorporated into cultivars by the 1930s (Bennett, 1971; Blickenstaff and Traveller, 1979). However, improving, or even maintaining, resistance is difficult given the quantitative nature of the inheritance of the known sources of resistance to curly top and the necessity of combining resistance to other diseases and the yield potential needed to maintain economic viability.

Curly top of sugarbeet is vectored by the beet leafhopper (*Circulifer tenellus*), a native of the Mediterranean region, that is common in arid and semi-arid regions of the western United States (Bennett, 1971). The California Department of Food and Agriculture's Curly Top Virus Control Program works to reduce the impact of curly top in California (Summers et al., 2005; Bryant, 2008; California Department of Food and Agriculture, 2008), but their aerial spray program to reduce the number of overwintering beet leafhopper in non-crop areas (i.e., on overwintering hosts) was suspended due to permitting issues in 2007 (Bryant, 2008). The impact of the loss of this program will not be clear for several years. If the incidence and severity of curly top in California increases, the need for better resistance will increase. Seed treatment with clothianidin and related compounds has shown promise in reducing curly top (Wang et al., 1999; Strausbaugh et al., 2006); however, even when combined with the best host resistance in commercial cultivars, there is still room for improvement.

New sources of curly top resistance from *B. corolliflora*, a wild relative of sugarbeet, especially if monogenic or oligogenic in expres-

sion and inheritance, would facilitate the development of curly top resistant cultivars. The genus *Beta* is divided into four sections, Beta, Corollinae, Nanae and Procumbentes. All cultivated sugarbeet belong to *B. vulgaris* subspecies *vulgaris* in section Beta (Lange et al., 1999). Section Corollinae is more closely related to section Beta than the other sections, and hybrids have been obtained between sugarbeet and *Beta corolliflora* Zos. (Savitsky, 1969; Coons, 1975; Gao and Jung, 2002). Hybrids with *B. corolliflora* have been difficult to work with due to apomixis (asexual reproduction) from *B. corolliflora*, low germination and high levels of sterility (Gao and Jung, 2002). Sugarbeet monosomic addition lines (2n+1) having one additional chromosome of a wild species can serve as a bridge for the introgression of traits have been produced using section Procumbentes (syn. Patellares) (*B. procumbens, B. webbiana, B. patellaris)* (Löptien, 1984) and *B. corolliflora* (Gao et al., 2001; Gao and Jung, 2002). The *B. corolliflora* monosomic addition lines are fertile and segregate for the *B. corolliflora* chromosome. Additionally, two of the lines described by Gao and Jung (2002) had partial resistance to Cercospora leaf spot caused by the fungus *Cercospora beticola,* Sacc.

H. Savitsky (1969) crossed tetraploid *B. corolliflora* (2n=2x=36) to a tetraploid *B. vulgaris* (2n=36) and used the F_1 as females in backcrosses to diploid sugarbeet (2n=18). Curly top resistance was evaluated by using the BC_2 plants that were symptomless after two rounds of inoculation by viruliferous beet leafhoppers as host for non-virus containing beet leafhoppers. These leafhoppers were then transferred to a young susceptible sugarbeet to confirm that the BC_2 did not contain the virus. From 257 BC_2 plants, they found 19 immune (symptomless and no virus transmission), 82 highly immune (symptomless, but transmitted virus), 138 susceptible and 18 died of the disease. All immune or highly immune plants contained *B. corolliflora* chromosomes with a range of 2-7 chromosomes in excess of the diploid number with the majority having 22 chromosomes (most likely 2n+4) and most of the susceptible plants had 18 chromosomes (2n), indicating no *B. corolliflora* chromosomes were present. Phenotypically the BC_2 resembled the sugarbeet parent, but Savitsky (1969) did not state if the resistant plants had thick waxy leaves, which is a characteristic of *B. corolliflora*, which may have reduced beet leafhopper feeding and consequently virus transmission. Unfortunately, seed from Savitsky's lines is not known to exist (R. Lewellen, personal communication).

The nature of curly top resistance from *B. corolliflora* seems to be reduced symptoms and/or resistance to viral accumulation. A positive correlation between viral accumulation (evaluated by ELISA testing)

and symptoms (Wintermantel and Kaffka, 2006), and negative correlations between symptom and yield (Strausbaugh et al., 2007) have been reported. This type of resistance would be of great utility for breeders. Neither resistance to transmission of the virus, nor resistance to beet leafhoppers has ever been reported in sugarbeet; therefore, such a finding would be of interest on many levels.

Savitsky (1969) reported that caged beet leafhoppers could transmit the virus to BC_2 plants containing *B. corolliflora* chromosomes. However, testing of *B. corolliflora* plants and off-spring from interspecific crosses in the field in conjunction with screening of sugarbeet germplasm is a more economical method of primary screening. Beet leafhoppers have a broad host range, and sugarbeet is considered to be a preferred host, resulting in efficient reproduction and development (Munyaneza and Upton, 2005). Beet leafhoppers tend to randomly sample plants, including non-preferred hosts, and continue to change plants until they find a desirable host on which to settle and feed (Thomas, 1972; Munyaneza and Upton, 2005). Their feeding behavior allows the spread of disease to plants that are less or non- accepted hosts. If the beet leafhoppers avoided *B. corolliflora*, this would make field screening difficult, yet it would be a useful resistance mechanism. Thomas and Boll (1977) showed that beet leafhoppers are capable of transmitting BCTV to tomato, a non-acceptable host, within the first hour of feeding. Based on feeding behavior, it is unlikely that beet leafhoppers would not at least probe a *B. corolliflora* plant.

The objective of this study was to determine if *B. corolliflora* accessions had field resistance to curly top, and if so, could the virus be detected via PCR amplification of the virus species known to be in Idaho. Accessions that had field resistance were inoculated using viruliferous beet leafhoppers in clip cages. Knowledge of the mechanism(s) of resistance will help determine the potential of these accessions; therefore, a preference test was conducted to determine if non-preference for *B. corolliflora* played a role in the results of the field trial.

MATERIALS AND METHODS

Plant materials

Seed from 14 accessions of *B. corolliflora* were obtained (Table 1) from Genebank Gatersleben, Foundation Liebniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany and the USDA-ARS-Western Regional Plant Introduction Station, Pullman WA (WRPIS). The *B. corolliflora* seedballs were soaked in 10% sodium hypochlorite for 2 minutes, rinsed twice for 3 minutes each in reverse

osmosis water, then soaked in hot (just below boiling) tap water and allowed to cool and continue soaking for 24 hr. The outer corky layer of the seedball was carefully scraped off, using a dissecting microscope and scalpel, in small patches to expose the seeds. Seedballs were placed either on filter paper moistened with autoclaved water in Petri dishes, or on the surface of a Petri dish containing water agar at pH 5.8. Dishes were covered, sealed with parafilm and incubated at room temperature on the bench top. When the radicle of a seedling was greater than 3

Table 1. Plant materials. The *Beta corolliflora* seed was obtained from Genebank Gatersleben, Foundation Liebniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany and the USDA-ARS-Western Regional Plant Introduction Station, Pullman WA (WRPIS). In addition to the IPK accession numbers (BETA XXX) the accession number for the International Database for Beta, Federal Centre for Breeding Research on Cultivated Plants, BAZ, Genebank Quedlinburg, Germany is given. Dry bean seed was obtained from Dr. Shree Singh of the University of Idaho and sugarbeet seed was obtained from Betaseed, Inc., Kimberly, Idaho.

Description	Species	Treatment Code	Source
BETA 401 (Accession 58248	*B. corolliflora*	8	IPK
BETA 408 (Accession 17812)	*B. corolliflora*	1	IPK
BETA 414 (Accession 35314)	*B. corolliflora*	6	IPK
BETA 501 (Accession 17862)	*B. corolliflora*	3	IPK
BETA 528 (Accession 58239)	*B. corolliflora*	7	IPK
BETA 601 (Accession 61216)	*B. corolliflora*	11	IPK
BETA 642 (Accession 58252)	*B. corolliflora*	10	IPK
BETA 690 (Accession 17867)	*B. corolliflora*	4	IPK
BETA 755 (Accession 61227)	*B. corolliflora*	12	IPK
BETA 805 (Accession 17813)	*B. corolliflora*	2	IPK
BETA 809 (Accession 18205)	*B. corolliflora*	5	IPK
BETA 846	*B. corolliflora*	9	IPK
PI 546495	*B. corolliflora*	13	WRPIS
PI 546496	*B. corolliflora*	14	WRPIS
'Le Baron'	*Phaseolus vulgaris*	15 and less-preferred control	Univ. of Idaho
'Beta 4773 R'	*B. vulgaris* spp. *vulgaris*	16 and preferred control	Betaseed Inc.

mm long, the entire seedball was planted in moist potting media in the greenhouse. For the field screening, emerged seedlings were transplanted to small pots and grown in the greenhouse until they were transplanted by hand to the field (June 22 to June 30, 2005) when they were approximately 4-6 weeks old. Late emerging plants were transplanted to the field directly from the germination trays when they reached the two- to four- leaf stage. Field transplants were watered daily by hand for approximately two weeks. For the clip-cage inoculation, plants were transplanted to small pots and grown in the greenhouse.

Field screening

The fields were subject to natural populations of beet leafhoppers and were inoculated with viruliferous beet leafhoppers in conjunction with the Beet Sugar Development Foundation (BSDF) curly top nurseries in 2005 (Strausbaugh et al., 2007). The nursery was managed using standard production practices. The experimental unit for the *B. corolliflora* entries in the BSDF nursery was a single-row 3 m plot. The nursery was arranged in a random complete block with three replications. The *B. corolliflora* plots were placed in contiguous plots within a replication of the BSDF curly top nursery to reduce the risk that they would be hoed out as weeds. The seedlings were planted June 22-24 in replications one and two and replication three was planted between June 24 and June 30. The goal was 11 plants per plot. Plants were not available for the third replications of some accessions due to poor germination (Table 2); in these cases, all plants that survived were scored. Plants were rated for curly top on August 26, 2005 and October 7, 2005 using a 0 (= healthy plant) to 9 (= dead plant) disease index (Mumford, 1974; Strausbaugh et al., 2007) and general disease pressure across the field was determined through evaluation of susceptible checks. Leaf samples of 3-4 leaf punch discs for DNA extraction were collected from all plants on August 26, 2005 and freeze-dried. The cap of the 1.5 ml microcentrifuge tube used to hold the sample was used as a punch to collect approximately 8 mm diameter leaf discs. Only samples from replication one were used for PCR analysis. Because of the possible genetic variation within *B. corolliflora* accessions and the extreme difficulty of germinating seed, we retained the exact plants that showed field resistance to obtain seed and leaves for the beet leafhopper choice assay. Eleven plants per entry (all of replication 1 and a few plants from replication 2 where required) for a total of 154 individually identified plants, were dug up on October 7, 2005. The plants were topped and replanted in the greenhouse.

Table 2. Results of field screening and clip-cage inoculation of *Beta corolliflora* accessions for resistance to curly top. Plants in the 2005 Beet Sugar Development Foundation curly top nursery in Kimberly, Idaho were visually evaluated for symptoms on August 26, 2005 and October 7, 2005 and the number of plants with any symptoms of curly top were noted. The number of plants from the first replicate which tested positive for PCR amplification of the BSCTV strain of curly top are reported. Seedlings were inoculated with viruliferous beet leafhoppers using clip cages on June 22, 2007. Leaf samples were collected for PCR evaluation 27 days after inoculation and the plants were visually evaluated at the end of August 2007 for curly top symptoms. All PCR reactions were done in duplicate and the number of plants that tested positive at least once for a PCR assay is given.

	Field nursery				**Clip cage inoculation**				
	Visual		PCR detection		Visual		PCR detection[†]		
Accession	Total plants	Curly top	Total plants	BSCTV	Total plants	Curly top	BSCTV	BMCTV	BCTV
BETA 401	41	0	10	0	1	0	-	+	+
BETA 408	30	0	10	0	1	0	-	-	-
BETA 414	39	0	10	0	1	0	-	-	-
BETA 501	18	0	10	0	1	0	+	-	-
BETA 528	36	0	10	0	2	0	-	-	-
BETA 601	21	0	9	0	.	.	.	.	.
BETA 642	20	0	11	1	1	0	-	-	-
BETA 690	36	0	10	0	2	0	-	-	-
BETA 755	16	0	11	0	.	.	.	.	.
BETA 805	33	0	11	0	1	0	-	-	-
BETA 809	32	0	10	1	1	0	-	-	-
BETA 846	42	0	11	3	2	0	-	-	-
PI 546495	41	0	11	6	2	slight	-	-	+[‡]
PI 546496	46	0	11	11	1	0	-	-	-

[†] BSCTV = *Beet severe curly top virus*, formerly CFH strain; BMCTV = *Beet mild curly top virus*, formerly Worland strain; BCTV = *Beet curly top virus*, formerly Cal/Logan strain.

[‡] One of two plants was positive for BCTV.

PCR detection of virus

Leaf samples were disrupted with a mixer mill (Retsch MM 301, Newtown, PA) for 2 min at 30 l s^{-1} rotated 180° and ran again at 2 min at 30 l s^{-1}. DNA was extracted using Qiagen DNeasy Plant Mini Isolation Kits (Qiagen 69106, Qiagen, Valencia, CA) as per the protocol of the manufacturer with the following modifications: In steps 11 and 12, 140μl AE Buffer and 60μl AE Buffer was used, respectively. The two elutions were not mixed and the second elution was used for PCR. PCR primers for differential detection of curtovirus species BSCTV (formerly CFH strain), *Beet mild curly top virus* (BMCTV; formerly Worland strain) and *Beet curly top virus* (BCTV; formerly Cal/Logan strain) were obtained from William Wintermantel, USDA-Agricultural Research Service, Salinas, CA (Strausbaugh et al., 2008). Positive virus control samples for BSCTV and BMCTV were obtained by extracting DNA from plants infected with well characterized species maintained by W. Wintermantel, USDA-ARS, Salinas, CA. Purified plasmids containing BCTV were obtained from Dr. Wintermantel. The positive control for the *B. corolliflora* genome is described below. PCR reaction conditions were 1x PCR Buffer (Amplitaq Gold Buffer II) 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.65 U (total amount) AmpliTaq Gold DNA polymerase, 0.2 μM each primer and 60ng total template DNA. PCR reaction conditions were 95°C for 5 min.; then 95°C for 1 min, 50ºC for 1 min., 72°C for 1 min. - 35 cycles; 72°C for 10 min, soak at 4°C. MJ Research PTC 200 thermal cycler was used. Products were analyzed using 2% agarose gel with TBE buffer and stained with ethidium bromide. Each primer set was tested individually against each control virus template and B. *corolliflora* genomic DNA. No cross amplification was observed.

Plants grown in the field study were tested only with primers for BSCTV and the positive control for *Beta* genomic DNA as described below. We originally thought that only BSCTV was present naturally in southern Idaho (Stenger and McMahon, 1997). In subsequently studies, all three species of curly top were present in southern Idaho (Strausbaugh et al., 2008). Therefore, the plants subjected to the clip-cage assay, conducted later, were screened with all three species-specific markers and the positive control for *Beta* genome described below. All PCR reactions were run in duplicate.

Positive control for Beta genome

A negative result of a PCR with the viral primers could be due to inhibitors in the DNA preparation or failure of the PCR reaction; therefore, as a positive control for amplification, PCR primers to the internal transcribed spacer (ITS) and 5.8S rRNA gene of *B. corolliflora*

and *B. vulgaris* were developed (Strausbaugh et al., 2008). Primer sequences ITS_Con_293F 5'- AGCGAAATGCGATACTTGGT and ITS_Con603R 5'- GGCAACATGTTAGGGTCCTG would amplify a 313 –bp fragment from Genbank accession AY858597 (*B. corolliflora* L. 5.8S ribosomal RNA gene). The PCR amplification conditions were the same as the viral amplification.

Clip cage inoculation

Remnant seed from the 2005 field screening was utilized. Ten seeds per accession were prepared as described above but germination was very poor. Despite expectations of having 5-6 plants per accession, only one to two plants per accession were obtained for testing. Viruliferous beet leafhoppers were obtained from Terry Brown (BSDF insectary, Kimberly, Idaho). Curtovirus (species unknown) infected plants were maintained over the winter by inoculating new healthy susceptible beets using clip cages as needed. The virus infected plants were used to increase the leaf hopper population before inoculation in the spring. The 2007 BSDF curly top nursery was inoculated with beet leafhoppers from this population and the plants had curly top symptoms within 10 days and tested positive (PCR amplification) for all three virus species (data not shown). *Beta corolliflora* plants were grown to the 6-leaf stage and inoculated using clip cages containing six viruliferous beet leafhoppers, one cage per plant, on June 22, 2007. A clip cage is a spring loaded clip glued to a small tube of plastic covered at each end with insect proof mesh. The cages were removed after one week. At least three beet leafhoppers were still alive in each cage at removal. On July 19, 2007, two leaf discs were removed from the plants and DNA was extracted as described above. The plants were grown in the greenhouse through the end of August 2007. All samples were tested for the presence of all three virus species and the positive control for the Beta genome in duplicate.

Leafhopper Choice Assay

The test was conducted in June 2006. Adult beet leafhoppers were obtained each morning from the BSDF insectary in Kimberly, Idaho. They were stored in cages with fresh beet leaves until they were used later that day. The experimental design was a balanced incomplete block with four incomplete blocks, 16 treatments and 30 replications. Each 15.24 cm diameter Petri dish was an incomplete block test unit containing four treatments and the preferred and less-preferred controls. There were 120 plates in the experiment and each treatment was present 30 times. The treatments in the study were the 14 accessions of *B. corollif-*

lora that had been used in the field trials, and the 'cultivated treatments' - sugarbeet and dry bean (*Phaseolus vulgaris* L.). The treatments and controls are listed in Table 1. Sugarbeet and bean were used as a treatment as well as a control in order to always provide the beet leafhopper with a choice between a preferred and less-preferred host, respectively. The Petri dish contained a damp filter paper marked with pencil into six equal sections. No free water was on the filters so that the beet leafhoppers would not adhere to the paper. The leaf samples were 1.905 cm freshly cut discs of leaf tissue which were placed adaxial side up evenly spaced around the perimeter of the plate. Leaves were collected in the morning, rinsed twice in distilled water and blotted dry. The discs were cut on clean paper towels using a punch. Towels were changed between samples, and the punch was washed in water containing a small amount of detergent and rinsed in tap water between entries.

Leaf samples were obtained from *B. corolliflora* plants that were over a year old. These had been grown in the field the previous year in the curly top nursery (see Field Screening), dug up and maintained without vernalization in the ARS greenhouse over the winter. Plants had been treated regularly with Safer insecticidal soap (Safer Inc., Newton, MA) (applied until run off; 2% potassium salts of fatty acids), Microthiol Disperss (Elf Atochem North America Inc., Philadelphia, PA) (4.74 kg/ha; 80% sulfur,) and Marathon 1% Granular (Olympic Horticultural Products, Mainland, PA) (2 g/pot; 1% imidacloprid) to control powdery mildew and insects. The last application of Marathon was over one month from the time of the trial, so it was not expected to have an effect on beet leafhopper behavior. The *B. vulgaris* (preferred control) was Beta 4773 R which was grown in a greenhouse and had been treated with Talstar (9.24 ml liter^{-1} water; 7.9% bifenthrin) to control thrips. A preliminary test showed that the newer inner leaves of these plants would not kill beet leafhoppers (data not shown); therefore, we used only the inner leaves for our assay. The dry bean cultivar, 'LeBaron', was grown in a growth chamber as a less-preferred control. Munyaneza and Upton (2005) reported that in caged plant studies beet leafhoppers initially showed no preference for various hosts and then later in the study they settled significantly less often on dry bean, *P. vulgaris* 'LeBaron' (56%), than on sugar beet, *B. vulgaris* 'Saccherifera' (100%) after the first 24 hr.

The containers of beet leafhoppers were put into a cold room at 6ºC for 5-10 minutes to immobilize them for easier handling, after which, they were transferred to Petri dishes containing damp filter paper. The dishes were allowed to sit for 1 hr at room temperature to starve the beet leafhoppers to ensure feeding once exposed to beet. The beet leafhop-

pers were then put into a freezer for 1-2 min to immobilize them and a homemade aspirator was used to transfer individual beet leafhoppers to the center of the Petri dish. Leaf tissue had been placed in dishes and dishes were quickly closed and put on a cart under black plastic to retain humidity in the growth chamber (22ºC, 50% relative humidity, 16 hr light/ 8 hr dark). The growth chamber program was set so that the lights went off after the 6 hr data was collected and turned on before the 19 hr data was collected.

Location of each beet leafhopper was noted at 1, 2, 3, 6, 19 and 24 hr as either on a leaf, hiding under a leaf or 'other'. A beet leafhopper was deemed to have made a 'choice' if it was resting on a leaf with its feet on the leaf surface, either top or bottom. We were not able from this method to determine if the hoppers actually fed on the leaves.

Results were pooled for the *B. corolliflora* treatments in order to compare the wild species to the known hosts of the beet leafhopper, i.e. the commercial sugarbeet and dry bean. The ratio tested was a comparison of the expected number of beet leafhoppers to settle on *B. corolliflora* (treatments 1-14), the cultivated treatments (treatments 15 and 16), and the controls. If the beet leafhoppers had selected randomly the expected percentages would be 58.3 on the *B. corolliflora* : 8.3 on the cultivated treatments: 16.7 on the less-preferred control : 16.7 on the preferred control. This is the expected ratio used for a second Chi-square goodness-of-fit test.

RESULTS

Field screening and PCR evaluation

No curly top symptoms were visible on either the August or October rating date. Plants that were later determined to have detectable virus did not have obvious curly top symptoms. Using PCR, all samples were positive for the *B. corolliflora* genome control but only five accessions were positive for BSCTV (Table 2).

Clip-cage inoculation and PCR evaluation

Due to poor germination of remnant seed, only one or two plants per accession were available for this study (Table 2). The sixteen plants screened exhibited no obvious symptoms of curly top through the end of August, 2007. Sugarbeet plants in the 2007 BSDF Curly Top nursery inoculated with beet leafhoppers from the same source had symptoms within 10 days of inoculation, and tested positive for all three curly top virus species (data not shown). The PCR positive control for the *Beta* genome was amplified from all templates, which indicated that all DNA

samples were amplifiable and that no inhibitors interfered with the reactions. Only 5 of 14 accessions had detectable BSCTV in the field trial (Table 2). Four of these five accessions were negative for all strains of virus in the cage assay. Only three accessions were positive for any virus strain in the clip cage assay. All three curly top species were detected and one accession was positive for BMCTV and BCTV species. This indicates that screening procedures need to test for the presence of all three virus species. We were not able to quantify the amount of virus accumulation in the plants, nor to compare the viral load in these plants to a susceptible or moderately resistant sugarbeet. Therefore, it is not known if resistance involves suppressed accumulation of virus and/or lack of symptom development. These plants did not show clear symptoms of the disease even when they had detectable levels of virus.

No virus was detected in the field study and the clip cage inoculation study for accessions BETA 408, BETA 414, BETA 528, BETA 690, and BETA 805.

Leaf Choice Assay

The beet leafhoppers tended to hop immediately upon placement in the dish and then they did not move frequently. After the dish was closed, a hopper would not move in response to lifting, moving, or gently tapping the dish. Covering or uncovering the plates with a black plastic did not elicit movement of the hoppers. After 24 hr, two beet leafhoppers had died before making any choice (observed settled on a leaf disc) and 64% (76/118) of the remaining beet leafhoppers made a choice at some point during the test period. At any one time point the percentage of beet leafhoppers that chose a leaf disc ranged from a low of 28% (1 hr) to a high of 47% (24 hr). Of the beet leafhoppers that made a choice, 22% (17/76) switched their choice at least once during the trial. Mortality at the end of the experiment was 4% (5/120).

We could not determine if beet leafhoppers had preferences among the treatments because the counts in each cell for each observation point when each treatment was considered separately were too low for a Chi-square goodness-of-fit test to be valid (data not shown). The count data did not indicate a preference for the sugarbeet treatment, though at 24 hr it had the highest count (data not shown). Some *B. corolliflora* treatments had consistently higher counts across the observation period and others had consistently lower counts, which may indicate that preferences exist (data not shown). The results of testing the ratio of counts of settling behavior for pooled treatments using Chi-square goodness-of-fit are presented in Table 3. The ratio tested was a comparison of the expected

Table 3. Chi-square for goodness-of-fit test for ratio of counts (number of insects settled on a leaf disc) assuming that the leafhopper randomly chose the leaf on which it settled. The counts for the treatments (Trt) were pooled to compare the *B. corolliflora* treatments (1-14) against the known host treatments (15 and 16) and the controls.

	Observed counts				**Expected ratio**				
Time of Observation	Trt 1-14	Trt 15-16	Less-preferred Control	Preferred Control	Trt 1-14	Trt 15-16	Less-preferred Control	Preferred Control	Chi-square[†]
Hour 1	20	1	10	2	19	3	6	6	7.05
Hour 2	24	2	11	3	23	3	7	7	5.38
Hour 3	23	2	11	1	22	3	6	6	8.59*
Hour 6	29	2	7	5	25	4	7	7	1.97
Hour 19	34	10	5	2	30	4	9	9	14.80*
Hour 24	40	8	6	2	33	5	9	9	10.98*

[†] Numbers marked with '*' do not fit the expected ratio at p= 0.05, *d.f.*=3

number of beet leafhoppers to settle on *B. corolliflora* (treatments 1-14), the cultivated treatments (treatments 15 and 16), and the controls. The ratio did not deviate from expectations for observations at 1 hr, 2 hr, and 6 hr, which indicates random selection by the insect. Observations at 3 hr, 19 hr and 24 hr are significantly different from random.

DISCUSSION

Over 70 years of breeding for resistance to curly top has produced cultivars with moderate levels of resistance which have sustained sugarbeet production in areas where the disease is endemic. However, curly top is a continuing major problem that reduces yield (Strausbaugh et al., 2007). Improving, or even maintaining, curly top resistance is difficult given the quantitative nature of the inheritance of resistance and the necessity of combining it with resistance to other diseases and other traits needed to maintain economic viability. The possibility of obtaining a high level of resistance to curly top from *B. corolliflora* is attractive to breeders.

In visual inspections, PCR evaluations of young leaves under field conditions, and in clip cage assays, accessions BETA 408, BETA 414, BETA 528, BETA 690, and BETA 805 showed no virus symptoms and PCR results were negative. Due to the small sample in the clip cage assay it is possible that these accessions may have shown evidence of virus accumulation if more plants had been used. These results are consistent with those of Savitsky (1969) who showed that BC_2 plants (usually 2n+4) appeared to have a very high level of resistance, if not immunity, to curly top. These accessions would be good parents to use to introgress this trait into sugarbeet. BETA 408, also known as accession 17813 (Table 1) is considered to be the international standard for molecular genetic analysis (Federal Centre for Breeding Research on Cultivated Plants (BAZ), 2007).

The lack of correlation between the clip cage assay and field trial results may be due to low numbers of samples in the clip cage test, the nature of resistance in *B. corolliflora*, and the test procedure. Escapes in the field were expected. It was not expected that four of the five accessions which had virus infected plants in the field, did not show virus accumulation in the clip cage assay. This may be due to the short period of time between inoculation and PCR evaluation in the assay. For example, if reduction in accumulation of virus is the mechanism, then a longer period of time may be required for the virus to reach a detectable level. We used 4 to 6 leaf plants for the clip cage assay because inoculation of young sugarbeet is very efficient. It is not known if the

age at innoculation, leaf morphology or epicuticular leaf wax composition of *B. corolliflora* affects infectivity and development of symptoms. It has been shown that epicuticular leaf wax is a factor in insect resistance in species as diverse as azalea (*Rhododendron* sp. L)(Chappell and Robacker, 2006), cabbage (Brassica oleracea var. capitata) (Eigenbrode et al., 1991), pea (*Pisum sativum* L.) (White and Eigenbrode, 2000) and poplar hybrids (*Populus* sp.)(Alfaro-Tapia et al., 2007). Savitsky (1969) used a much higher number of beet leafhoppers and a longer exposure period to the vector than is normally necessary to transfer the virus in her study, which indicates that resistance to initial infection may be a possible mechanism of resistance.

The low level of homology between the sugarbeet and *B. corolliflora* genomes is evidenced in the F_1 hybrids by low chromosome pairing during meiosis I (4 of 70 pollen mother cell had one or two trivalents) and the loss of wild chromosomes with each back cross (Savitsky, 1969). However, Savitsky (1969) found that irregularities, such as chromosome breakage and closed ring formation, were minimal during meiosis and the BC_1 and BC_2 were fertile and vigorous. There is no evidence for gene transfer from Corollinae to *B. vulgaris* through natural recombination (Gao et al., 2001; Gao and Jung, 2002), which will complicate introgression of new resistance genes in to the sugarbeet genome. Molecular tools that were not available in 1969 can now be used to increase the efficiency of breeding by using molecular markers to detect wild chromosomes in a sugarbeet background. A species specific set of highly repetitive DNA sequences which can detect and in some cases differentiate among *B. corolliflora* chromosomes in a sugar beet background have been developed and shown to be useful in squash dot screening (Gao et al., 2000; Gao et al., 2001; Gao and Jung, 2002). This will facilitate screening for plants with wild beet chromosomes in a backcrossing program. Molecular markers are not abundant for the sugarbeet genome, but work is continuing to increase genomic coverage of maps made with publicly available markers (McGrath et al., 2007). Sugarbeet markers that differentiate the two genomes need to be established, since detecting rare recombinants between the genomes will be critical to stabilize the trait. Fluorescent in-situ hybridization (FISH), which allows the visualization of recombinant chromosomes, could also be used to increase efficiency of selection. Apomixis, which is common in *B. corolliflora*, is localized to chromosome 9 (Ge et al., 2007) and should not be a large problem unless resistance is located on the same chromosome. The gene or genes underlying this trait, such as an R gene, may function when transformed into sugarbeet, or native genes in sugarbeet may be modified based on information from *B. corolliflora* to create resistance or tolerance which

would overcome the problem of finding rare recombinants between the *B. corolliflora* and *B. vulgaris* genomes.

The nature of the resistance is of interest to plant breeders. The results of the insect choice assay did not allow us to differentiate between feeding on a host and merely testing the suitability of a potential host. Because a large percentage of the insects were not observed to settle on a leaf disc, this may not be the best assay to determine their preferences. Observations at 1, 2, and 3 hr showed an excess of settling on the less-preferred control (bean) though only 3 hr ratio of counts significantly deviated from the expectation of randomness. The bean plants were grown in the growth chamber and their leaves were thinner and softer than the other leaf samples, which may explain the initial preference of the beet leafhopper for them. As the experiment progressed, the bean leaves became flaccid whereas the other treatments remained turgid, which may explain why they lost their appeal later in the experiment. Later time points (19 hr and 24 hr) indicate that the insects favored the *B. corolliflora* and pooled cultivated treatments 15 and 16. It is not clear why the cultivated treatments would have an excess number of counts, and the less preferred and preferred controls have a deficit. Additionally, starving the beet leafhoppers for a greater period of time may have increased settling behavior.

It was clear that the beet leafhopper did not have a strong aversion to *B. corolliflora* and would most likely have at least sampled the plants in the field. Field screening using PCR identified 5 of 14 accessions as susceptible, which indicates that field screening is acceptable, at least in early generations. At least half of the beet leafhoppers survived a week in the clip cage assay which indicates *B. corolliflora* may be a poor host. A mortality rate of 50% after one week for confined beet leafhoppers is similar to results for tomato and dry beans, which are poor hosts (Munyaneza and Upton, 2005). The sample size was small; therefore, more studies are needed to confirm this observation. Unlike sugarbeet, dry bean, or tomato, *B. corolliflora* has very thick waxy leaves which may have contributed to the lack of transmission of the virus. It is unlikely that the very high levels of resistance found in the BC_2 by Savitsky (1969) can be mainly attributed to leaf morphology. The possibility that *B. corolliflora* may also reduce reproductive success of the beet leafhopper has not been ruled out and may warrant further investigation. The lack of curly top symptoms and virus accumulation in the field trial and clip cage assay are likely due to resistance to transmission of the virus and repressed viral accumulation, rather than non-preference for the host. Even if the level of resistance from *B. corolliflora* is reduced through introgression, it potentially is monogenic, and therefore easier to use for breeding than the current sources of resistance.

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