

Length of Efficacy for Control of Curly Top in Sugar Beet With Seed and Foliar Insecticides

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Abstract

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Curly top in sugar beet caused by *Beet curly top virus* (BCTV) is an important yield-limiting disease that can be reduced via neonicotinoid and pyrethroid insecticides. The length of efficacy of these insecticides is poorly understood; therefore, field experiments were conducted with the seed treatment Poncho Beta (clothianidin at 60 g a.i. + beta-cyfluthrin at 8 g a.i. per 100,000 seed) and foliar treatment Asana (esfenvalerate at 55.48 g a.i./ha). A series of four experiments at different locations in the same field were conducted in 2014 and repeated in a neighboring field in 2015, with four treatments (untreated check, Poncho Beta, Asana, and Poncho Beta + Asana) which were arranged in a randomized complete block design with eight replications. To evaluate efficacy, viruliferous (contain BCTV strains) beet leafhoppers were released 8, 9, 10, or 11 weeks after planting for each experiment, which corresponded to 1, 2, 3, and 4 weeks after Asana application. Over both years, in 30 of 32 observation dates for treatments with

Poncho Beta and 14 of 16 observation dates for Asana, visual curly top ratings decreased an average of 41 and 24%, respectively, with insecticide treatments compared with the untreated check. Over both years, in eight of eight experiments for treatments with Poncho Beta and six of eight experiments for Asana, root yields increased an average of 39 and 32%, respectively, with treatment compared with the untreated check. Over both years, the Poncho Beta treatments increased estimated recoverable sucrose (ERS) yield by 75% compared with the untreated check for weeks 8 and 9. By week 10, only the Poncho Beta + Asana treatment led to increases in ERS in both years, while the influence of increasing host resistance may have made other treatments more difficult to separate. When considering curly top symptoms, root yield, and ERS among all weeks and years, there was a tendency for the insecticides in the Poncho Beta + Asana treatment to complement each other to improve efficacy.

Curly top is an important yield-limiting disease problem caused by a number of *Beet curly top virus* (BCTV) strains in semiarid sugar beet production areas (Bennett 1971; Gharouni Kardani et al. 2013; Harveson 2015; Stenger and McMahon 1997; Strausbaugh et al. 2008b; Varsani et al. 2014; Yazdi et al. 2008). BCTV is vectored by the beet leafhopper, *Circulifer tenellus* Baker (Hemiptera: Cicadellidae), which moves into crop production areas from desert areas when the weed hosts begin to desiccate (Bennett 1971; Blickenstaff and Traveller 1979; Creamer et al. 1996). If plants desiccate in early spring from lack of rainfall, the early migration into sugar beet fields will lead to greater yield loss because plants will become infected at an earlier growth stage (Duffus and Skoyen 1977; Ritenour et al. 1970; Wintermantel and Kaffka 2006). Yield loss from curly top was so great in the 1920s and early 1930s that sugar factories were not operated during some years (Bennett 1971; Panella et al. 2014). By the mid-1930s, the first resistant sugar beet cultivars became available to sugar beet growers (Bennett 1971). However, curly top resistance in sugar beet is quantitatively inherited and, therefore, difficult to maintain in parental lines used to make hybrid crosses for commercial seed (Gillen et al. 2008; Kaffka et al. 2002; Panella et al. 2014). Thus, most sugar beet commercial cultivars today only have

low to moderate resistance to curly top (Strausbaugh et al. 2007). The low resistance is particularly evident in specialty cultivars focused on other traits (ex. nematode and Rhizoctonia root rot resistance) or cultivars rapidly brought into production such as rhizomania-resistant Idaho cultivars in 1996 or glyphosate-resistant cultivars in 2008 (Panella et al. 2014). In the future, genetic engineering may also offer opportunities for controlling BCTV in sugar beet (Aregger et al. 2012; Golenberg et al. 2009; Horn et al. 2011; Ji et al. 2015; Lee et al. 2013; Sahu and Prasad 2015). However, until transgenic virus resistance or better host resistance becomes commercially available, additional management options are needed for the control of curly top in sugar beet in semiarid production areas.

In order to supplement host resistance, the use of in-furrow, foliar, and seed-treatment insecticides has also been investigated (Kaffka et al. 2002; Malm and Finkner 1968; Mumford and Griffin 1973; Ritenour et al. 1970; Strausbaugh et al. 2006, 2008a, 2010a,b, 2012, 2014; Wang et al. 1999). The best supplement to host resistance has been the neonicotinoid seed treatments Poncho and NipsIt (clothianidin at 60 g a.i. per 100,000 seed) and Cruiser (thiamethoxam at 60 g a.i. per 100,000 seed), because they can increase yields 17% or more in commercial fields with moderate to severe curly top pressure (Strausbaugh et al. 2006, 2012, 2014). Recently, the use of pyrethroid foliar insecticides Asana (esfenvalerate at 55.48 g a.i./ha) and Mustang (zeta-cypermethrin at 56 g a.i./ha) were shown to be an effective means to supplement the early- to midseason control provided by the seed treatments (Strausbaugh et al. 2014). Earlier investigations suggested that the neonicotinoid seed treatments were able to protect sugar beet plants from curly top via control of the beet leafhopper for at least the first 55 to 59 days of the growing season (Strausbaugh et al. 2012, 2014). However, the length of efficacy against the beet leafhopper in sugar beet of both the neonicotinoid seed and pyrethroid foliar treatments has not been thoroughly investigated. Thus, the present study was conducted to provide more information on the length of efficacy of the neonicotinoid seed and pyrethroid foliar treatments to aid sugar beet management decisions. The change in host resistance to curly top

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in the field with a susceptible commercial sugar beet cultivar also has not been thoroughly investigated, particularly at late growth stages. Thus, a susceptible commercial sugar beet cultivar developed for high sucrose content was utilized in the investigation.

Materials and Methods

Treatments. An untreated check and three insecticide treatments were included in the study: an insecticide seed treatment, Poncho Beta (clothianidin at 60 g a.i. + β -cyfluthrin at 8 g a.i. per 100,000 seed; Bayer CropScience, Research Triangle Park, NC); an insecticide foliar treatment, Asana (esfenvalerate at 55.48 g a.i./ha; DuPont Crop Protection, Newark, DE); and Poncho Beta + Asana applied at the same rates used individually. The seed and foliar treatments represent two insecticide chemical classes: neonicotinoid (Poncho; IRAC group 4A), and pyrethroid (Asana and Beta, IRAC group 3). The β -cyfluthrin in Beta included with Poncho in the seed treatment is a nonsystemic insecticide and, therefore, should not have influenced the beet leafhoppers feeding on plant leaves. The Poncho Beta seed treatment was applied by Betaseed, Inc., Kimberly, ID. The Asana foliar spray was applied with a CO₂-powered backpack sprayer at 2.1 kg/cm² using a boom with a 8002VS spray nozzle (TeeJet Technologies, Wheaton, IL) centered over each row. The sprayer was calibrated to release a volume of 168 liters/ha. To limit the influence of fungal pathogens and allow for good stand establishment, the fungicides Allegiance FL (metalaxyl at 15.6 g a.i. per 100 kg of seed; Bayer CropScience) and Thiram 42S (thiram at 250 g a.i. per 100 kg of seed; Bayer CropScience) were applied to the seed.

Field study: 2014. To test the length of efficacy of the three insecticide treatments (Poncho Beta, Asana, and Poncho Beta + Asana), these treatments plus an untreated check were evaluated in a series of four experiments planted side by side and managed with the same techniques and timing for all field operations. The 2014 study was established in a field with Portneuf silt loam soil located in Twin Falls County on the United States Department of Agriculture–Agricultural Research Service North Farm (42°33.264' N 114°21.253' W, elevation 1,185 m) near Kimberly, ID. The field had been in barley the previous year and was disked and plowed in fall 2013. Fertilizer (N at 100.8 kg/ha and P₂O₅ at 123.3 kg/ha) was applied on 11 April and incorporated with a roller harrow. The treatments were arranged in a randomized complete block design with eight replications. Four-row plots were 10.4 m long, with 56 cm between rows. The plot layout was repeated three more times down the field (with different randomizations) to establish a series for four experiments (2014Wk8 to 2014Wk11), with each experiment differing in the date on which they were infested with beet leafhoppers. Plots were established by planting seed of commercial 'B-57' sugar beet (Betaseed Inc., Kimberly, ID) on 21 April to a density of 352,272 seed/ha into moist soil and thinned to 117,424 plants/ha on 31 May. B-57 was a high-sugar cultivar with almost no curly top resistance (based on the last ratings in two different nurseries in 2014, this cultivar was not significantly [$P < 0.0001$, $\alpha = 0.05$] different from the susceptible check; C. A. Strausbaugh, unpublished data). Prior to thinning, a stand count was taken on 12 May, when the plants had only cotyledons and no true leaves. The stand count was conducted by counting the number of plants in a 3-m section in the middle of one of the center rows. Percent stand was determined by comparing the number of plants versus what was planted. Irrigation water was applied through hand lines as needed to replace evapotranspiration (ET) based on data from the Twin Falls AgriMet station (station TWFI; elevation 1,197 m; 42°32.746' N 114°20.762' W; 1.18 km from plots). The crop was managed according to standard cultural practices mentioned in the 2014 Sugar Beet Grower's Guide Book (The Amalgamated Sugar Company, LLC, Boise, ID). The foliar spray was applied on 9 June (7 weeks after planting) to all four experiments. In experiment 2014Wk8 on 16 June (8 weeks after planting, 1 week after foliar treatment), approximately six viruliferous beet leafhoppers (reared on plants containing BCTV) per plant were released by shaking them from cages over the whole plot area. This ensured good curly top disease pressure, because only trace levels of natural curly top pressure were evident in this production area. The viruliferous beet

leafhoppers came from the Beet Sugar Development Foundation insectary maintained in Twin Falls, ID. In the other three experiments, the beet leafhoppers were released at weekly intervals on subsequent weeks: 23 June (experiment 2014Wk9), 30 June (2014Wk10), and 7 July (2014Wk11). In late July, 10 symptomatic plants were arbitrarily sampled (three leaf punches per plant with the cap of a 2-ml microcentrifuge tube) to determine the BCTV strains present in the plants (see "BCTV strain evaluation" below). On 4 June at the eight-leaf growth stage, the percentage of plants infested with beet leafminer (*Pegomya betae* Curtis; Diptera: Anthomyiidae) was determined by visually inspecting all plants in the center two rows. On 29 July, the plants in the center two rows were visually evaluated to determine the percentage of plants with black bean aphids (*Aphis fabae* Scopoli; Hemiptera: Aphididae) in the center whorl. On 12 August and 15 September, all plants in the center two rows of each plot were considered when giving a curly top rating using a disease index (Mumford 1974) of 0 to 9 (Table 1) in a continuous manner (all numbers between 0 and 9 were possible). Other disease and pest problems were not evident in the field during the growing season. The center two rows were mechanically topped on 22 September and harvested with a small plot harvester. During harvest, two eight-beet samples per plot were collected and submitted to the Snake River Sugar Company Tare Lab in Paul, ID for sugar analysis. Percent sucrose, conductivity, and estimated recoverable sucrose (ERS) were determined as described previously (Strausbaugh et al. 2014). The mean of the two samples from each plot was used for analyses.

Field plots: 2015. In 2015, the study conducted in 2014 was repeated in an adjacent field (42°33.166' N 114°21.200' W, elevation 1,187 m) on the same farm and 0.989 km from the Twin Falls AgriMet station. The trial was fertilized (N at 100.8 kg/ha and P₂O₅ at 123.3 kg/ha) on 9 April and planted with the commercial B-57 sugar beet (the same seed lot used in 2014) on 20 April into dry soil. Because the seed was planted into dry soil and remained dry until the first irrigation on 4 May, the timing for this study is based on the first irrigation and not the planting date. The stand was assessed on 26 May and thinned on 29 May. There were only trace levels of beet leafminer, no black bean aphids, and no other pest infestations; therefore, no insect pest evaluations were conducted in 2015. The foliar spray was applied on 22 June. The beet leafhoppers were released 8, 9, 10, and 11 weeks after first watering on the following dates: 29 June and 6, 13, and 20 July. Ten symptomatic plants were sampled for strain determination in late July. Plants were given a curly top rating on 10 August and 9 September. The roots were harvested on 24 September.

BCTV strain evaluation. Ten symptomatic plants were arbitrarily selected and sampled from both studies (20 plants total) in late July of each year to determine the BCTV strains present. The DNA

Table 1. Beet curly top disease rating system utilized by the Beet Sugar Development Foundation

Rating	Description of plant symptoms ^z
0	Healthy; no symptoms
1	Vein clearing of heart leaves, slight pimpling of veins on the underside of leaves
2	Slight leaf curl of the edges of new leaves; pimpling on the veins of the underside of the leaves
3	Center few whorls of leaves with curling edges
4	Most leaves moderately curling; more than half of the upper surface of the leaf visible
5	Slight stunting, severe leaf curling; less than half of the upper leaf surface visible due to curling; most of the larger leaves still erect
6	Stunting, slight yellowing; most leaves becoming prostrate
7	Severe stunting, yellowing; leaves prostrate and some leaves dead
8	Only the center few whorls of leaves green and alive
9	Plant dead

^z Rating system was published by David Mumford (1974). The rating system was utilized in a continuous manner rather than categorically. Thus, any number, including decimal numbers, between zero and nine were possible when scoring the plants.

extraction and polymerase chain reactions (PCR) with primers BSCTV-C1 2315F, BSCTV-C1 2740R, BMCTV-C1 2213F, BMCTV-C1 2609R, BCTV-C1 2097F, and BCTV-C1 2387R were conducted as described previously (Strausbaugh et al. 2008b). Amplification products were electrophoresed through agarose gels (2% wt/vol) supplemented with ethidium bromide (0.0002 mg/ml) in Tris-borate-EDTA buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA). DNA from CTS07-11ID (which contains strains CA/Logan, Severe, and Worland) served as a positive control (Strausbaugh et al. 2008b). Reactions without template DNA served as negative controls.

Data analysis. The SAS (version 9.2; SAS Institute Inc., Cary, NC) Univariate procedure was used to test for normality and Levene's test (HOVTEST = Levene) was used to determine homogeneity of variance. Data were subjected to analysis of variance using the SAS generalized linear models procedure (Proc GLM). Mean comparisons were conducted using Fisher's protected least significant difference (LSD; $\alpha = 0.05$). When means are followed by $\pm x$, x refers to the standard error.

Table 2. Length of efficacy of the Asana (1 to 4 weeks after application) and Poncho Beta (8 to 11 weeks after planting) insecticide treatments based on curly top symptoms using the commercial sugar beet B-57 during the 2014 and 2015 growing seasons near Kimberly, ID^y

Treatment ^z	August		September	
	2014	2015	2014	2015
Wk8-1				
Untreated	6.9 a	5.5 a	8.0 a	7.1 a
Asana	3.9 b	4.5 b	4.7 bc	5.8 b
Poncho Beta	4.0 b	3.6 c	5.2 b	4.2 c
Poncho Beta + Asana	2.0 c	3.3 c	3.9 c	4.1 c
$P > F$	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.2	0.4	1.0	0.6
Wk9-2				
Untreated	6.6 a	4.7 a	7.7 a	5.1 a
Asana	5.4 b	3.9 b	6.6 b	4.2 b
Poncho Beta	4.2 c	3.3 c	5.9 b	3.6 c
Poncho Beta + Asana	3.7 c	2.6 d	5.0 c	3.1 c
$P > F$	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.9	0.3	0.8	0.6
Wk10-3				
Untreated	5.3 a	4.6 a	7.2 a	5.1 a
Asana	3.7 b	4.0 b	6.6 ab	4.0 b
Poncho Beta	3.1 bc	3.4 c	5.9 bc	3.5 c
Poncho Beta + Asana	2.1 c	2.8 d	5.0 c	3.0 d
$P > F$	0.0002	<0.0001	0.0077	<0.0001
LSD	1.2	0.3	1.2	0.3
Wk11-4				
Untreated	2.1 a	4.3 a	6.4	4.9 a
Asana	0.9 b	3.9 b	5.5	4.0 b
Poncho Beta	0.4 b	3.5 c	4.8	3.2 c
Poncho Beta + Asana	0.2 b	3.0 d	5.1	2.8 d
$P > F$	0.0005	<0.0001	0.0678	<0.0001
LSD	0.8	0.2	NS	0.3

^y Curly top in the center two rows was rated on a linear scale of 0 to 9 (0 = healthy and 9 = dead) in a noncategorical manner. $P > F$ was the probability associated with the F value. Means followed by the same letter did not differ based on Fisher's protected least significant difference (LSD) value with $\alpha = 0.05$. NS = not significantly different.

^z Insecticide treatments were Untreated = no insecticide treatment, Poncho Beta = clothianidin at 60 g a.i. + β -cyfluthrin at 8 g. a.i. as a seed treatment per 100,000 seed, Asana = esfenvalerate at 55.48 g a.i./ha as a foliar treatment, and Poncho Beta + Asana = at the same rates as when used individually. Treatments were evaluated in four separate experiments: Wk8-1 (viruliferous beet leafhoppers released 8 weeks after planting for the seed treatment and 1 week after foliar treatment), Wk9-2 (9 weeks after planting and 2 weeks after foliar treatment), Wk10-3 (10 weeks after planting and 3 weeks after foliar), and Wk11-4 (11 weeks after planting and 4 weeks after foliar). In 2015, the seed was planted into dry ground; therefore, the timing for 2015 was initiated based on first irrigation.

Results

Growing degree days. In 2014, there were 1,293 growing degree days (gdd; based on 10°C base at the Twin Falls AgriMet station) from 21 April through 21 September. In 2015, there were 1,288 gdd accumulated from 4 May through 23 September. Overall gdd were similar between years but June 2015 was warmer by 70.5 gdd than June 2014 and July 2014 was warmer by 56.9 gdd than July 2015.

Stand. In 2014, the stand across all four experiments could be compared because there were no differences among experiments ($P = 0.1531$) and both the experiment-block and experiment-treatment interactions were not significant ($P = 0.1679$ and 0.9081 , respectively; data not shown). Also, there were no significant ($P = 0.9908$) stand differences among treatments, with the percent stand ranging from 67 to 68%. In 2015, the stand across all four experiments could be compared because there were no differences among experiments ($P = 0.8943$) and both the experiment-block and experiment-treatment interactions were not significant ($P = 0.4741$ and 0.6187 , respectively). Also, there were no significant ($P = 0.9778$) stand differences among treatments, with the percent stand ranging from 66 to 67%. Thus, during both years, there were sufficient plants to allow for thinning to the desired plant spacing of 15.24 cm.

Beet leafminer and black bean aphids. In the 2014 plots, there were natural infestations of both beet leafminer and black bean aphids. All plants in the center two rows of the untreated check plots and the Asana plots (the same as untreated check, because the foliar insecticide Asana was not applied until 9 June) were infested with beet leafminer. On the other hand, none of the plants in the plots with the seed treatment Poncho Beta had any damage from the beet leafminer. In the 2015 plots, only a trace (<1% in all plots) of beet leafminer was detected at this same growth stage. In the 2014 plots, the black bean aphid infestation was not significantly different among the four experiments ($P = 0.2766$) and the experiment-block and experiment-treatment interactions were not significant ($P = 0.1362$ and 0.1982 , respectively). Therefore, the treatment means could be compared and were found to be significantly different ($P < 0.0001$; $LSD_{\alpha = 0.05} = 4$). The untreated check had 14% of the plants in the center two rows infested with black bean aphids, which was significantly higher than the other three treatments: Asana (7%), Poncho Beta (4%), and Poncho Beta + Asana (2%). The Poncho Beta + Asana treatment had fewer black bean aphids than the Asana treatment but was not different from the Poncho Beta treatment. In the 2015 plots, no black bean aphids were detected.

Curly top symptoms. In both 2014 and 2015, the visual ratings differed among the experiments in August ($P < 0.0001$ and $P = 0.0005$, respectively) and September ($P < 0.0001$). Therefore, the analyses for visual ratings were conducted by experiment (Table 2). The August visual ratings for the untreated checks in 2014 and 2015 showed a reduction (70 and 22% less, respectively) from Wk8-1 (6.9 ± 0.4 and 5.5 ± 0.4 , respectively) to Wk11-4 (2.1 ± 1.1 , and 4.3 ± 0.2 , respectively). With the September visual ratings for the untreated checks in 2014 and 2015, there was a reduction in symptoms (20 and 31% less, respectively) from Wk8-1 (8.0 ± 0.5 and 7.1 ± 0.4 , respectively) to Wk11-4 (6.4 ± 1.4 , and 4.9 ± 0.2 , respectively). With the two treatments containing Poncho Beta, the visual ratings were reduced by an average of 41% compared with the untreated check in 30 of 32 comparisons across the experiments. With the Asana treatment, there was an average of 24% fewer symptoms than the untreated check in 14 of 16 comparisons. In 22 of the 32 comparisons, the treatments with Poncho Beta had an average of 25% fewer symptoms than the Asana treatment. In 7 of 16 comparisons, the Poncho Beta + Asana treatment had fewer symptoms than the other three treatments. Based on the 21 comparisons in these seven experiments, the Poncho Beta + Asana treatment averaged 32% fewer symptoms than all other treatments. Based on Wk11-4 visual ratings, there were significant differences among treatments in three of four comparisons, indicating that the active ingredients were still making a difference 77 days after planting.

Curly top strains. In 2014, the 10 strain evaluation samples were determined to be 100, 90, and 50% positive for the CA/Logan,

Severe, and Worland primers, respectively. In 2015, the 10 strain evaluation samples were determined to be 100, 90, and 70% positive for the CA/Logan, Severe, and Worland primers, respectively. The positive and negative checks for the primers performed as expected.

Root yield. In both 2014 and 2015, the root yield differed among experiments ($P < 0.0001$). Therefore, the analyses for root yield were conducted by experiment (Table 3). Root yield among the untreated checks increased from 37 to 102% from the Wk8-1 to the Wk11-4 experiments in 2014 (37.79 ± 9.86 to 76.24 ± 14.32 t/ha, respectively) and 2015 (62.74 ± 7.21 to 86.19 ± 3.11 t/ha, respectively). Root yield for Wk8-1 in 2015 was higher than root yields in 2014 but, by Wk11-4, the standard errors for the means overlap when comparing years. In all eight experiments, the root yield for both treatments with Poncho Beta (16 comparisons) was an average of 39% higher than the untreated check (Table 3). In six of eight experiments, Asana had an average of 32% more root yield than the untreated check. In 10 of 16 comparisons, the Poncho Beta treatments had an average of 18% more root yield than the Asana treatments.

Sucrose content. In both 2014 and 2015, the root sucrose content differed among experiments ($P = 0.0010$ and 0.0004 , respectively). Therefore, the analyses for root sucrose content were conducted by experiment (Table 3). In four of eight comparisons, there was no significant difference in sucrose content among treatments. However, the Asana, Poncho Beta, and Poncho Beta + Asana treatments always ranked better for sucrose content than the untreated check in all eight experiments, with the only exception being the Poncho Beta treatment in Wk10-3 in 2015.

ERS. In both 2014 and 2015, the ERS differed among experiments ($P < 0.0001$). Therefore, the analyses for ERS were conducted by

experiment (Table 3). ERS among the untreated checks increased 126% from Wk8-1 to Wk11-4 in 2014 ($4,592 \pm 1,301$ to $10,370 \pm 2,222$ kg/ha, respectively) and 47% in 2015 ($8,486 \pm 1,148$ to $12,476 \pm 607$ kg/ha, respectively). ERS for Wk8-1 in 2015 was higher than ERS in 2014 but, by Wk11-4, the standard errors for the means overlapped when comparing years. For Wk8-1 and Wk9-2 in 2014 and 2015, both treatments with Poncho Beta had an average of 75% more ERS than the untreated check. In Wk10-3, only the Poncho Beta + Asana treatment had more ERS than the untreated check in both years. There were significant differences among treatments at Wk11-3 in 2015 but not in 2014. The Asana treatment had an average of 46% more ERS than the check in five of eight comparisons but there was no consistent week-to-week trend. In five of eight comparisons, the Poncho Beta + Asana treatment had an average of 25% more ERS than the Asana treatment. In four of eight comparisons, the treatment with Poncho Beta only had an average of 18% more ERS than the Asana treatment.

Discussion

Insecticidal activity against beet leafhoppers was still evident when significant differences were present 11 weeks after planting for Poncho Beta and 4 weeks after application of Asana when compared with the untreated check. Over both years, in 30 of 32 comparisons for treatments with Poncho Beta and 14 of 16 comparisons for Asana, visual ratings decreased an average of 41 and 24%, respectively, compared with the untreated check. Over both years, in eight of eight experiments for treatments with Poncho Beta and six of eight experiments for Asana, root yields were increased an average of

Table 3. Length of efficacy of the Asana (1 to 4 weeks after application) and Poncho Beta (8 to 11 weeks after planting) insecticide treatments based on yield variables using the commercial sugar beet B-57 during the 2014 and 2015 growing seasons near Kimberly, ID^x

Treatment ^z	Root yield (t/ha)		Sucrose content (%)		ERS (kg/ha) ^y	
	2014	2015	2014	2015	2014	2015
Wk8-1						
Untreated	37.79 c	62.74 b	14.16 b	15.92 b	4,592 c	8,486 b
Asana	82.49 ab	65.41 b	15.86 a	16.32 b	11,226 ab	9,161 b
Poncho Beta	75.63 b	81.82 a	15.70 a	17.09 a	10,176 b	11,913 a
Poncho Beta + Asana	89.82 a	82.85 a	16.31 a	16.38 ab	12,524 a	11,541 a
$P > F$	<0.0001	<0.0001	<0.0001	0.0223	<0.0001	<0.0001
LSD	8.20	7.60	0.65	0.72	1,301	1,279
Wk9-2						
Untreated	40.46 d	79.96 c	14.08 b	16.44	4,847 d	11,188 c
Asana	53.51 c	87.20 b	14.85 a	16.56	6,864 c	12,356 b
Poncho Beta	67.63 b	90.47 ab	15.16 a	17.00	8,802 b	13,247 a
Poncho Beta + Asana	76.44 a	93.93 a	15.32 a	17.07	10,048 a	13,799 a
$P > F$	<0.0001	<0.0001	0.0056	0.1691	<0.0001	<0.0001
LSD	8.38	4.71	0.68	NS	1,196	766
Wk10-3						
Untreated	57.05 c	86.39 c	14.63 b	16.80	7,244 c	12,534 b
Asana	68.28 b	88.12 c	15.20 ab	17.14	9,024 b	13,050 b
Poncho Beta	73.39 b	92.27 b	15.62 a	16.47	9,978 b	12,976 b
Poncho Beta + Asana	86.80 a	96.71 a	15.67 a	16.96	11,796 a	14,037 a
$P > F$	<0.0001	<0.0001	0.0134	0.2114	<0.0001	0.0030
LSD	9.98	3.41	0.66	NS	1,553	738
Wk11-4						
Untreated	76.24 b	86.19 c	15.60	16.85	10,370	12,476 c
Asana	84.92 a	90.50 b	15.76	17.30	11,573	13,488 b
Poncho Beta	87.67 a	96.12 a	16.10	17.29	12,231	14,284 a
Poncho Beta + Asana	82.00 a	92.29 a	16.25	17.18	11,594	13,556 ab
$P > F$	0.0317	0.0001	0.2384	0.4739	0.0814	0.0006
LSD	7.62	3.61	NS	NS	NS	741

^x $P > F$ was the probability associated with the F value. Means followed by the same letter did not differ based on Fisher's protected least significant difference (LSD) value with $\alpha = 0.05$. NS = not significantly different.

^y ERS = estimated recoverable sucrose.

^z Insecticide treatments were Untreated = no insecticide treatment, Poncho Beta = clothianidin at 60 g a.i. + β -cyfluthrin at 8 g. a.i. as a seed treatment per 100,000 seed, Asana = esfenvalerate at 55.48 g a.i./ha as a foliar treatment, and Poncho Beta + Asana = at the same rates as when used individually. Treatments were evaluated in four separate experiments: Wk8-1 (viruliferous beet leafhoppers released 8 weeks after planting for the seed treatment and 1 week after foliar treatment), Wk9-2 (9 weeks after planting and 2 weeks after foliar treatment), Wk10-3 (10 weeks after planting and 3 weeks after foliar), and Wk11-4 (11 weeks after planting and 4 weeks after foliar). In 2015, the seed was planted into dry ground; therefore, the timing for 2015 was initiated based on first irrigation.

39 and 32%, respectively, compared with the untreated check. When considering ERS over both years, the Poncho Beta treatments increased yield by 75% compared with the untreated check in weeks 8 and 9. By week 10, only the Poncho Beta + Asana treatment led to increases in ERS in both years. By week 11, there were only differences in ERS among treatments in 2015. Based on sucrose content, there was a trend for all three insecticide treatments to rank better than the untreated check in all eight experiments, with only one exception. When considering curly top symptoms, root yield, and ERS data over weeks and years, there was a tendency for the insecticides in the Poncho Beta + Asana treatment to complement each other to improve efficacy.

The clothianidin residue on or in plants will persist for a variable period of time depending on plant, growth stage, and amount applied (Simon-Delso et al. 2015). The metabolism of clothianidin in plants occurs in two phases leading to a number of metabolites, some of which can exhibit a long-lasting action against pests, particularly plant-sucking pests (Simon-Delso et al. 2015). However, the concentrations of these metabolites are rarely measured (Bonmatin et al. 2015). These long-lasting metabolites potentially explain why the Poncho Beta seed treatments are effective for a long period of time in sugar beet. In plant tissues and sap, between 5 and 10 ppb are generally regarded as the concentrations necessary to provide protection from insect pests (Byrne and Toscano 2006; Castle et al. 2005; Goulson 2013). In aqueous solutions, clothianidin will degrade via photolysis in less than 2 h (Žabar et al. 2012). In another study, clothianidin was found to be stable under direct sunlight and environmentally realistic pH and temperatures (Bonmatin et al. 2015; Peña et al. 2011). Toxicity and regulatory data indicate that the clothianidin in Poncho Beta has a half-life in anaerobic soil, hydrolysis, and aerobic soil of 27, 33, and 214 days, respectively (Kegley et al. 2014). When clothianidin was applied as a foliar application to peach, the biological half-life was determined to be 5.2 days for single treatments and 7.0 days for triple treatments (Park et al. 2012). In tomato samples with a foliar application of 120 g a.i./ha, the half-life for clothianidin was 7 to 12 days, depending on location (Li et al. 2012). The half-life of clothianidin applied to tea leaves at 60 g. a.i./ha ranged from 3.71 to 4.49 days (Chowdhury et al. 2012). When thiamethoxam (10% WG sprayed at 10 g per 20 liters; metabolized in one step to clothianidin) was applied to Swiss chard (*Beta vulgaris*, like sugar beet) leaves, the half-life ranged from 4.2 to 6.3 days (Rahman et al. 2015). However, these studies were focused on residues on the plant surface as opposed to systemic uptake via seed treatment. When evaluated as a seed treatment in sugar beet, Poncho Beta has been shown to limit curly top symptoms for at least 55 to 59 days; however, the limits of the length of efficacy were never determined (Strausbaugh et al. 2012, 2014). In the present study, Poncho Beta when tested alone reduced curly top symptoms in 15 of 16 ratings; thus, there appears to still be some insecticidal activity even 77 days after planting. When tested alone, Poncho Beta also increased root yield in all experiments and increased ERS in six of eight comparisons. As mentioned earlier, when Poncho Beta was evaluated in combination with Asana, the combination treatment had a tendency to provide the best protection and increase root and sucrose yield. The combination treatment should also reduce the chance of resistance appearing to these two insecticides in the beet leafhopper populations, because Poncho Beta is a neonicotinoid (IRAC group 4A) and Asana is a pyrethroid (IRAC group 3).

Based on a number of studies, the Asana residue on plants will persist for a variable period of time depending on plant, growth stage, and amount applied (Antonious 2001; Antonious and Byers 1994; Walgenbach et al. 1991). When Asana was evaluated on broccoli against the flea beetle (*Phyllotreta cruciferae* Goeze; Coleoptera: Chrysomelidae), residue levels 14 days after spraying at 7.0 g a.i./ha were 0.002 $\mu\text{g}/\text{cm}^2$, which still reduced the *P. cruciferae* population by 88% (Antonious 2001). When Asana was evaluated on green pepper and pumpkin plants at 7.0 g a.i./ha, the half-life on leaves and fruit varied from 1 to 3 days (Antonious and Byers 1994). After 21 days, only trace levels (0.0001 ppm) were detectable on pepper fruit (Antonious and Byers 1994). When esfenvalerate was

evaluated on tomato foliage for control of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), there was still >65% mortality 14 days after application (Walgenbach et al. 1991). Thus, although Asana is rapidly degraded on plant surfaces, the insecticidal activity can still remain effective for weeks after application. A recent sugar beet study showed that Asana, when applied 1 week before viruliferous beet leafhopper release, could reduce curly top symptoms and increase yields (Strausbaugh et al. 2014). The present study confirms these results and shows that one Asana foliar spray could still reduce curly top symptoms 4 weeks after application, except for the Wk10-3 and Wk11-4 September ratings in 2014. Asana also increased root yield in six of eight comparisons, including the two Wk11-4 comparisons, and increased ERS in five of eight comparisons, including one of the two Wk11-4 comparisons. On sugar beet, this residual activity of Asana is even more effective when combined with the residual activity associated with the Poncho Beta seed treatment, as mentioned previously.

Neonicotinoids are active against a broad spectrum of economically important crop pests, including members of the families Aphidae (aphids), Aleyrodidae (whitefly), Cicadellidae (leafhoppers), Chrysomelidae (western corn rootworm), Elateridae (wireworms), Fulgoroidea (planthoppers), and Pseudococcidae (mealybugs), and phytophagous mites (Elbert et al. 2008; Jeschke et al. 2011; Simon-Delso et al. 2015). This broad spectrum of activity is an important reason why clothianidin is now registered for use on 146 agricultural crops and was applied to about 18.6 million ha annually between 2009 and 2011 (Brassard 2012). In 2014, beet leafminers were evaluated on the 4 June (eight-leaf growth stage) in the plot areas prior to the foliar application of Asana. Plants in plots with the Poncho Beta seed treatment had no beet leafminers, while all plants without the seed treatment had beet leafminers. These data are consistent with beet leafminer efficacy data collected in previous studies (Strausbaugh et al. 2006, 2010a, 2012, 2014). Although the beet leafminer infestation was high in 2014 and at only trace levels in 2015, the influence on data collected should be minor when compared with losses caused by BCTV, as noted in a previous study (Strausbaugh et al. 2012). The losses associated with beet leafminer in sugar beet in Idaho are poorly understood and should be investigated without the overwhelming influence of BCTV. In 2014, a July infestation of black bean aphids was evaluated whereas, in 2015, no black bean aphids were evident. When plants with aphids were evaluated on the 29 July, all insecticide treatments had fewer infested plants (2 to 7% infested) than the untreated check (14% infested). The treatment with both Poncho Beta and Asana ranked as the best treatment for aphid control but only performed significantly better than Asana, not the Poncho Beta treatment. The ability of Poncho Beta to provide protection from aphids into July on sugar beet has been noted previously (Strausbaugh et al. 2010a). Like the beet leafminers, the influence of black bean aphids on sugar beet yield is poorly understood and should be evaluated without the overwhelming influence of BCTV. One can also argue that the influence of beet leafminers and black bean aphids in 2014 must have been inconsequential compared with the overwhelming influence of curly top, because the conclusions from both the 2014 and 2015 datasets were the same.

A field study (Duffus and Skoyen 1977) and a greenhouse study (Wintermantel and Kaffka 2006) evaluated sugar beet plant age and host resistance to curly top using clip cages and the CA/Logan strain of BCTV. In the field study, plants were inoculated up to 12 weeks after planting, which established that the percentage of plants infected decreased and incubation period increased with increasing plant age (Duffus and Skoyen 1977). In the present study, plants were inoculated with unrestricted (released and not restricted to clip cages) beet leafhoppers that were carrying multiple strains of BCTV and the disease assessments were based on a disease index, not the percentage of infected plants. Nevertheless, there was a 20 to 70% decrease in symptoms from untreated check plants inoculated in week 8 to those inoculated in week 11. Likewise, there was a corresponding 37 to 102% increase in root yield and a 47 to 126% increase in ERS across the untreated plots from week 8 to week 11. Even though resistance increased with plant age, there was a tendency

for plants to still benefit from insecticide applications even though the insecticides were potentially approaching the end of their efficacy.

After nearly 2 decades of use, resistance among pest populations to the neonicotinoids had begun to appear (Jeschke et al. 2011). Some examples of resistance include the brown planthopper (*Nilaparvata lugens*; Hemiptera: Delphacidae), Colorado potato beetle (*Leptinotarsa decemlineata*; Coleoptera: Chrysomelidae), greenhouse whitefly (*Trialeurodes vaporariorum*; Hemiptera: Aleyrodidae), green peach aphid (*Myzus persicae*; Hemiptera: Aphididae), and silverleaf whitefly (*Bemisia tabaci*; Hemiptera: Aleyrodidae) (Alyokhin et al. 2007; Cahill et al. 1996; Foster et al. 2008; Gorman et al. 2008; Jeschke and Nauen 2008; Karatolos et al. 2010; Nauen and Denholm 2005; Nauen et al. 2008; Prabhakar et al. 1997; Slater et al. 2012; Szendrei et al. 2012; Wang et al. 2008). Cross resistance among the different generations of neonicotinoids may also occur, but not always (Alyokhin et al. 2007; Elbert and Nauen 2000; Shi et al. 2011). Given the widespread use of neonicotinoids, finding resistance after almost 2 decades of use should not be surprising. Neonicotinoids are likely to remain important for agriculture because, for many of the most important crops grown in North America (particularly corn), there are no nonneonicotinoid seed alternatives readily available to growers in the marketplace (Simon-Delso et al. 2015). In sugar beet in the United States, the same is true for control of curly top. Thus, given the strong selection pressure provided by the widespread use of neonicotinoid seed treatments (likely 100% usage in sugar beet areas with a history of BCTV pressure), growers should consider a foliar pyrethroid application to not only extend the early- to midseason curly top control but also to limit the potential for the buildup of resistance in the beet leafhopper population.

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