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Population Dynamics of the Beet Leafhopper in Northeastern Oregon and Incidence of the Beet Leafhopper-Transmitted Virescence Agent Phytoplasma

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Abstract Beet leafhoppers were collected weekly on yellow sticky traps placed at 36 locations in Morrow and Umatilla Counties in northeastern Oregon in April through November 2007, 2008, and 2009. Insects were counted, collected, and a subset of the insects was tested for the presence of the beet leafhopper-transmitted virescence agent phytoplasma, the causal agent of potato purple top disease in this region. Beet leafhoppers were present throughout the sampling period and the number of insects collected peaked in June of each year with smaller peaks in July and October. Of the 804 insects tested for phytoplasma in 2007, 2008, and 2009, 21, 18, and 22% tested positive for phytoplasma, respectively. Most of the phytoplasma-positive insects were collected from mid-June through July. Positive insects, however, were collected as late as 13 November in 2007 and 2008. These data indicate that a relatively high proportion of the beet leafhoppers in this area are harboring the phytoplasma. Therefore, the potential for development of purple top disease of potatoes from migrating beet leafhoppers in this important potato producing region is quite high and measures to control this pest throughout the growing season are probably necessary in order to reduce disease pressure.

Resumen Se colectaron chicharritas de la remolacha semanalmente en trampas amarillas pegajosas ubicadas en 36 localidades en los Condados de Morrow y Umatilla en el noreste de Oregon desde abril hasta noviembre de 2007, 2008 y 2009. Se contaron los insectos, se colectaron y se probó un sub-juego de ellos para la presencia del fitoplasma, agente de la virescencia transmitido por la chicharrita de la remolacha, que es el agente causal de la enfermedad de la punta morada de la papa en esta región. Las chicharritas de la remolacha estuvieron presentes a lo largo del período del muestreo y el número de insectos colectados alcanzó un máximo en junio de cada año con pequeños picos en julio y octubre. De los 804 insectos probados para fitoplasma en 2007, 2008 y 2009, 21, 18 y 22% fueron positivos para el fitoplasma, respectivamente. La mayoría de los insectos positivos se colectaron desde la mitad de junio y todo julio. No obstante, se colectaron positivos hasta tan tarde como el 13 de noviembre de 2007 y 2008. Estos datos indican que una alta proporción relativa de las chicharritas de la remolacha en esta área están manteniendo al fitoplasma. De aquí que el potencial de desarrollo de la enfermedad de la punta morada de la papa proveniente de chicharritas migratorias en esta región tan importante en la producción de papa es muy alto y las medidas de control de esta plaga a lo largo del ciclo de cultivo son probablemente necesarias a fin de reducir la presión de la enfermedad.

Keywords Plant diseases · Phytoplasma · BLTVA

Introduction

Phytoplasmas are single-celled prokaryotes that are associated with a number of serious plant diseases worldwide (Davis and Sinclair 1998; Lee et al. 2000). These pathogens are transmitted to plants by insects with piercing-sucking mouthparts, especially leafhoppers, plant hoppers, and psyllids. In the Columbia Basin region of Washington and Oregon the beet leafhopper, *Circulifer tenellus* Baker; (Hemiptera: Cicadellidae) transmits a phytoplasma termed

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the beet leafhopper-transmitted virescence agent, BLTVA (Golino et al. 1987), a.k.a. the Columbia Basin potato purple top phytoplasma (Munyaneza et al. 2005), to potatoes and other vegetable and field crops (Shaw et al. 1990, 1993; Crosslin et al. 2005; Munyaneza et al. 2006, 2007). Phytoplasma-infected potatoes exhibit "purple top" symptoms, which include upright growth habit, rolling of leaflets, purple and/or yellow leaf discolorations, and elongation of the axial bud, often resulting in aerial tubers, and a bushy appearance (Lee et al. 2004; Munyaneza et al. 2006). In 2002, there was a major outbreak of BLTVA-induced potato purple top disease in the Columbia Basin which resulted in losses in potato yield and quality (Thomas et al. 2003; Munyaneza et al. 2009). The disease was also observed in 2003 and 2004, especially on organic potatoes, in this region (Munyaneza et al. 2005). More recently, anecdotal reports from growers, fieldmen, and Extension agents in the Columbia Basin suggest that the incidence of purple top disease is relatively low, however, there is a need to increase our knowledge of the biology of the beet leafhopper. Diseases caused by this phytoplasma can be economically important either by reducing yield, quality, or effects upon flowering and subsequent seed-set (Golino et al. 1989; Schultz and Shaw 1991). Recently, research showed that all of the economically important potato cultivars grown in the Columbia Basin are susceptible to potato purple top disease, although differences in susceptibility among cultivars were noted by Munyaneza et al. (2009).

The beet leafhopper is a common insect in the Columbia Basin region (Cook 1967). The beet leafhopper also transmits *Beet curly top virus* (Thomas and Mink 1979) and *Spiroplasma citri* (Lee et al. 2006). These two pathogens are also found on various crops in the Columbia Basin of Washington and Oregon. Research from Washington indicated that the beet leafhopper is capable of overwintering as an adult in this location and that 29.6% of the overwintering insects carried BLTVA (Munyaneza et al. 2010b). Monitoring the occurrence and incidence of phytoplasma in vector insects provides useful information on the potential risk to a crop by these pathogens (Goodwin et al. 1999). Recently, data was published on the seasonal occurrence of the beet leafhopper in central Washington at a single location and on the incidence of phytoplasma in these insects (Munyaneza et al. 2010b). The timing of arrival of potentially infective beet leafhoppers can be important since potatoes are most susceptible to purple top disease when infected early in the season (Munyaneza et al. 2010a). Also, if potentially infective leafhoppers are only present during a portion of the growing season, then control efforts can be focused on these periods rather than the entire growing period. The objective of the work described herein was to investigate the seasonal occurrence and abundance of the beet leafhopper at multiple locations from within two counties in northeastern Oregon and determine the incidence of phytoplasma in a portion of the insects collected in this region where more than half of Oregon potatoes are grown.

Materials and Methods

Beet Leafhopper Collections

Yellow double-sided sticky traps (10×16 cm; Cascade AgServices, Mount Vernon, WA) were positioned in 36 areas in Morrow and Umatilla Counties, OR (Fig. 1). The detailed location coordinates are shown in Table 1. All locations were near potato fields, among native weedy areas, and away from dusty roads. There was one trap per location and approximately the same location was used for each of the 3 years. Traps were kept low to the ground (the bottom edge of the trap was approximately 15 cm from the soil surface) since beet leafhoppers are most active at that height. Sticky traps were collected and replaced weekly from early April until 15 November in 2007, 6 November in 2008, and 30 October in 2009. Traps were taken to the entomology laboratory at the Oregon State University, Hermiston Agricultural and Research and Extension Center where samples were identified, counted and the numbers of beet leafhoppers recorded. Beet leafhoppers were identified by the second author. A subsample of beet leafhoppers was carefully removed from the traps each week and placed into vials with 95% ethanol. Traps were collected on Thursday of each week, so the actual sampling dates varied slightly between years.

Detection of Phytoplasma

A subset of insects collected from four sites out of the 36 in 2007 and seven sites in each of 2008 and 2009 were selected and tested for the presence of phytoplasma. The trapping sites that were selected represented locations that provided the most sampling times throughout the season where at least five insects were collected during that week. One to five beet leafhoppers per trap per collection date were removed from the traps and placed into ethanol for phytoplasma testing.

Insects were removed from the ethanol and placed individually into 1.5 ml microcentrifuge tubes with 600 µl of CTAB extraction buffer (Zhang et al. 1998). Insects were ground with a sterile micropestle (Research Products International, Mount Prospect, Illinois) and nucleic acids were extracted as described (Zhang et al. 1998). Five micrograms of glycogen (Roche Diagnostics, Mannheim, Germany) was added along with the isopropanol to aid precipitation of the nucleic acids. Nucleic acid pellets were resuspended in 50 µl of UltraPure water (Invitrogen). A nested polymerase chain reaction (PCR) procedure (Gundersen and Lee 1996) with primers P1 and P7 followed by primers fU5 and BLTVA-int

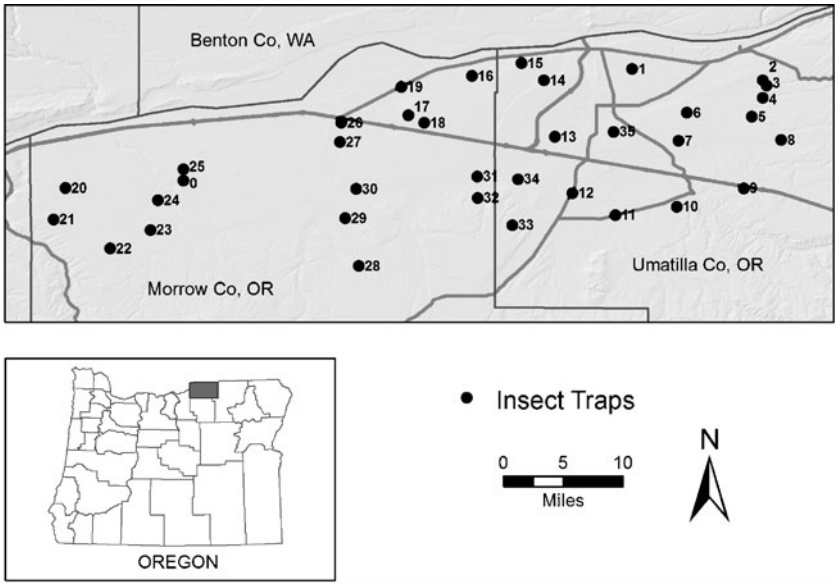

Fig. 1 Location of beet leafhopper trapping sites in northeastern Oregon in 2007, 2008, and 2009

(Table 2) was used for testing 2 μl of the nucleic acid extracts for BLTVA according to the procedures of Crosslin et al. (2006). Presence of the predicted c. 1,200 bp amplicon after agarose gel electrophoresis indicated a BLTVA phytoplasma-positive sample.

Data Analysis

To determine if seasonal captures differed over time, a repeated measures ANOVA was conducted. Data consisted of number of beet leafhoppers over 21 comparable weeks for each year individually and combined. Also, to determine if infectivity rate was similar from site to site, data where enough beet leafhoppers were found, ranging from zero to five insects was subjected to repeated measures ANOVA. Data was analyzed assuming an underlying normal distribution model since the data contained many zero values.

Results

Beet Leafhopper Population Dynamics

Figure 1 identifies the 36 trap locations used in all years. Beet leafhoppers were detected from April or early May through October or November in each of the three years (Fig. 2). In 2007, the average beet leafhopper counts in this portion of northeastern Oregon steadily increased from late April through early June. The highest average number of beet leafhoppers trapped per week was on 5 June (64 beet leafhoppers per trap per week) (Fig. 2). The lowest counts were recorded in early April and then in August to early September. In 2008, the highest counts occurred on 19 June (25 beet leafhoppers per trap per week) and 23 October (20 beet leafhoppers per trap per week). Insect numbers peaked later in June of 2008 compared to June of 2007. In 2009, the highest beet leafhopper numbers were reached between 5 June and 7 July. After 7 August, the average number of beet leafhoppers caught on the traps steadily declined then increased again somewhat in October.

Statistical analysis showed that there was a significant effect of time on beet leafhopper captures (Wilks' lambda, $F=6.32$, df=20, 10, $P<0.0024$). Additionally, leafhopper captures did not vary significantly from site to site ($F=1.04$, df=14, 29, $P>0.4439$). Moreover, there was not a significant interaction of site with time (Wilks' lambda, $F=0.89$, df=280, 154.7, 10, $P<0.8046$).

Incidence of Phytoplasma in Beet Leafhoppers

Figure 3 shows the percentage of beet leafhoppers that tested positive for BLTVA averaged for all trapping locations. Of 250 insects tested in 2007 from trapping locations 4, 10, 31, and 35, 52 of them (21%) were positive for BLTVA phytoplasma. In 2008, 269 insects were collected and tested from these four sites plus locations 0, 14, and 19 and a total of 49 (18%) were positive for the phytoplasma. In 2009, 64 of 285 insects (22%) tested from the same trapping locations as those in 2008 were positive for the phytoplasma. Among all trapping sites and years, leafhopper infection rates varied from 0 to 65%. The highest phytoplasma infection rate occurred in insects collected the first week of July 2007 (Fig. 3). Most of the phytoplasma-positive insects were collected in late June through July as shown in Fig. 3. Phytoplasma-positive insects were collected as early as 28 May in 2007 and as late as 13 November in each of 2007 and 2008.

Table 1 Global positioning system (GPS) coordinates of beet leafhopper trapping locations

Trap Number	GPS Coordinates
0	N 45.45.461 W 119.48.790
1	N 45.53.608 W 119.16.077
2	N 45.52.781 W 119.06.539
3	N 45.52.416 W 119.06.267
4	N 45.51.508 W 119.06.546
5	N 45.50.119 W 119.07.336
6	N 45.50.423 W 119.12.075
7	N 45.48.369 W 119.12.672
8	N 45.48.437 W 119.05.187
9	N 45.44.901 W 119.07.929
10	N 45.43.561 W 119.12.827
11	N 45.42.951 W 119.17.290
12	N 45.44.552 W 119.20.427
13	N 45.48.652 W 119.21.705
14	N 45.52.773 W 119.22.505
15	N 45.54.028 W 119.24.119
16	N 45.53.109 W 119.27.743
17	N 45.50.237 W 119.32.393
18	N 45.49.672 W 119.31.246
19	N 45.52.305 W 119.32.903
20	N 45.44.921 W 119.57.393
21	N 45.42.623 W 119.58.263
22	N 45.40.502 W 119.54.129
23	N 45.41.866 W 119.51.169
24	N 45.44.010 W 119.50.652
25	N 45.46.290 W 119.48.784
26	N 45.49.686 W 119.37.276
27	N 45.48.270 W 119.37.373
28	N 45.39.232 W 119.35.986
29	N 45.42.717 W 119.36.987
30	N 45.44.848 W 119.36.193
31	N 45.45.765 W 119.27.364
32	N 45.44.197 W 119.27.323
33	N 45.42.209 W 119.24.810
34	N 45.45.566 W 119.24.397
35	N 45.49.028 W 119.17.411

Table 3 shows the phytoplasma infection rate summarized by trap location. Nearly all leafhopper collections showed some level of BLTVA infection. None of the ten insects tested from trap location 0 in 2008 tested positive for phytoplasma. This was the only beet leafhopper collection tested that showed no phytoplasma infection. Infection rates for the other locations and years ranged from approximately 9–40%. The 3 year average infection rate per location ranged from 11 to 25%. Repeated ANOVA did not yield a robust conclusion since many dates had zero leafhoppers. Although we tested leafhoppers for BLTVA from only 4 sites out of 36 in 2007, and 7 sites in 2008 and 2009, there was a year by location interaction (F=1.66, df= 7, p>0.0995). This indicates there were significant differences among beet leafhoppers collected in various locations when compared by year.

Discussion

Our data on the timing and number of beet leafhoppers present in this geographic region is consistent with leafhopper data collected in Washington and Oregon in 2003–2005 (Munyaneza et al. 2008). These researchers reported the peak in leafhopper numbers occurred in late May through July. Thereafter, the numbers generally declined through the summer and showed a slight increase in October, similar to what we observed in 2007, 2008 and 2009 at the Oregon trapping locations. The statistical analysis conducted on the 21 weeks by 15 location data set indicated no significant difference among trapping locations. This result confirms that the beet leafhopper is widespread in this portion of northeastern Oregon. Additionally, this result would indicate that sampling for beet leafhoppers in a relatively few locations within a given geographic area may provide important information on beet leafhopper population dynamics.

Our data shows that phytoplasma-infected beet leafhoppers were present for most of the growing season in numerous locations in Morrow and Umatilla counties. The percentages of BLTVA phytoplasma-positive insects observed in 2007–2009 are similar to those reported at one site in Washington State (Munyaneza et al. 2010b). These workers reported that the incidence of BLTVA in beet leafhoppers near a potato field averaged approximately 29, 35, and 9% in 2005, 2006, and 2007, respectively.

An action threshold for phytoplasma-infected beet leafhoppers in the Columbia Basin region of Washington and

Table 2 Oligonucleotide primers used for detection of phytoplasma in beet leafhoppers by nested PCR

Primer	Sequence (5′ to 3′)	Reference
P1	AAGAGTTTGATCCTGGCTCAGGATT	Deng and Hiruki 1991
P7	CGTCCTTCATCGGCTCTT	Schneider et al. 1995
fU5	CGGCAATGGAGGAAACT	Lorenz et al. 1995
BLTVA-int	GATGATTTTAGTATATATAGTCC	Smart et al. 1996

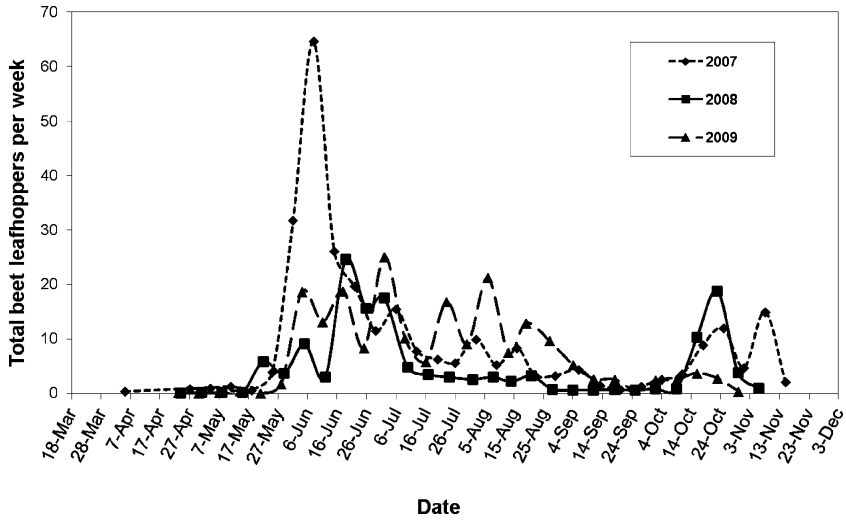

Fig. 2 Population dynamics of beet leafhoppers in northeastern Oregon in 2007, 2008, and 2009. Numbers are averaged across all 36 trapping locations

Oregon has yet to be firmly established. Yet it is clear that the high infection rates consistently observed in the two Oregon counties reported here and in the single Washington location reported previously (Munyaneza et al. 2010b) suggest that if this insect is present then it is likely the phytoplasma is also present. Detecting the presence of the leafhopper with sticky traps provides an easy method for growers to monitor the influx of leafhoppers in the early summer. Identifying insects that are carrying phytoplasma helps to identify potential vectors among a large number of species (Klein et al. 2001; Crosslin et al. 2005). Determining the percentage of phytoplasma-infected leafhoppers in a population can also aid growers in making decisions on insect control measures (Burkness et al. 1999). Affordable services to determine beet leafhopper infectivity are not currently available to growers, but sampling leafhopper densities using sticky traps is straightforward. Assistance in identifying insects can be obtained from county extension offices and the Washington State Potato Commission also has resources to aid collection and identification of leafhoppers (www.potatoes.com/pdfs/Beet%20Leafhopper%20card.pdf.) . Additionally, the second author offers insect identification training.

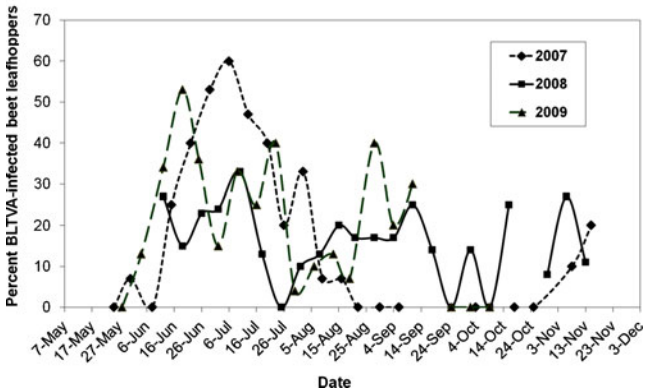

Fig. 3 Percentage of beet leafhoppers collected in northeastern Oregon in 2007, 2008, and 2009 that tested positive for BLTVA phytoplasma by nested PCR. These data are combined for insects collected at locations 4, 10, 31 and 35 in 2007 and locations 0, 4, 14, 10, 19, 31, and 35 in both 2008 and 2009. Sampling dates varied slightly between years. Line discontinuities indicate that no insects were available for testing for that period

Of the 18 year by location data points shown in Table 3, the incidence of BLTVA-infected leafhoppers exceeded 10% in 16 of these and exceeded 20% in 10 of the cases. These data show that BLTVA-infected beet leafhoppers were widespread within the trapping area in 2007, 2008, and 2009. The trapping locations (Fig. 1) roughly corresponded to an area measuring 85 km east to west and 25 km north to south. Although the percentage of BLTVA phytoplasma-positive insects ranged from 0 to 40% throughout the sampling area, most (10 of 18) of the annual collections showed 20% or more BLTVA-positive insects.

Table 3 Phytoplasma positive beet leafhoppers by trapping location in 2007, 2008, and 2009

Trap location	Year			
	2007	2008	2009	Total
00	Nt[a]	0/10 (0)[b]	5/35 (14)	5/45 (11)
4	21/75 (28)	6/64 (9)	15/60 (25)	42/199 (21)
10	10/80 (13)	3/20 (15)	10/25 (40)	23/125 (18)
14	Nt	3/30 (15)	6/35 (17)	9/55 (16)
19	Nt	8/35 (23)	16/60 (27)	24/95 (25)
31	12/50 (24)	12/57 (21)	4/20 (20)	28/127 (22)
35	10/45 (22)	17/63 (27)	8/50 (16)	35/158 (22)

[a] Nt is none tested

[b] Number phytoplasma-positive beet leafhoppers over number tested. Percent positive in parentheses.

Earlier work showed that most of the economically important potato cultivars grown in the Columbia Basin were susceptible to BLTVA (Munyaneza et al. 2009). Recent evidence also indicates that BLTVA is transmitted to tubers and subsequently to daughter plants of many important potato cultivars (Crosslin et al. 2011). The presence of phytoplasma-positive beet leafhoppers throughout most of the growing season in each of the 3 years suggests that there is a significant risk of infection to potato crops in this area. In particular, the presence of phytoplasma-infected leafhoppers early in the season poses the greatest risk since younger potato plants are more likely to become infected (Munyaneza et al. 2010a) and because of longer exposure, results in the greatest possible yield and/or quality loss. Additionally, because the beet leafhopper feeds on and transmits phytoplasma to diverse species of plants (Golino et al. 1987; Shaw et al. 1990, 1993; Munyaneza et al. 2006) there would seem to be a need to monitor and control this insect. The beet leafhopper transmits not only phytoplasma but also *Beet curly top virus* (Thomas and Mink 1979) and *Spiroplasma citri* (Lee et al. 2006) to other vegetable and field crops. Therefore, this insect poses a threat not only to potatoes, but to irrigated agriculture in general in northeastern Oregon.

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