



## Study of distribution pattern and population variation of leafhopper vectors of beet curly top disease in Hamadan province

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### ABSTRACT

Curly top disease is one of the important diseases of sugar beet. Spatial pattern and population variation of leafhoppers *Circulifer haematoceps*, and *C. tenellus*, vectors of sugar beet curly top virus, were studied in Hamadan province, Iran for three years (2007-9). Sampling was carried out at the early stage of plant growth, in accordance with planting date for 9 weeks at 7- 10 day intervals. For doing this, four fields of five hectares each spaced approximately one kilometer from each other were selected in Asadabad region, Hamedan province. The insect sweep net was considered as sampling unit. By using the data obtained, the RV (a measure of sampling accuracy) factor for the three years was 12.2, 6.52, and 16.65%, respectively. Because of finding *C. haematoceps* as a vector of the disease in this region, variance to mean ratio ( $S^2 / \bar{x}$ ) was used for determination of the spatial pattern of *C. haematoceps* population. The numerical value of Z for the whole year and total of three years was more than 1.96 which indicates the cumulative spatial distribution of the vector. The proportion of *C. haematoceps* population to total population of other leafhopper species in 2007, 2008, and 2009 was 5.68, 1.97 and 2.43%, respectively. Average of infected sugar beet plants to curly top disease in 2007, 2008, and 2009 was 5.2, 8.6, and 5%, respectively. In 2007, the number and proportion of leafhopper vector population was higher than the total population of other species but owing to late planting, infection percentage was low, while in 2008, due to early planting and slow growth of the plants, vectors had more time for distribution. Despite the low number and proportion of leafhopper vector population to total population of other species in 2008, the average of plants infection (8.6%) was higher than the two other years. Also, a linear positive and significant relationship was found between leafhopper vector population frequency and the frequency of plants infection throughout the season. The coefficient of determination ( $R^2$ ) for the three years were 96, 90, and 94%, respectively, indicating the existence of a strong relation between the curly top occurrence and vector population in the field.

**Keywords:** *Circulifer haematoceps*, Curly top, Leafhopper, Population distribution pattern, Sugar beet

### INTRODUCTION

Leafhoppers injure plants either directly, through feeding which can damage plant tissue and rob the plant of essential nutrients, or indirectly, through the transmission of plant pathogens such as beet curly top virus. Beet curly top virus is one of the main viral diseases in sugar beet production areas in the world which has been reported in different regions in Iran including Isfahan, Khorasan, Kerman, and Hamedan (Al-e Yassin

et al. 1995). The virus has a broad host range including 300 plant species from 44 families (Bennett 1971).

The infection rate reported in Fasa city was 100% (Kheyri 1991). While 80% of the plants were infected, the disease damage was reported to 40%. Because of short proliferation period, high frequency of vectors, and their feeding ability of various plants, the disease widespread quickly in the region. At least, five species in *Curtovirus* genus are characterized as sugar beet curly top virus in which Beet curly top Iran virus (BCTIV) and Beet

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severe curly top virus (BSCTV) are reported in Iran (Taheri et al. 2012). Two leafhopper species including *Circulifer haematoceps* and *C. tenellus* are involved in curly top virus transmission. Based on Fattahi et al. (2012) and Taheri et al. (2012) reports, the transmission of BCTV and CSCTV is carried out by *C. haematoceps* leafhopper. Ashrafmansoori et al. (2010) studied the effect of planting date on curly top infection by using two susceptible and resistant cultivars in Fasa city. They showed that early sugar beet planting decreased crop damage. Strausbaugh et al. (2006) and Wang et al. (1999) reported that early planting together with systemic insecticides application such as Furat, Aldicarb, Imidacloprid, and Clothianidin delayed the emergence of disease symptoms and decreased the damage. In west of the USA, the greatest curly top damage was observed in the fields in which overwintered leafhoppers migrated early spring. Therefore, using resistant cultivars and proper planting date contributes to reduction in infection widespread (Thresh 1974). Because of curly top history in Iran, compared with rhizomania disease, more breeding studies were allocated to curly top. Domestic and foreign germplasm evaluation under natural or controlled (in greenhouse) infection conditions resulted in the release of resistant parental lines (Farsinejad et al. 1991). In a study by Ashrafmansoori et al. (2010), tetraploid lines with considerable resistance (under natural infection) were generated through the treatment of diploid lines with colchicine. The key factor associated with disease outbreak is the frequency and distribution of the vectors (insects) in the fields and a rapid growth of their population during the critical stages of the plant growth. Therefore, determination of the vectors distribution pattern and their populations' fluctuation during the critical stages of the sugar beet growth is important. By determining the leafhopper vectors population frequency at different growth stages, the selection of proper planting date and chemical control against vectors can be performed readily. Limited studies were carried out to evaluate curly top vectors in Iran which need to be reviewed and repeated again to overcome previous limitations such as insufficient region and sampling number, or lack of accuracy. Insect net is one of the most common methods for leafhopper sampling which requires accuracy in the case of samples sufficiency and proper sampling (Pedigo and Buntin 1994). Beet curly top was reported in 1966 in Marvdasht and Zarghan regions by Gibson (1979). *C. tenellus* leafhopper was

identified as a natural vector of beet curly top in the USA (Oman 1970). Furthermore, *C. haematoceps* was identified as the most important vector of this disease in Asia and Europe (M and R) (Kheyri 1991). In some Middle-East countries and also in Iran, both the aforesaid species are involved in curly top transmission (Oman 1970; Kheyri 1991). The presence of *C. tenellus* and *C. haematoceps* in sugar beet fields in Fars province was reported in previous studies (Kheyri 1991; Fattahi et al. 2012; Taheri et al. 2012). Based on other studies performed in different countries, the rate of beet curly top damage is associated with vectors frequency. Therefore, one of the practical methods to reduce the damage is to conduct conducting a control program against vectors and their population in sugar beet fields (Bennett 1957). Some studies evaluated the *C. tenellus* population distribution and fluctuation in sugar beet fields in the USA but because of different factors such as ecological condition, existence of only one vector species in the fields, and also vast migration of this species in several states, the results are largely limited to the USA (Flock and Deal 1959). This study was performed to evaluate spatial distribution and population frequency of *C. tenellus* and *C. haematoceps* vectors as the most important curly top vectors for three years (2007-10) in sugar beet fields in Hamedan province. In addition, linear correlation between leafhopper population density and disease severity was evaluated.

## MATERIALS AND METHODS

Field evaluation was performed for three years (2007-10) in Asadabad (Musa Abad village) sugar beet fields. This area has experienced severe beet curly top disease. For this purpose, samples were taken from four fields of 5 hectares area spaced approximately one kilometre from each other. Monogerm cultivar was planted in selected fields and furrow irrigation method was used.

### 1- Sampling

#### A) Sampling unit

Because of remarkable mobility of curly top vectors, each sweep-net application was considered as a sampling unit (Fleischer et al. 1985). Sweep net advantage is in its simplicity, standardized application, and frequency of the data obtained. Sweep nets, each with a 38 cm diameter net hoop and 1 m long handle were used. Sweep-

ing was performed in such a way that the net edge was pulled at the top of plants and on rows and sweeping pattern was repeated several times to be able to compare different fields and sampling dates.

Each sampling unit was considered as the number of sweeps equal to 1 m<sup>2</sup>. For this purpose, absolute density of leafhopper population was determined by counting the mature leafhoppers on the leaves and shaking all plants located in 1 m<sup>2</sup> into the bag and counting the mature insects and nymphs. At the next stage, sweeping was performed. After each sweeping, number of mature insects and leafhopper nymphs were counted and sweeping was repeated. Sweeping was continued until the equality between the number of leafhoppers in the net with the number of leafhoppers counted in a shaking method was achieved. The average number of sweeping in 1 m<sup>2</sup> was considered as the sampling unit. Comparing absolute sampling (plants shaking in 1 m<sup>2</sup>) with relative sampling (sweeping in the field) during three years showed that in each sampling, five sweeps in the field was equal to one sampling unit. Therefore, the average of 10 samples which included 50 sweeps was used and in general, 200 sweeps were performed at each sampling date in all four fields.

#### B) Sample number

Sampling is used as a decision making tool in pest management. Pest spatial distribution has an effective role in the design of appropriate sampling programs. Information regarding spatial and temporal distribution of the pest contributes in better understanding of their interaction with environment, estimation of their frequency, and application of this information in conducting control programs (Jafari et al. 2003; Kianpur et al. 2009). The difference between initial sampling data is a key factor in the determination of sample size or number. In order to determine the proper sample number, an initial sampling including five sweeps with 10 samples was performed. Then, the relative variation (R.V.) was estimated using the following formula:

$$RV = 100 \left( \frac{SE}{\bar{x}} \right)$$

where  $\bar{x}$  is the mean of data and SE is standard error of the initial sampling data. In pest management and spatial distribution pattern determination, up to 25% RV value is acceptable. Number of samples was estimated using the following equation:

$$N = \left( \frac{tS}{D\bar{x}} \right)^2$$

where N is the proper number of samples, t is the value from student's t-test Table based on sample number degrees of freedom, S is standard error of the initial sampling data, D is accepted error, and  $\bar{x}$  is the mean of initial sampling data (Jafari et al. 2003).

#### C) Sampling time

Planting date in Asadabad fields varied based on rainfall status in early season. As a result, seed emergence and sampling date was also varied in each year and began approximately one month after planting. In some years, sampling was performed with one month delay compared with previous year. For example, first sampling date in 2007, 2008 and 2009 was in mid-June, mid-May, and late May, respectively. Sampling was carried out from 4-leaf stage for 9 weeks at approximately 7-day intervals between 10 a.m. and 12 p.m. Through randomised sampling, necessary information regarding distribution pattern, frequency, and population variation were obtained.

#### 2- Spatial distribution pattern of pest population

Leafhopper density in each field and sampling date was considered as a population density index in the field and the average field results in each region was considered as population density of the region. Therefore, to determine the spatial distribution pattern of *C. haematoceps* population in sugar beet fields, variance to mean ratio ( $S^2/\bar{x}$ ) was used. In this ratio, values greater than one indicates aggregated distribution, equal to one indicates random distribution, and less than one shows uniform spatial distribution. Deviation from a random distribution can be tested by calculating the index of dispersion ( $I_D$ ):

$$I_D = \frac{(n-1)S^2}{\bar{x}}$$

where  $S^2$  denotes the variance and  $\bar{x}$  is mean data. This index can be tested by Z value as follows:

$$Z = \sqrt{n \cdot I_D} - \sqrt{(2V-1)}$$

where n is the number of samples and  $V = n-1$ .

If  $1.96 \geq Z \geq -1.96$ , the spatial distribution would be random but if  $Z < -1.96$  or  $Z > 1.96$ , it would be uniform and aggregated, respectively. In this method, data from each year and also the total of three years were analyzed together.

**Table 1.** ANOVA results for leafhopper population and R.V. values in different years

Year	Mean (leafhopper number)	Sample number	Variance	Standard deviation	Standard error	Calculated standard error	Result
2007	8.00	10	9.50	3.082	0.974	12.2	Acceptable
2008	18.77	10	4.944	2.223	0.703	6.52	Acceptable
2009	3.77	10	3.94	1.984	0.627	16.65	Acceptable

**Table 2.** ANOVA results for determination of *C. haematoceps* spatial distribution pattern using variance to mean ratio

Year	Mean (m)	Variance ( $S^2$ )	Variance to mean ratio ( $S^2/m$ )	Index of dispersion ( $I_b$ )	Z
2007	7.66	16.25	2.12	16.97	2.08
2008	7.66	20	2.61	20.88	2.72
2009	29.44	262.03	8.90	71.20	8.19
Mean	14.74	59.03	4.00	32.03	4.26

### 3- Leafhopper population fluctuation

All specimens collected were killed in killing bottle containing potassium cyanide to immobilize insects, or after shaking the net, insects were transferred into the water tub and leafhoppers were separated using a brush, or trapped leafhoppers inside the net were transferred into a plastic bag and were taken to the laboratory. Leafhoppers collected inside plastic bags were immobilized for 10 minutes inside freezer and after separation and recording were temporally preserved in 75% ethyl alcohol. Identification was done following Nielsen (1985) and Khajehali et al. (2001) methods. Diagrams related to leafhopper population variation at early stage of sugar beet growth were drawn using total population frequency of leafhoppers. Population variation of *C. haematoceps* species was compared with the total population of leafhoppers.

### 4- Determination of infected plants to curly top disease in the field

At the beginning of the growing season, the field infection to curly top was determined by walking inside the field (in M shape pattern), placing 10 quadrates ( $1 \text{ m}^2$ ) at different sites, and counting number of healthy and infected plants per quadrate. For evaluation and determination of infected plants percentage, MacFarlane and Bennett (1968) method was used. In early growing stages, the symptoms and intensity of the disease were recorded. Disease severity included the following conditions:

1) vein clearing, 2) vein clearing and slight leaf curl, 3) small enations on the underside veins of the leaves with curling edges, 4) enations on the undersides veins of the leaves with curling edges and dramatic growth reduction, 5) plant dead.

### 5- Determination of linear relationship between disease severity and vectors population density

To draw graph, vectors population frequency was placed in horizontal axis and infection to curly top was depicted in vertical axis.

Based on the ANOVA results, RV values for all three years were in acceptable range.

## RESULTS AND DISCUSSION

### 1- Leafhopper population sampling in the field

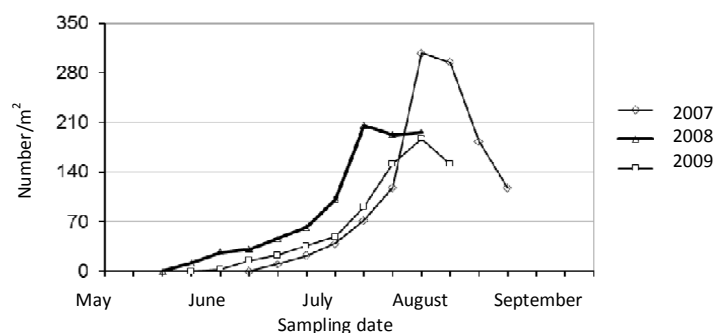
### 2- Determination of the spatial distribution pattern of pest population

Using variance to mean ratio, ANOVA results for 2007, 2008 and 2009 and also the total of three years showed that Z value for each year and also total of three years was more than 1.96 indicating cumulative spatial distribution (Table 2).

### 3- Population fluctuation in the field and identification of leafhoppers

Figure 1 indicates leafhopper population fluctuation for three years.

First leafhopper appearance was observed at 4-leaf stage and by increase in temperature, their population increased in the following days. In late July, leafhopper population was several times higher than June. Identification and separation of leafhopper population for three years resulted in the identification of eight genus and two species from Cicadellidae and Delphasidae families (Table 3, 4, and 5). *Empoasca decipiens* (Paoli) was determined as a dominant species in the region and from the beginning to the end of the growing season, high population of it was observed. This species had a 69-91% frequency and although it doesn't have any role in curly top transmission but because of high density and its activity in all sugar



**Figure 1.** Total leafhopper population fluctuation for three years

**Table 3.** Frequency and distribution of leafhopper species in sugar beet planting fields in Asadabad, Hamedan (2007)

Identified genus and species	Sampling date										Number	Population (%)
	12.06.2007	20.06.2007	26.06.2007	03.07.2007	10.07.2007	17.07.2007	23.07.2007	29.07.2007	05.08.2007			
<i>Empoasca decipiens</i>	31	69	93	171	211	924	896	4878	328	3210	68.8	
<i>Psammotettix alienus</i>	2	2	33	48	83	103	104	85	41	501	10.7	
<i>Laodelphax striatellus</i>	0	0	2	25	87	98	92	85	52	441	9	
<i>Zyginidia sohrab</i>	5	4	7	14	41	45	36	42	25	219	5	
<i>Circulifer haematoceps</i>	3	13	23	27	46	51	47	31	24	265	5.68	
<i>Euscelis insisus</i>	0	0	0	2	2	3	2	1	0	10	0.2	
<i>Macrosteles laevis</i>	0	0	0	1	2	5	3	1	0	8	0.2	
<i>Platymetpius rosteratus</i>	0	0	0	0	0	2	1	0	0	3	0.06	
Matured leafhopper number (male and female)	41	88	158	288	427	1231	1181	732	470	4661	100	

**Table 4.** Frequency and distribution of leafhopper species in sugar beet planting fields in Asadabad, Hamedan (2008)

Identified genus and species	Sampling date										Number	Population (%)
	14.05.2008	22.05.2008	31.05.2008	08.06.2008	18.06.2008	01.07.2008	09.07.2008	16.07.2008	22.07.2008			
<i>Empoasca decipiens</i>	39	95	102	140	195	364	792	752	770	3249	92.7	
<i>Psammotettix alienus</i>	0	0	0	2	5	3	3	2	0	15	0.41	
<i>Laodelphax striatellus</i>	0	2	5	12	25	17	10	5	3	79	2.19	
<i>Zyginidia sohrab</i>	2	3	12	3	5	2	2	8	8	47	1.3	
<i>Circulifer haematoceps</i>	3	5	5	9	8	12	14	12	1	69	1.97	
<i>Euscelis insisus</i>	0	0	0	6	4	4	0	0	0	14	0.38	
<i>Macrosteles laevis</i>	0	0	0	1	3	6	5	3	0	18	0.5	
<i>Platymetpius rosteratus</i>	0	0	0	0	0	0	0	0	0	0	0	
Matured leafhopper number (male and female)	44	105	124	185	247	408	824	772	782	3491	100	

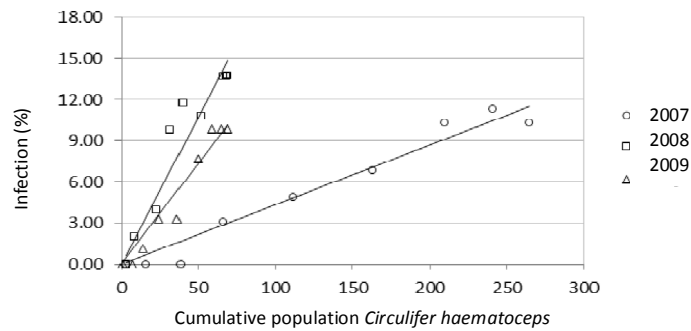
beet growth stages, it imposes a considerable damage to the crop. *C. haematoceps* (M and R) is identified as the main vector for curly top (Nielsen 1968) and its population frequency in 2007, 2008, and 2009 was 5.68, 1.97, and 2.43%, respectively. In this study, from the beginning of sampling, *C. haematoceps* population percentage was low and its activity continued until the last week of sampling. However, *C. tenellus* species, which is one of the most important vectors for beet curly top transformation, was not found.

#### 4- Evaluation of sugar beet fields infection to curly top

In 2007, *C. haematoceps* population had the highest frequency (5.68%) compared with other years. However, irrespective of high population frequency, infection rate was lower than other years. The reason for this difference might be due to the delay in planting in 2007 which increased the initial growth rate of the plants and prepared them for field resistance. In 2008, the proportion

**Table 5.** Frequency and distribution of leafhopper species in sugar beet planting fields in Asadabad, Hamedan (2009)

Identified genus and species	Sampling date									Number	Population (%)
	24.05.2009	31.05.2009	07.06.2009	21.06.2009	30.06.2009	04.07.2009	09.07.2009	19.07.2009	26.07.2009		
<i>Empoasca decipiens</i>	9	47	81	122	152	310	563	694	582	2580	91
<i>Psammotettix alienus</i>	0	0	2	3	2	2	4	3	2	18	0.6
<i>Laodelphax striatellus</i>	0	0	2	4	11	8	5	6	3	39	1.4
<i>Zyginidia sohrab</i>	2	7	8	5	15	30	27	19	13	126	4.5
<i>Circulifer haematoceps</i>	1	6	7	10	12	14	9	6	4	69	2.43
<i>Euscelis insisus</i>	0	0	0	0	0	0	0	0	0	0	0
<i>Macrostelus laevis</i>	0	0	0	0	0	0	0	0	0	0	0
<i>Platymetpius rosteratus</i>	0	0	0	0	0	0	0	0	0	0	0
Matured leafhopper number (male and female)	12	60	100	144	192	364	608	748	604	2832	100

**Figure 2.** Leafhopper cumulative frequency correlation with plant infection for three years

of leafhopper population compared to total population was 1.97% which was lower than 2007 and 2009. However, in 2007 and 2008, the total leafhopper population was equal. In 2008, the average infection (8.6%) was higher than 2007. In 2008, due to early planting and slow growth of the plants, vectors had more time for distribution. In 2009, the proportion of leafhopper population compared to total population was 2.43% (with an average of 5%) which was lower than 2007. In general, different plant densities and dates influenced infection percentage. In early planting, due to the slow growth rate and coincidence of this stage with leafhopper vectors activity, infection percentage was high. In a study by Jalali et al. (2006), the effect of planting date and sugar beet variety on curly top outbreak and vectors population was evaluated in Isfahan province. Results showed that the infection was significantly high in first planting date compared with second and third ones. Kheyri (1991) showed that leafhoppers migration occurred in early April and in early planting dates in Fars province. In this study, due to different reasons, seed germination percentage was not uniform. Because of low plant density,

proper sun radiation into canopy, better ventilation, and relative humidity reduction, leafhopper population increased quickly than other fields and as a result infection rate was high. This is in accordance with the earlier study of Ashrafmansoori et al. (2010). Therefore, delay in planting, increase in environment temperature, and as a result increase in plant growth rate and field uniformity, decreased leafhopper migration from outside areas into the field. On the other hand, with a reduction in plant growing period, planting dates and plant growth rate in each region become similar and vectors may not have the opportunity to transfer the disease into other fields. However, because of the probability of yield reduction, late planting is not recommended.

##### 5- Determination of linear relationship between leafhopper population and virus frequency

At this stage, data obtained for vectors frequency in each field was compared with curly top frequency. In Figure 2, regression line and related equation is presented. Coefficient of determination coefficient ( $R^2$ ) for three consecutive years was 96, 90, and 94%, respectively indicating

**Table 6.** Average of *C. haematoceps* frequency and plant infection to curly top in simultaneous sampling

Sampling number	Average of leafhopper number and plant infection percentage					
	2007		2008		2009	
	Leafhopper number	Contamination (%)	Leafhopper number	Contamination (%)	Leafhopper number	Contamination (%)
1	3	0	3	0	1	0
2	13	0	5	1.96	6	0
3	23	0	5	3.92	7	1.09
4	27	3.09	9	9.8	10	3.29
5	46	4.9	8	11.76	12	3.29
6	51	6.86	12	10.78	14	7.69
7	47	10.3	14	13.72	9	9.89
8	31	11.34	12	13.72	6	9.89
9	24	10.3	1	13.76	4	9.89
Total leafhopper vectors number	265	-	69	-	69	-
Total leafhopper vectors number to total species ratio (%)	5.68	-	1.97	-	2.43	-
Average plant contamination to beet curly top disease		5.2		8.6		5

strong association between leafhopper population frequency and disease occurrence and distribution in the growing season. According to the results, reduction in leafhopper population is one of the ways to decrease disease damage. Also, determination of vectors spatial distribution pattern has an effective role in the design of appropriate sampling programs.

Generally, curly top can be controlled by different methods, among which breeding and resistant cultivar application rank first. Following resistant cultivar application, conducting a control program against vectors in their natural habitat and also in the field area is one of the effective methods. Results showed that using proper insecticide and as a result delay in the contamination of young seedlings, significantly decreased disease severity. Conducting a control program against leafhopper population during winter, contributes to the reduction of the disease outbreak. As weed population decreases, leafhoppers overwintering on weed debris can be controlled by spraying. Using systematic insecticide is another way to control leafhopper, although, sprayed plants may be contaminated by leafhopper feeding but the insect will die before contaminating other plants. The following studies are recommended for further evaluation (from the perspective of plant protection): evaluation of leafhopper vectors in natural habitat during off-season, determination of the natural leafhopper habitat location and distribution in the country, identification of leafhopper hosts in off-season, and conducting a control program against leafhopper vectors before their migration. In early planting or in other words on-time planting, with increase in plant age, resistance to contamination increases and the dis-

ease damage's decreases. In early planting, plants have enough time for growing before leafhopper migration from mountainous regions; therefore, the damage percentage will be low. In general, every factor causing a rapid growth and development of sugar beet plant would be useful in the disease control and reduction of its damage. Early infection increases the damage percentage. Proper plant density may also reduce disease severity.

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