NARDI FUNDULEA, ROMANIA
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ROMANIAN AGRICULTURAL RESEARCH, NO. 34, 2017
Print ISSN 1222-4227; Online ISSN 2067-5720

EFFECT OF SEEDLING TRANSPLANTATION AND POST-EMERGENCE HERBICIDES APPLICATION ON FIELD DODDER (*CUSCUTA CAMPESTRIS*) CONTROL IN SUGAR BEET

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ABSTRACT

In order to control field dodder (*Cuscuta campestris* Y.) in sugar beet, experiments were done in greenhouse and field of Sugar Beet Research Institute, Karaj, Iran. In greenhouse test, the impact of direct seed sowing, transplanting of seedlings at two and eight-leaf stages (factor A) and dodder seed sowing with no distance and at 7 cm distance from the host (factor B) on control of the weed was studied in a factorial experiment based on completely randomized design with three replications. In the field test, the effects of direct seed sowing and transplanting of sugar beet seedlings (factor A) and application of four post-emergence herbicides (factor B) on control of the weed were evaluated in a factorial experiment based on randomized complete block design with four replications. In the greenhouse experiment, percentage of infection was decreased by increasing the distance between the parasite and the host. In the field experiment, the degree of host infection by dodder was recorded before and after application of the herbicides. Fresh and dry weight of dodder and root yield of sugar beet was measured. The method of cultivation was significantly effective on the ability to deploy of dodder on the host so that in direct sowing of sugar beet seed, the ability of dodder to colonize the host was found to be 3.5 times higher than that of transplanting method. Root yield was significantly influenced by the sowing method and showed 2.5 times increase in transplanting method. The biomass of the parasite established on the host demonstrated 7.5 times decrease compared to the direct seed sowing method.

Key words: direct sowing dodder, herbicide, sugar beet, transplantation.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important industrial crops in the world which plays a strategic role in supplying the energy needed by human. Due to the increased demand for sucrose worldwide, the areas of sugar beet cultivation increased rapidly from its origin in central Europe to the world and it is now produced as an industrial crop in all continents except Australia (Cooke and Scott, 2011). In 2014, the total area of sugar beet cultivated by 50 countries was 3442745 ha with a total production of 205874329 t root and average yield of 59.80 t ha^{-1}; among these countries, the total cultivated area, average root yield and total production in Iran were 77720 ha, 42.6 $t.ha^{-1}$ and 3309890 t, respectively (FAO STAT, 2014).

Although weeds comprise only 1% of the xerophilic plants, they have been among the factors causing yield loss of field crops in agro-ecological systems and impose latent but remarkable economic losses. Bandegi and Armin (2014) reported 57% decrease in root yield and 84% decrease in sugar yield of sugar beet due to complete interference of weeds with the crop. Among the weeds, Field dodder is considered as a challenge (Parker, 2012) and major threat (Sandler, 2010) to sugar beet production.

The dodder weed is a plant of Cuscutaceae family which has been assigned

Received 12 November 2016; accepted 7 February 2017. First online: March, 2017. DII 2067-5720 RAR 2017-119

to Convolvulaceae in some references. It is an annual, obligatory parasitic plant without any leaf and green tissue and with a high power for seed production (Zaroug et al., 2014a, b). So far, 200 species of this plant in the world (Lanini and Kogan, 2005) and 18 species in Iran (Tajdoost et al., 2013) have been identified. All Cuscuta species have been adapted towards the parasitic life (Sharifi et al., 2013).This parasitic species has been introduced as a problematic weed in over 50 countries in the world (Costea and Tardif, 2006).

Field dodder (*Cuscuta campestris*) is one of the 10 important problem weeds in the world (Nadler-Hassar and Rubin, 2003). Wide diversity of the biotypes of the Field dodder has caused different responses of this parasitic weed to herbicides (Nadler-Hassar et al., 2009). As the sugar beet parasitic weed, the Field dodder is of special importance in Iran as well (Mousavi, 2015) which infects a high proportion of sugar beet fields annually (Jafarzadeh et al., 2015a).

Melander (2000) reported that seedling transplantation, five days after transplantation, provides proper conditions for mechanical weeding and that transplantation decreases the competition ability of the weeds and the costs of mechanical weeding in production of organic sugar beet. A study on the effect of seedling transplantation on uniformity of plant establishment and control of weeds demonstrated that seedling transplantation decreased the costs of weed control remarkably (Khaembah and Nelson, 2016).

MATERIAL AND METHODS

This research was conducted in two independent field and greenhouse experiments in 2014. In the greenhouse test, which was carried out at Sugar Beet Seed Institute (SBSI), Karaj, Iran, the impact of direct seed sowing (a_1), seedling transplantation at two-leaf (cotyledonous) (a_2) and eight-leaf (a_3) stages (factor A) and dodder seed sowing with no distance (b_1) and 8 cm distance (b_2) from the hostcrop (factor B) on control of the weed was studied in a factorial experiment based on completely randomized design with three replications. Each treatment contained 48 pots per replication and the temperature of the greenhouse was maintained at 25±2°C. The measured traits included percent of germination of dodder seed, percent of attachment of the weed to the host crop and percent of penetration of the weed seedlings into the host tissue and ability to infect it. Firstly, the dodder seeds were treated with concentrated (98%) sulfuric acid for 30 m to break the seed dormancy. The host treatments (factor A) were employed to the pots filled with proper soil mixture and the dodder seeds were sown at 0.5 cm depth on the basis of the predetermined treatments (with and without distance from the host).

The sugar beet seedlings for the treatments of the greenhouse experiment were prepared by using the naked-root seedlings method (Yousefabadi et al., 2015). From the second day after sowing on, the status of the germination of the dodder seed, success of the parasite to reach the host and also penetration into the host tissue and creation of infection were recorded daily.

In the field test, the effects of sowing method and application of post-emergence herbicides on control of the Field dodder were evaluated in sugar beet field. In this experiment, direct seed sowing and transplanting of sugar beet seedlings (factor A) and four post-emergence herbicides including the two formulations of propizamid, Wettable powder (WP 50%, Kerb) for 2.5 $kg.ha^{-1}$, and Suspension concentrate (SC 50%, Burst) for 2.5 $l.ha^{-1}$, Autofomezat with concentrated suspension formulation (SC500, Auto) for 2.5 $l.ha^{-1}$and Glyphosate (Roundup) with water-soluble liquid formulation (SL42%) for 0.1 $l.ha^{-1}$ along with the parasite-infected controls without herbicide and without infection by the parasites (factor B) were evaluated in a factorial experiment based on randomized complete block design with four replications in the microplots provided.

After the microplots were uniformly infected with the dodder seed, the sugar beet seeds and naked-root seedlings were simultaneously sown in the microplots. In the seedling transplantation method, firstly the required seedlings were prepared in the nursery free from any infection to the weed seed including Field dodder. The seedlings were then transplanted to the main field at proper time (Yousefabadi, 2017) at final density (20 cm distance from each other). The irrigation was conducted by drip, tape irrigation method. The plots directly sown were thinned at 2-4-leaf stage with 15 cm on-row spacing. Before applying the chemical control treatments, the weeds of the experimental plots, except the field dodder, were controlled by mechanical method. The rate of infection of the plots to the dodder was assessed by visual scoring (Wang et al., 1995) before applying the herbicide treatments. In this method, the scores 1 to 9 were considered for the lowest and highest infection to the parasite, respectively. The specific leaf area (SLA) of the host in each treatment was measured at the middle of the growth period. In order to measure this trait on the host at direct seed sowing and seedling transplantation methods, the leaf blades of the host plants were randomly sampled before applying the herbicide treatments. The leaf area was determined using leaf area meter (Delta T, Cambridge, UK). In order to measure the dry weight of the samples, they were oven dried at 75°C for 24 h and then the dry weight of the leaves was measured by a digital balance. The specific leaf area ($cm^2.g^{-1}$ dry matter) was calculated using the equation 1:

$$SLA=LA/LDW \quad (1)$$

where: LA and LDW are the leaf area and leaf dry weight, respectively.

After the parasite attached to the host (40 days after sowing), the herbicide treatments were applied in the microplots as foliar spraying. Measurement of the rate of infection of the host to the parasite was repeated one month after application of the herbicide treatments. Before harvesting the crop, the parasite was collected from 1 m^2 area per plot on the host and its biomass per unit area was determined. At the end of the growing season, each plot was harvested separately. After dissecting the above-ground part from the root, root yield, raw sugar yield, white sugar yield and weight of the above-ground part were measured. Analysis of variance and mean comparisons were performed by SAS 9.1 software.

RESULTS AND DISCUSSION

Results of analysis of variance in greenhouse experiment (Table 1) showed that the germination of the field dodder seed was not affected by sugar beet sowing method and sowing distance from the host. This is in line with the findings of Ganbari et al. (2012). In contrary to the parasitic broom rape plant which needs host plant and the stimulating material released from it, the dodder seed needs no special host and stimulating material released from it for germination and emergence.

Table 1. Mean of squares for germination percent, percent of seedling attachment to host, and percent of penetration of the dodder seedling into the host tissue

Source of variation	df	Mean Square		
		Percent of germination	Percent of attachment	Percent of penetration and establishment
Sowing method (A)	2	0.39ns	172.7**	3394.9**
Parasites distance from host (B)	1	112.5ns	6013.0**	4449.0**
A×B	2	29.2ns	9.6ns	310.2*
Error	12	26.2	29.2	54.4
Coeff. Var. (%)		5.8	8.3	15.6

^{ns}, * and ** show non-significance and significance at 5 and 1% probability levels, respectively.

Percent of dodder seedling attachment to the host and also percent of penetration of the dodder seedling into the host tissue and creation of effective infection were significantly ($p \leq 0.01$) affected by sowing method and parasite distance from the host.

The method of sugar beet sowing influenced the ability of the dodder seedlings to attach the host and the means were separated into two groups. In the sugar beet direct seed sowing treatment, 64.8% of the parasite seedlings were able to penetrate into the host tissue and create effective infection. The figure was 58.5% in the two-leaf seedlings (transplants) which formed the same group with the direct sowing treatment. In the eight-leaf seedling transplantation treatment, only 20.8% of the parasite seedlings were able to penetrate into the host tissue and create effective infection and the related means were placed in independent groups (Table 2).

Mean comparison indicated that 83.8% of the dodder seedlings which were sown with no distance from the host reached the host, whereas only 47.2% of the seedlings sown with a distance of 8 cm from the host could reach the host.

The extents of penetration of the parasite haustorium into the host tissue and creation of effective infection in the treatments sown with and without distance from the host were 32.3 and 63.8%, respectively (Table 2). In the treatments sown with and without distance from the host, 68.4 and 76.1%of the seedlings which reached the host were able to create effective infection. This means that the dodder seedlings consume some of their food reserves to reach the host tissue, so that the longer the distance of the dodder seedling from the host, the higher the consumption of food reserves is and so less energy is left for the formation and penetration of haustoria into the host tissue and creation of effective infection. This is in accordance with the results of Benvenuti et al. (2005).

Table 2. Mean comparison of the levels of factors for the traits not affected by the interaction of factors

Factors	Germination of dodder seed	Attachment of dodder to the host	Haustorium penetration onto the host tissue
	%		
Sowing method (A)			
Direct seed sowing	87.8a	71.2a	64.8a
Two-leaf seedling	87.5a	64.0b	58.5a
Eight-leaf seedling	87.3a	60.5b	20.8b
LSD (5%)	6.4	6.8	9.5
Distance of dodder from the host (B)			
No distance	90.1a	83.7a	63.8a
8 cm distance	85.1a	47.2b	32.3b
LSD (5%)	5.3	5.6	7.5

Analysis of variance for the data obtained from the final harvest of the field-grown sugar beet showed that the method of sowing significantly ($p<0.01$) influenced root yield, raw sugar yield, white sugar yield and specific leaf area of the host. The post-emergence herbicides also significantly ($p<0.01$) influenced the traits except specific leaf area. The interaction of sowing method and herbicide application was non-significant for the above-mentioned traits (Table 3).

Mean comparisons showed that seedling transplantation as compared with direct sowing decreased specific leaf area from 210 to 161 $cm^{2}\cdot g^{-1}$ dry weight of sugar beet leaf. This indicates that the leaves of transplanted sugar beet plants at early growing season which coincides with germination of dodder seed and attack of its seedlings to the host, are thicker and woodier than the leaves of the directly sown plants and have lower specific leaf area. These characteristics can provide resistance to penetration of the dodder haustoria into the host tissue and unsuccessful establishment of the parasite on the transplanted plants compared with the directly sown plants of sugar beet and can be the reason for lowered infection rate to the parasite (Table 4). The other main reason for the lower infection to the dodder weed in the seedling transplantation treatment could be assigned to the plant population per unit area and/or on-row spacing of the sugar beet plants.

Table 3. Analysis of variance for root yield, sugar yield, white sugar yield and specific leaf area in sugar beet

Source of variation	df	Mean Square			
		Root yield	Sugar yield	White sugar yield	Specific leaf area
Replication	3	37.0	1.17	0.61	618.7
Sowing method (A)	1	10384.0**	309.69**	190.0**	14686.0**
Herbicides (B)	3	243.0**	9.16**	6.26**	280.4ns
A×B	3	30.0ns	0.55ns	0.47ns	498.6ns
Error	21	11.0	0.37	0.28	367.0
Coeff. Var. (%)		7.8	9.2	10.4	10.0

^{ns}, * and ** show non-significance and significance at 5 and 1% probability levels, respectivel

In the seedling transplantation treatment, the seedlings were transplanted on the rows at final density (with 15 cm distance), whereas in the direct sowing treatment, the sugar beet seeds were first sown on the rows with more density and then thinned at 4-6-leaf stage (Cooke and Scott, 2011). Therefore, in the direct sowing treatment, the higher plant density and lower distance between the host plants greatly increase the chance of the parasite seedlings to reach and successfully attach the host compared with the seedling transplantation treatment. Benvenuti et al. (2005) reported that the distance between the dodder weed and the host affects the rate of infection of the host which is decreased when the distance is increased. Jafarzadeh et al. (2015b) reported that when the distance between the parasite and host increased to 15 cm, the rate of infection of sugar beet by the parasite decreased by 47%.

Table 4. Mean comparisons for the host specific leaf area, root yield, raw sugar yield and white sugar yield

Treatment		Specific leaf area $cm^2.g^{-1}$	Root yield	Raw sugar yield	White sugar yield
			$t.ha^{-1}$		
Levels of factor A					
Direct sowing		210^{a}	23.4^{b}	3.6^{b}	2.6^{b}
Seedling transplantation		167^{b}	59.4^{a}	9.8^{a}	7.5^{a}
Levels of factor B					
Auto		192^{ab}	33.4^{b}	5.14^{c}	3.8^{c}
Propizamide (Kerb)		180^{b}	45.8^{a}	7.5^{a}	5.6^{ab}
Propizamide (Burst)		194^{ab}	42.2^{a}	6.7^{b}	5.1^{b}
Glyphosate (Roundup)		189^{ab}	44.2^{a}	7.4^{ab}	5.7^{a}
Direct sowing	Infected to parasite (control 1)	183.7	16.2	2.5	1.8
	Non-infected to parasite (control 2)	301.6	35	5.12	3.7
Seedling transplantation	Infected to parasite (control 3)	182.8	54	8.9	6.9
	Non-infected to parasite (control 4)	170.5	63.3	11.1	8.8

In the sugar beet direct seed sowing method, root yield, raw sugar yield and white sugar yield were 23.4, 3.6 and 2.6 $t.ha^{-1}$, respectively, whereas in the transplantation method, the figures were 59.4, 9.8 and 7.5 $t.ha^{-1}$, respectively. This indicates that sugar beet seedling transplantation method increased root yield, raw sugar yield and white sugar yield 2.5, 2.7 and 2.9 times as compared with the direct seed sowing method. In this research, two reasons could be mentioned for the increased yield of sugar beet in the seedling transplantation method. First, results showed that seedling transplantation increases sugar beet yield. Second, seedling transplantation significantly decreased the rate of infection of sugar beet to the dodder weed so that the weed infection scores in the control, infected seedling transplantation (control 3) and direct sowing (control 1) methods were 1.8 and 7.3, respectively. In other words, the rate of infection to the dodder weed in the seedling transplantation method was four times lower that in the direct sowing method (Table 6).

In a three-year field study conducted by Yousefabadi et al. (2014), the increase in root yield and white sugar yield in sugar beet seedling transplantation method was 40 and 35.5% higher than that in the direct seed sowing method. The rate of increase in sugar beet yield by seedling transplantation method was reported to be 31% (Karbalaei et al., 2012) and 70% (Nasri et al., 2011).

The effect of herbicides on yield traits of sugar beet was also significant (Table 3). The highest root yield (45.8 $t.ha^{-1}$) was obtained by application of propizamide with wettable powder formulation (WP 50%). The yield difference of this treatment with Glyphosate and propizamide with concentrated suspension formulation (SC 50%) treatments was not significant and the means were placed in one group (a), but it had significant difference with Autofomezat. The lowest root and sugar yields were obtained by the application of Autofomezat herbicide (Table 4). So, it can be said that this treatment does not have any significant effect on control of the Field dodder weed in sugar beet cultivation. The sugar beet seedling transplantation treatment with no infection to the parasite and without application of herbicide (control 4) produced the highest root yield (63.3 $t.\ ha^{-1}$), raw sugar yield (11.1 $t.\ ha^{-1}$) and white sugar yield (8.8 $t.ha^{-1}$) and the lowest specific leaf area (170 $cm^2.g^{-1}$ leaf dry weight). The lowestroot yield (16.2 $t.\ ha^{-1}$), raw sugar yield (2.5 $t.\ ha^{-1}$) and white sugar yield (1.8 $t.ha^{-1}$) were obtained by application of the parasite-infected direct sowing treatment (control 1) (Table 4).

The effect of sowing method, herbicide application and interaction of the factors on the infection ability of the dodder weed and its biomass (fresh and dry weight of the parasite tissue established on the host) is shown in Table 5. Results show that the sowing method, either before or after the application of herbicide treatments, significantly ($p<0.01$) influenced the ability of the dodder weed to infect the host. The sowing method had also significant effect ($p<0.01$) on the biomass of the parasite tissue established on the host. The interaction of sowing method and application of post-emergence herbicides was also significant for the fresh and dry weight of the parasite tissue and its infection ability (Table 5).

Table 5. Analysis of variance for the traits measured on the dodder weed established on the host (sugar beet)

Source of variation	df	Mean Square			
		Infection score		Biomass of parasite	
		Before treatment	After treatment	Fresh weight	Dry weight
Replication	3	3.28	3.01	185.4	11.28
Sowing method (A)	1	270**	63.28**	30634**	2568**
Herbicides (B)	3	2.53ns	53.26**	33306**	1592**
A×B	3	0.86ns	8.21**	5494**	537.5**
Error	21	0.9	0.84	1620.6	112.8
Coeff. Var. (%)		18	20	8.2	8.3

^{ns},* and ** show non-significance and significance at 5 and 1% probability levels, respectively.

At early growing stages and before thinning, the lower distance between the host plants in the direct seed sowing method increased the chance for the dodder seedlings to be placed close to the host. As a result, the percent of attachment of the parasite seedlings to the host and creation of infection to the parasite was increased (Table 6). These results are in accord with the findings of Benvenuti et al. (2005) and Jafarzadeh et al. (2015b).

The highest infection rate of the dodder weed (score 8.5) was obtained in the direct seed sowing and application of Autofomezat treatment. This treatment formed a separate statistical group (group a). The lowest infection rate (score 1.5) was observed in the sugar beet seedling transplantation and application of the Propizamide (Kerb) herbicide treatment which was grouped with the application of the Propizamide (Brest) herbicide treatment in the seedling transplantation (score 1.6), the Propizamide (Kerb) herbicide treatment in the direct sowing treatment (score 1.8) and Glyphosate (score 2.5) in one statistical group (group c).

The highest biomass of the parasite tissue (178 g.m^{-2} fresh weight and 49.5 g.m^{2} dry weight) was obtained in the sugar beet direct seed sowing and application of the Autofomezat herbicide and formed a separate statistical group (group a). Also, this treatment, after the application of herbicide in different treatments, gained the highest parasite infection score (8.5) which did not have a significant difference with the infected control.

Table 6. Mean comparison of the interaction of the levels of factors for the traits affected by the interaction of the two factors

Sowing method	Herbicide	Parasite infection score		Parasite fresh weight	Parasite dry weight
		Before treatment	After treatment	g.m^{-2}	
Direct sowing	Auto	8.5^{a}	8.5^{a}	178^{a}	49.5^{a}
	Propizamide (Kerb)	7.5^{a}	1.8^{c}	33.7^{cd}	7.8^{b}
	Propizamide (Burst)	7.5^{a}	6.9^{b}	65^{bc}	$15.^{bc}$
	Glyphosate (Roundup)	8.8^{a}	6.9^{b}	104.5^{b}	28.9^{b}
Seedling transplantation	Auto	3.3^{b}	6.8^{b}	66.5^{bc}	13.9^{bc}
	Propizamide (Kerb)	1.3^{c}	1.5^{c}	0.0^{d}	0.0^{c}
	Propizamide (Burst)	2.3^{bc}	1.6^{c}	0.0^{d}	0.0^{c}
	Glyphosate (Roundup)	2.3^{bc}	2.5^{c}	5.1^{d}	1.4^{c}
Direct sowing	Infected to parasite (control 1)	7.3	9.0	164	45.3
	Non-infected to parasite (control 2)	1.0	1.0	0.0	0.0
Seedling transplantation	Infected to parasite (control 3)	1.8	5.0	66.2	15.7
	Non-infected to parasite (control 4)	1.0	1.0	0.0	0.0

CONCLUSION

In the greenhouse experiment, germination of the field dodder seed was not affected by sugar beet sowing method and sowing distance from the host, whereas percent of dodder seedling attachment and its penetration into the host tissue were significantly affected by sowing method and parasite distance from the host. In the field experiment, the method of cultivation was significantly effective on the ability of dodder to colonize the host so that in direct sowing of sugar beet seed, the ability of dodder to colonize the host was 3.5 times higher than that of transplanting method. Root yield was significantly influenced by the sowing method and showed 2.5 times increase in transplanting method. Also, the biomass of the parasite established on the host demonstrated 7.5 times decrease compared to the direct seed sowing method. The lowest infection rate (score 1.5) was observed in the sugar beet seedling transplantation and application of the Propizamide (Kerb) herbicide treatment.

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