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Impact of *Cuscuta* spp. seed size variability on machine operation during seed finishing of natural alfalfa seeds

Original Article

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Abstract:
The alfalfa seeds intended for marketing must not contain any seeds of the dodder (*Cuscuta* spp.), because it is a quarantine weed in Serbia and the world. *Cuscuta* spp. are frequently found in natural alfalfa seeds; nevertheless and because of this, the seed must be processed, i.e. cleaned of weeds. The paper presents a study of seed size variability of *Cuscuta* spp. of four seed lots of natural alfalfa seeds originating from Banat, Serbia. From each of the four examined seed lots of natural alfalfa seeds, a difference in seed size was determined for 30 seeds of *Cuscuta* spp. per lot. The difference in seed length of *Cuscuta* spp. was 371 µm, and the difference in seed width was 330 µm. The coefficient of variation varied from CV=8.356% to 12.00% for seed width; and from CV=9.383% to 10.69% for seed length. Seed size of *Cuscuta* spp. did not significantly affect the efficiency of magnetic separator work in extracting *Cuscuta* spp. seeds from natural alfalfa seeds.

Key words:
Cuscuta spp., variability, seed width and seed length, seed processing

Apstrakt:
Uticaj varijabilnosti veličine semena *Cuscuta* spp. na rad mašina pri doradi naturalnog semena lucerke
Seme viline kosice (*Cuscuta* spp.) je karantinski korov u Srbiji i svetu i zato se u semenu lucerke koje je namenjeno prodaji, ne sme naći seme korova *Cuscuta* spp. Međutim, seme *Cuscuta* spp. se relativno često može naći u naturalnom semenu lucerke i zbog toga se seme mora doraditi, odnosno očistiti od korova. U radu je prikazano ispitivanje varijabilnosti veličine semena *Cuscuta* spp. koja je izdvojena iz naturalnog semena četiri partije semena lucerke poreklom iz Banata, Srbija. Na po 30 semena *Cuscuta* spp., koja su izdvojena iz svake od četiri ispitivane partije naturalnog semena lucerke, uvrđena je varijabilnost za veličini semena. Razlika po dužini semena *Cuscuta* spp. je bila 371 µm, a po širini 330 µm. Izraženo kroz varijabilnost, koeficijent varijacije se kretao od CV=8.356 do 12.0% za širinu semena i od CV=9.383 do 10.69% za dužinu semena. Veličina semena *Cuscuta* spp. nije uticala značajno na efikasnost rada magnetnog separatora prilikom izdvajanja iz naturalnog semena lucerke

Ključne reči:
Cuscuta spp., varijabilnost, širina i dužina semena, proces dorade

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Introduction

Dodder (*Cuscuta* spp.) belongs to the genus *Convolvulaceae* which includes about 200 mostly parasitic species (Costea et al., 2015 a,b). It is a parasitic plant that causes significant economic damage around the world (Dawson et al., 1994; Costea & Tardif, 2006; Sarić-Krsmanović et al., 2020). It is especially harmful in alfalfa seed crops (Dawson et al., 1994; Sarić-Krsmanović et al., 2020).

Dodder seed causes damage by extracting nutrients from the parent plant; thus, the parent plant weakens, becomes exhausted and lags behind in development. About ten species of *Cuscuta* spp. were detected in Serbia in the 1960s and 1970s, and they can cause significant damage to agricultural crops (Stojanović et al., 1973), with a particularly large problem in alfalfa (Mijatović & Stojanović, 1968). In agronomic practice, it is considered that it is best to control *Cuscuta* spp. in the seed crop before harvesting. Nevertheless, it happens that alfalfa seed crops often contain *Cuscuta* spp. According to Janjic et al. (2005), in Serbia, protection from *Cuscuta* spp. began with weed control in crops that had

already been infected. However, a better approach is to apply preventive measures that should be the basic way of weed control. The properties of the dodder seed, and above all, its vitality in the soil, enables the weeded parcels to become a source of further infection during the next ten or more years. Therefore, the basic strategy in the fight must be to prevent infestation of parcels (Janjić et al., 2005).

Regardless of the numerous ways of testing and experience in biology and agronomic practice to study its characteristics and control to this day, it is widespread and causes significant damage in the world (Abdel-Khalik, 2006; Córdoba et al., 2021) and Serbia (Sarić-Krsmanović et al., 2022). Due to the close resemblance in size between *Cuscuta* spp. and alfalfa seeds, the presence of *Cuscuta* spp. in the seed crop not only directly affects seed production but also presents substantial challenges for seed finishing. The dispersal of *Cuscuta* spp. in the alfalfa seed-growing region is another major issue. Also, a significant issue is the ability of *Cuscuta* spp. seeds to retain germination in the soil for decades and germinate again after alfalfa resowing (Jayasuriya et al., 2008; Olszewski et al., 2020). Especially this was a problem for seed production in the eighties of the last century (Čuturilo & Nikolić, 1986). It is necessary to study the advantages of extracting the seeds of *Cuscuta* sp. from natural alfalfa seeds on a laboratory machine Laboratory magnetic separator by German manufacturer Emceka Gompper and compare it with the efficiency of extracting *Cuscuta* sp. seeds in a conventional way. Further studies of weeds *Cuscuta* spp. in Serbia have given some solutions in some aspects, but the question of *Cuscuta* spp. is not close to being fully solved (Sarić-Krsmanović et al., 2020; 2022). It should be added that the seeds of *Cuscuta* spp. in alfalfa seed are considered quarantine weeds which means that such seed cannot be placed on the market Gazette of SFRY, 1987 (47/87), Gazette of the Republic of Serbia, 45 2005, and supplement to 2021 which is in line with ISTA rules (ISTA 2021), which mainly deals with quality and seed trade in Europe.

The aim of this research was to extract *Cuscuta* spp. seeds from four seed lots of natural alfalfa seed, and examine its variability in seed size. Then, to determine whether the size of *Cuscuta* spp seeds affects the efficiency of seed finishing.

Additionally, to contrast the effectiveness of *Cuscuta* spp. seed separation from natural alfalfa seeds on a laboratory magnetic separator made by German company Emceka Gompper with the effectiveness of *Cuscuta* spp. seed separation on equipment used in the industrial-standard process of processing natural alfalfa seeds.

Materials and Methods

Seeds of four seed lots of alfalfa were collected from the Banat region (the part of Serbia where alfalfa for seed production is grown the most). The seed lots were selected on the basis of a test sample, according to which the seed lots (I to IV) of natural alfalfa seed had approximately the same amount of quarantine weed *Cuscuta* spp.

The analyzes were performed in an accredited laboratory for seed quality control, Institute of Plant Protection and Environment Belgrade. A precise electronic scale was used to measure the samples. The seed samples for analysis weighed 5 g (working) samples in accordance with the regulations of the Law on Seeds and Planting Material. All conditions related to the method of production, processing, use, circulation, import and testing are prescribed seeds of agricultural plants (Gazette of the SFRY No. 47, 1987 and Official Gazette of the Republic of Serbia No. 45, 2005). The quality of alfalfa seeds should correspond to the Regulation on the quality of seeds of agricultural plants, which is harmonized with the international regulation on the seed quality ISTA 2021.

Subsample I: A sample of 5 grams of natural alfalfa seed was obtained from each seed lot. The length and width of the dodder seeds from this weed were measured using the random selection method on a sample of 30 seeds. The length and width of the seeds were examined with an Olympus BH-2 microscope.

For each seed lot, the average, lowest and highest value (Min. and Max.), standard deviation (StDev), coefficient of variation (CV%), and standard error of mean were determined to determine the variation in seed size of *Cuscuta* spp. (SEM). For each seed lot, the correlation coefficients (r) between *Cuscuta* spp. seeds' length and width were computed.

Subsample IIa: From each seed lot, 1 kg of seeds was divided into four parts (repetitions) and cleaned on a laboratory magnetic separator.

Subsample IIb: In the seed processing facility of the company „Herba" d.o.o. Belgrade, the seed lots are processed in the traditional industrial manner, also in four repetitions.

All seed batches of natural alfalfa were processed using a selector from the CIMBRIA brand, model DELTA super 102. This selector features three shaker shoes with two 625 mm x 800 mm sieves each, for a total of six 3 m^2 sieves. I shaker shoe has two sieves with round holes of 2.25 mm and 2.25 mm, II shaker shoe has two sieves with round openings of 1.4 mm and 1.4 mm, and III shaker shoe has two sieves with rectangular openings of 0.7 mm and 0.5 mm (#-wire). Sieves with the following

perforations are used in practice to remove *Cuscuta* spp. quarantine weeds from alfalfa: 1.85 mm, 2.00 mm, and 2.25 mm. Other seeds (like *Lolium* sp.) are typically sieved using sieves with openings smaller than 4.5 mm. The sieves are arranged in size order from top to bottom, with larger opening sieves at the top and smaller opening sieves at the bottom. Seeds are divided into length and width using rectangular and round sieves, respectively. based on the results of the purity test of the natural seed sampled (determination of the type of inert substances, the presence of weeds and quarantine weeds *Cuscuta* sp., *Rumex* sp., *Ambrosia* sp. and other impurities).

Depending on the level of purity of the natural seed achieved after the primary seed finishing on the CIMBRIA selector, a subsequent step of seed processing on the CIMBRIA trier model HSR 4020 RL may be required. A total of two segments, one for round seed and the other for long seed, make up the mantle, which is about 600 mm in diameter and 2,000 mm long. A mantle with alveoli with a diameter of less than 2 millimetres was employed in this instance of completing alfalfa seeds. A CIMBRIA gravity table can be used in the seed processing procedure if necessary.

The last, final phase of alfalfa seed processing is the passage through the magnetic separator, where the seeds of the dodder are separated, as well as other harmful weeds and impurities that are undesirable in alfalfa seeds. Separation of weeds, especially weeds with wrinkled and unsmooth seeds and other impurities, on this machine is done by electromagnetic force. A certain quantity of steel powder with water is used for the technological process of weed separation on a magnetic separator.

Between the results of the separation of *Cuscuta* spp. seeds, obtained by industrial and laboratory processing, the correlation coefficient (r) was calculated for each seed lot of natural alfalfa seeds. Statistical analysis was done via the freeware software package Minitab 16.

Results and discussion

In our experiments, dodder seed, isolated from natural seed lots, showed variability in terms of average seed size (**Tab. 1**). The highest average length and width of seeds of *Cuscuta* spp. was in the third seed lot (1,361 µm and 1,197 µm), which is 371 µm more in length, and 330 µm more in width, compared to the seed lot with the smallest seed (lot IV: length 990 µm and width 867 µm).

Table 1. The seed size variability of *Cuscuta* spp. detected in four seed lots of natural alfalfa seed

***Cuscuta* spp. seeds (serial number)**	**Seed dimensions of *Cuscuta* spp. detected in a seed lots of natural alfalfa seeds (µm)**							
	I		**II**		**III**		**IV**	
	Length	**Width**	**Length**	**Width**	**Length**	**Width**	**Length**	**Width**
1	1053	951	1275	879	1256	1156	1189	1025
2	1062	988	1283	1157	1325	1265	956	845
3	971	1109	1495	1530	1256	1253	899	802
4	1254	1086	1210	1169	1152	989	1075	895
5	1225	1114	1340	1156	1155	1156	1172	956
6	1102	978	1380	1155	1456	1356	856	802
7	1221	1121	1434	1423	1352	1302	848	832
8	1489	1178	1340	1049	1299	1255	1025	1002
9	1164	1014	1183	1127	1158	1199	975	887
10	1206	1066	1196	1073	1522	1325	887	799
11	1381	1114	1298	1181	1632	1025	926	802
12	1094	1026	1564	1346	1452	1152	1175	1011
13	1145	1128	1090	971	1289	1136	956	789
14	1178	1078	1116	1098	1325	1256	875	803
15	1301	1082	1278	1151	1256	1302	1125	1033
16	1236	1027	1198	1140	1488	1235	963	805
17	1341	1341	1313	1051	1502	1156	1023	921
18	1131	1131	1309	1155	1558	1235	985	875

***Cuscuta* spp. seeds (serial number)**	**Seed dimensions of *Cuscuta* spp. detected in a seed lots of natural alfalfa seeds (µm)**							
	I		**II**		**III**		**IV**	
	Length	**Width**	**Length**	**Width**	**Length**	**Width**	**Length**	**Width**
19	1143	1143	1211	1068	1365	1099	885	756
20	1208	1208	1619	1398	1236	1156	1025	901
21	1078	1078	1201	1030	1320	1299	1125	1023
22	1347	1347	1330	1272	1398	1005	982	789
23	1214	1214	1193	971	1299	1125	976	821
24	1379	1379	1488	1084	1365	1233	1087	902
25	1242	1242	1211	1063	1455	1302	965	842
26	1314	1314	1269	1163	1266	1235	875	702
27	1347	1347	1448	1172	1566	1099	1023	825
28	1231	1231	1373	1196	1488	1263	986	756
29	1306	1021	1188	1051	1259	1198	831	799
30	1221	956	1626	1221	1368	1022	1025	1002
Average	1219	1134	1315	1150	1361	1197	990	867
Min	971	951	1090	879	1152	989	831	702
Max	1489	1379	1626	1530	1632	1356	1189	1033
StDev	115.2	123.1	140.6	138.0	127.7	99.99	100.7	92.59
CV (%)	9.447	10.86	10.69	12.00	9.383	8.356	10.17	10.68
SEM	13.97	14.93	17.05	16.74	15.48	12.13	12.21	11.23

For the dodder seed width, the highest variability was in the second seed lot (CV=12.00%), and the lowest was in the third seed lot (CV=8.356%). The variability of seed size that occurred in different seed lots can be statistically considered low (Hadživuković, 1991). Ho & Costea (2018) stated significantly higher variability for the seed size of *Cuscuta* spp. However, this study was done at different geographical distances and was different from the region where our experiment had been placed. Within the genus *Convolvulaceae*, the seed size of *Cuscuta* spp. is most affected by the species (Costea et al., 2015a,b). The following physical properties are considered to be the most important for seed finishing: humidity, shape, dimensions, sphericity, mass of 1,000 seeds, volume and porosity, volume-hectolitre mass, density, static and dynamic angle of internal friction (free fall angle), and external condition seed surfaces (Copeland & McDonald, 2004; Black et al., 2006; Babić & Babić, 2012; Baskakov et al., 2018).

In our trials, all seed lots showed a positive correlation between seed length and width, whereas three seed lots (I, II and IV) had a very high correlation between these features ($p \leq 0.001$). In seed lot III, seed length and width did not correlate significantly ($p \geq 0.05$). Also, positive correlative interdependence was high in all seed lots ($p \leq 0.001$) (**Tab. 2**).

Healthy alfalfa seeds have a smooth surface, and after mixing the seeds with steel powder and water in a certain proportion, the steel powder does not stay on that surface. In contrast, the dodder seed has a grain that is porous, and steel powder remains on it, which allows it to separate from alfalfa seeds (**Fig. 1**, **Fig. 2**).

Table 2. Simple correlations (r) between length and width of *Cuscuta* spp. seeds (n=30)

Seed dimensions of *Cuscuta* spp. originating from lots of natural alfalfa seed (µm)							
I		**II**		**III**		**IV**	
Length	**Width**	**Length**	**Width**	**Length**	**Width**	**Length**	**Width**
r=0.579***		r=0.672***		r=0.046 ns		r=0.818***	

* p≤0.05, ** p≤0.01, *** p≤0.001, ns – not significant p≥0.05

Fig. 1. Laboratory magnetic separator German manufacturer Emceka Gompper

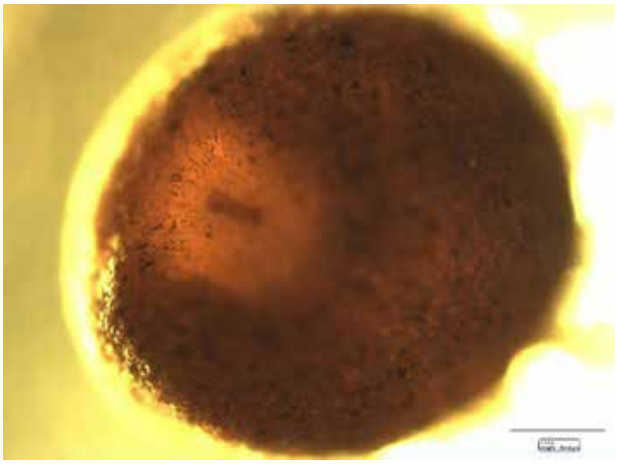

Fig. 2. Seed of *Cuscuta* spp.

The electromagnetic cleaning machine is used for the final cleaning of alfalfa seeds from the seed of weeds. The quality of weed cleaning on this machine depends on the correct ratio of water and magnetic seed cleaning powder and the amount of seed passed through the magnetized cleaning rollers (Đokić & Stanisavljević, 2012; Uhlarik et al., 2018; Đokić et al., 2020).

In our experiment, a simple correlation coefficient between the length and width of *Cuscuta* spp. seeds was very significant (r=0.784; **Tab. 3**).

Table 3. Simple correlations (r) between length and width of *Cuscuta* spp. seeds separated from all seed lots of natural seed (n=120)

Length	Width
r=0.784***	

***, p≤0.001

The principle of operation of electromagnetic separators (trifolin machine-detector) is based on electromagnetic action. They are intended for separating weed seeds with wrinkled and unsmooth surfaces and other impurities. The success of removing the dodder from alfalfa seeds also depends on the method of applying the appropriate metal powder (Milošević et al., 1996). Electromagnetic seed cleaning machines are very important due to their high-quality cleaning performance. Such a high quality of cleanliness cannot be achieved by pneumatic cleaning, triers or selector screens (Kozlov, 2013.).

The seed of *Cuscuta* spp. was completely isolated from all seed lots of alfalfa seeds in our laboratory tests. Also, the seed size did not affect the separation efficiency (**Tab. 4**).

Table 4. Efficiency (%) of separating *Cuscuta* spp. seeds on a laboratory magnetic separator

Seed size *Cuscuta* spp. (length - D µm and width W µm) separated from seed lots of natural seed of alfalfa							
I		II		III		IV	
L	W	L	W	L	W	L	W
100a	100A	100a	100A	100a	100A	100a	100A

a - Tukey's test (p≤0.05) for length, A - Tukey's test (p≤0.0) for width

In seed finishing practice, the assumption is that laboratory and industrial processing of alfalfa seeds should be equally or similarly effective in removing *Cuscuta* spp. from natural alfalfa seeds.

Table 5. Simple correlations (r) between the isolation of *Cuscuta* spp. from four seed lots of natural alfalfa seeds in laboratory conditions and in conventional alfalfa seed processing (n=4).

Seed lots of natural alfalfa seed			
I	II	III	IV
r=0.984**	r=0.931*	r=0.999***	r=0.966**

* p≤0.05, ** p≤0.01, *** p≤0.001, ns – not significant (p≥0.05)

In our study, there was a positive and highly correlated relationship ($p \leq 0.05$ to $p \leq 0.001$) between the efficiency of magnetic separators used in the laboratory and those used in industry (**Tab. 5**), which is in agreement with the results of Lin et al. (2022).

Conclusion

An average sample of thirty seeds of *Cuscuta* spp. taken from four seed lots, differed by 371 μm in length and by 330 μm in width. Dodder seed isolated from all four seed lots showed variability for width from CV=8.356 (seed from the third lot) to CV=12.00% (seed from the second lot). Between the length of the seeds of *Cuscuta* spp. and width was a positive correlation, but at a different level of statistical significance ($p \geq 0.05$ to $p \leq 0.001$). The influence of *Cuscuta* spp. seed size in the process of its separating from natural alfalfa seeds was not significant. There was a positive and significant correlation ($p \leq 0.05$ to $p \leq 0.001$) between the separation of *Cuscuta* spp. seeds by laboratory and industrial magnetic separator.

According to the law on seed material, processed alfalfa seeds must not contain any seeds of quarantined weed *Cuscuta* spp, regardless of the type and dimensions of *Cuscuta* spp. With electromagnetic cleaning machines, the seeds of the *Cuscuta* spp. must be completely removed from the alfalfa seeds that are being processed.

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