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# Temperature Effects on *Cuscuta campestris* Yunk. Seed Germination

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**SUMMARY**

Studies of biological characteristics of seeds and conditions for their germination have a major importance for planning and executing rational measures of weed control. The aim of this study was to investigate the effect of different temperatures on germination of *C. campestris* seeds. Three treatments ($T_1$- storage at room temperature; $T_2$ – exposure to 4°C for 30 days; $T_3$ – scarification by concentrated sulphuric acid) differing in manipulation with seeds before germination were tested at different temperatures (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C). Germinated seeds were counted daily for ten days and the length of seedlings was measured on the last day. The results showed that differences in germination of *C. campestris* seeds were very prominent between temperatures, as well as between treatments $T_1$, $T_2$ and $T_3$. Seeds failed to germinate at 5°C and 45°C in all treatments ($T_1$, $T_2$, $T_3$). Germination ranged from 6.25 at 10°C to 96.88%, the highest percentage, achieved at 30°C.

**Keywords:** *Cuscuta campestris*; Germination rate; Seedling length; Temperature

## INTRODUCTION

As an obligate parasite, *Cuscuta campestris* Yunk. (field dodder) reduces the fitness of its hosts. Plants parasitized by *Cuscuta* become weak, their growth is limited and they produce very small yields (Tsivion, 1981; Koskela et al., 2001). When *C. campestris* is not removed, it usually causes a complete destruction of its host. Therefore, the species is a serious threat to alfalfa crops, especially when it occurs as a major infestation (Parker and Riches, 1993; Dawson et al., 1994). Apart from alfalfa, common hosts for this species are also sugar beet, potato and tomato (Haidar and Bibi, 1995), as well as a number of weed species, such as: *Polygonum aviculare*, *Convolvulus arvensis*, *Chenopodium album*, *Amaranthus retroflexus,* etc. (Parker and Riches, 1993; Holm et al., 1997).

Seed dormancy is an important feature of *C. campestris* that ensures its survival as a parasite of crops (Hutchison and Ashton, 1980). There are three different types of seed dormancy (morphological, physical and physiological), at least two of which have evolved on several separate occasions (Baskin and Baskin, 1998). Dormancy of *C. campestris* occurs owing to its hard seed coat (Lyshede, 1992). The percentage of hard seeds at dispersal varies among *C. campestris* (Hutchison and Ashton, 1979) and *C. chinensis* plants (Marambe et al., 2002). Dormancy can be broken by the activity of soil microorganisms or by tillage, causing scarification of seed coat (Haidar et al., 1999). The dynamics of germination of *C. campestris* depends on a double mechanism of dormancy. After a period of primary dormancy (additional maturation caused by coat impermeability), the seed goes into an annual cycle of secondary dormancy. In *C. campestris*, secondary dormancy occurs at the end of summer and it prevents germination during the following autumn and winter in order to avoid the season in which potential hosts of the temperate region would be scarce due to low temperatures. Secondary dormancy ends at the end of winter when temperature begins to grow and overall conditions for germination and growth of host plants improve (Benvenuti et al., 2005). Physical dormancy has been reported for seeds of several *Cuscuta* species: *C. campestris* (Benvenuti et al., 2005; Hutchison and Ashton, 1980), *C. trifolii* (Lados, 1999), *C. monogyna* and *C. planiflora* (Salimi and Shahraeen, 2000), *C. chinensis* (Marambe et al., 2002), *C. gronovii*, *C. umbrosa*, *C. epithymum* and *C. epilinum* (Costea and Tardif, 2006). However, it is not common for *Cuscuta pedicellata* (Lyshede, 1984) because seeds of that species are readily water permeable due to a specific structure of their epidermis and endosperm.

Unlike the important holoparasitic weeds of the genus *Orobanche* and some hemiparasitic weeds in the genera *Striga*, *Cuscuta* spp. do not require host-root exudates to stimulate germination (Vail et al., 1990; Benvenuti et al., 2002). This indicates that seed dormancy is the most important factor for *C. campestris* survival and spreading in agroecosystems. Predicting the start and duration of seedling emergence can contribute to making better weed control decisions (Berti et al., 1996) and facilitate optimal timing of control practices (Grundy, 2003). Data about the effects of environmental factors on germination and emergence can be very useful in that context. The aim of this study was to investigate the effect of different temperatures on seed germination and seedling growth of *C. campestris*.

## MATERIAL AND METHODS

Seeds of *C. campestris,* collected in fields around Šabac during August 2008 were purified and kept in the laboratory at room temperature, 22-25°C. Seed germination was examined at the following temperatures: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C for 10 days. Three treatments were included, differing in seed manipulation before the germination study: $T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid ($H_2SO_4$) for 30 minutes. Seeds from treatment $T_3$ were kept in distilled water for 15 minutes after scarification. Immediately before germination, seeds were sterilized with a solution of sodium hypochlorite and distilled water at 1:1 ratio for 10 minutes and then rinsed three times with distilled water. Any possibility of microorganisms being still present was thus eliminated. Twenty seeds of *C. campestris* were placed in each Petri dish and 5 ml of distilled water was added. Germination took place in the dark in an incubator (Binder CE, Germany). Germinated seeds were counted every day over a ten-day period and the length of seedlings was measured on the last day. All treatments were done in four replications, and the whole experiment was repeated twice.

Germination rate (GR, sum of germinated seeds per day) was calculated using the formula described by Maguire (1962):

$$GR = n_1/t_1+n_2/t_2+....+n_x/t_x,$$

where $n_1$, $n_2$, ....$n_x$ are the numbers of germinated seeds at times $t_1$, $t_2$, ....$t_x$ in days.

All data were analyzed by a one-way ANOVA (F-values) using the statistical software Statistica 8.0. Differences between populations were tested using t-test.

## RESULTS AND DISSCUSION

The germination results (Figures 1-7) show that the examined temperature range (5-45°C) had a significant effect on seed germination of *C. campestris* in each of the tested treatments ($T_1$, $T_2$, $T_3$) (Table 1). The seeds could not germinate at 5°C and 45°C, and only those scarified with sulphuric acid germinated at 10°C (6.25%), starting on the 6$^{th}$ day (Figure 1). At 15°C, 7.5% of the seeds previously cold stratified (4°C) germinated, as well as 15.63% of those scarified

with sulphuric acid, while seeds from the $T_1$ treatment did not germinate (Figure 2). At all other test temperatures, germination ranged from 13.75 to 96.88% (Figures 1-7) and the highest germination rate was recorded at 30°C ($T_1$: 20.63%, $T_2$: 38.75%, $T_3$: 96.88%). Similar results had been reported by Hutchison and Ashton (1979) under laboratory conditions with *C. campestris* seeds, whose dormancy was broken by mechanical scarification using abrasive paper dipped in concentrated sulphuric acid. The recorded germination was negligible at 10°C and the highest at 30°C. The authors concluded that the overwintering period or storage at low temperature had broken dormancy. Benvenuti et al. (2005) examined the influence of temperature on *C. campestris* germination and found that 60% and 80% of the seeds scarified with sulphuric acid germinated at 20°C and 30°C, respectively, while the germination of unscarified seeds was significantly lower (20%).

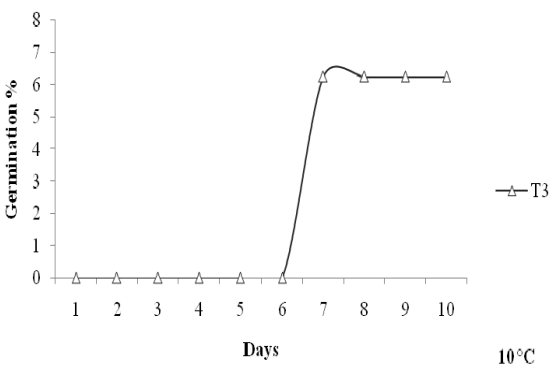


**Figure 1.** Dynamic of seed germination of *C. campestris* at 10°C ($T_3$ – seeds were scarified by concentrated sulphuric acid)

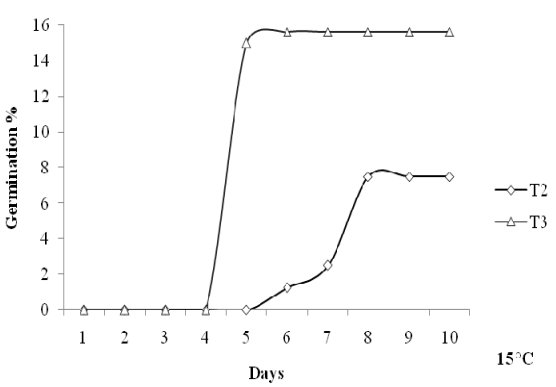


**Figure 2.** Dynamic of seed germination of *C. campestris* at 15°C ($T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

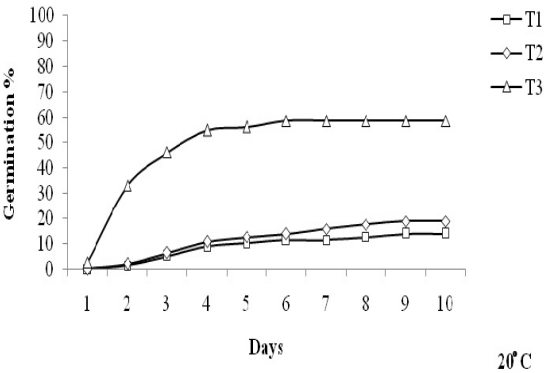


**Figure 3**. Dynamic of seed germination of *C. campestris* at 20°C ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

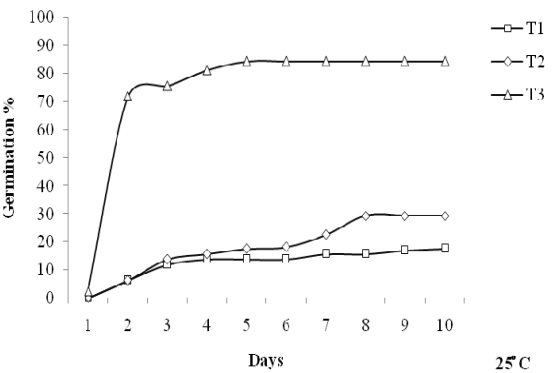


**Figure 4**. Dynamic of seed germination of *C. campestris* at 25°C ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ - seeds were exposed to 4°C for 30 days; $T_3$ - seeds were scarified by concentrated sulphuric acid)

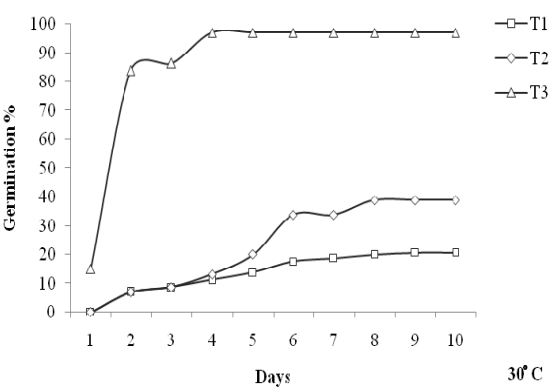


**Figure 5.** Dynamic of seed germination of *C. campestris* at 30°C ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

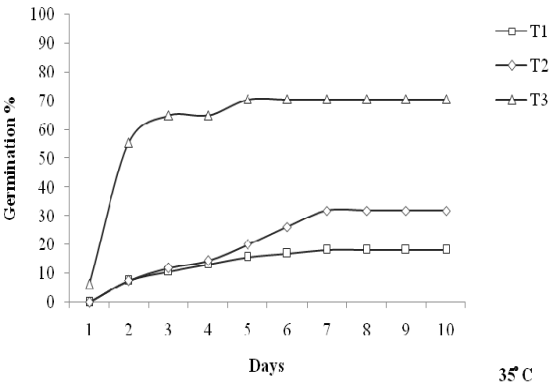


**Figure 6.** Dynamic of seed germination of *C. campestris* at 35°C ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

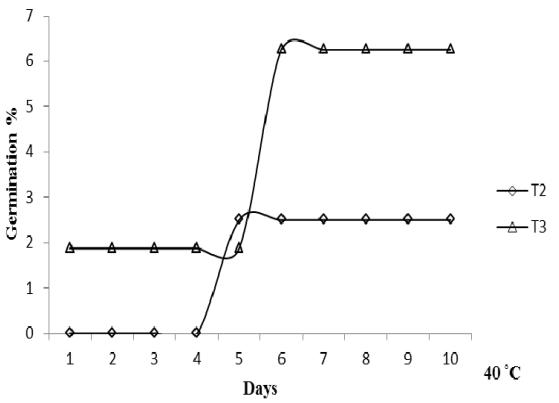


**Figure 7.** Dynamic of seed germination of *C. campestris* at 40°C ($T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

**Table 1.** Results of one-way ANOVA (F-values) for the main effects of temperature on germination percentage, germination rate and seedling length in treatments $T_1$, $T_2$ and $T_3$ ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

| Parameter | $T_1$ | $T_2$ | $T_3$ |
|---|---|---|---|
| Germination (%) | 67.65833** | 130.3518** | 178.6567** |
| Germination rate (no. day-1) | 45.97119** | 70.06563** | 194.8619** |
| Seedling length (cm) | 75.88997** | 117.9717** | 409.3811** |

** ($p < 0.01$)

At all temperatures other than 5°C and 45°C, *C. campestris* seeds best germinated after scarification with sulphuric acid (Figures 1-7), and exposure to low temperature also contributed to an increase in germination ($T_2$) but this increase was significantly lower than it was after scarification with sulphuric acid ($T_3$). Specifically, the germination of seeds at the optimal temperature (30°C) after exposure to low temperature (30 days at 4°C) was 38.75%, while seeds scarified with sulphuric acid reached 96.88% germination. Gaertner (1950) studied seed germination of several *Cuscuta* species and concluded that the water impermeable seed coat (along with physiological dormancy in some species) was responsible for dormancy in all of them. However, germination of freshly collected seeds of *C. epilinum*, *C. epithymum* and *C. europea* was not facilitated by scarification with concentrated sulphuric acid. Meulebrouck et al. (2008) found that scarified seeds of *C. epithymum* required a period of cold stratification in order to break physiological dormancy of the embryo. Thus, they assumed that the seeds of that species have a combined dormancy (physical plus physiological). Tingey and Allred (1961) clearly showed that seeds of *C. approximata* also combined their dormancy as scarified seeds required two weeks of stratification at 5°C to germinate. In our present study, the lowest germination, ranging from 1.25 to 20.63%, was found in seeds previously stored at room temperature ($T_1$). No germination of those seeds was recorded at low temperatures (5°C, 10°C and 15°C) or high temperatures (40°C and 45°C) and the percentage of germination was highest at 30°C (20.63%). On the other hand, dry storage for two months had been found in another study to result in 65% germinated seeds of *Cuscuta australis*, while weak germination (8-9%) was found after moist storage (Jayasuriya et al., 2008). The authors found that *C. australis* seeds dipped in boiling water for a period of 10 s increased germination to 75%, while the percentage of germinated seeds decreased with increasing duration of dipping (15 s and 20 s). In our study, statistical analysis of the results (Table 2) showed that differences in seed germination were statistically significant between treatments $T_1$ and $T_2$ at temperatures 15°C, 25°C, 30°C and 35°C, as well as between treatments $T_1$ and $T_3$ at temperatures 15°C, 20°C, 25°C, 30°C and 35°C, and between treatments $T_2$ and $T_3$ at temperatures 20°C, 25°C, 30°C and 35°C.

**Table 2.** Differences between treatments (ANOVA, t-test) at different temperatures for germination percentage, germination rate and seedling length in treatments $T_1$, $T_2$ and $T_3$ ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

| | Treatment | 5°C | 10°C | 15°C | 20°C | 25°C | 30°C | 35°C | 40°C | 45°C |
|---|---|---|---|---|---|---|---|---|---|---|
| Germination (%) | $T_1$:$T_2$ | NG | NG | 0.003203** | 0.056848$^{NS}$ | 0.000035** | 0.000005** | 0.000032** | 0.148904$^{NS}$ | NG |
| | $T_1$:$T_3$ | NG | 0.015389* | 0.000000** | 0.000000** | 0.000000** | 0.000000** | 0.000000** | 0.032322* | NG |
| | $T_2$:$T_3$ | NG | 0.015389* | 0.00705** | 0.000000** | 0.000000** | 0.000000** | 0.000009** | 0.246158$^{NS}$ | NG |
| Germination rate (no. day$^{-1}$) | $T_1$:$T_2$ | NG | NG | 0.00147** | 0.306844$^{NS}$ | 0.09318$^{NS}$ | 0.002199** | 0.012679* | 0.148904$^{NS}$ | NG |
| | $T_1$:$T_3$ | NG | 0.015389* | 0.000000** | 0.000000** | 0.000000** | 0.000000** | 0.000000** | 0.017801* | NG |
| | $T_2$:$T_3$ | NG | 0.015389* | 0.000014** | 0.000000** | 0.000000** | 0.000000** | 0.000000** | 0.088974$^{NS}$ | NG |
| Seedling length (cm) | $T_1$:$T_2$ | NG | NG | 0.000507** | 0.000612** | 0.048457* | 0.002119** | 0.026000* | 0.014477* | NG |
| | $T_1$:$T_3$ | NG | 0.006386** | 0.000000** | 0.00000** | 0.000033** | 0.000003** | 0.000481** | 0.001131** | NG |
| | $T_2$:$T_3$ | NG | 0.006386** | 0.699785$^{NS}$ | 0.000042** | 0.000005** | 0.000000** | 0.002408** | 0.17086$^{NS}$ | NG |

NG- no germination; NS- no significant differences ($p > 0.05$); * ($0.01 < p < 0.05$); ** ($p < 0.01$).

In treatments $T_1$ and $T_2$, germination rates of *C. campestris* seeds were very low or equalling zero (0.00 to 7.42), while $T_3$ was the treatment in which germination rate was zero only at 5°C and 45°C, and ranged from 0.60 to 38.35 at the other temperatures. The maximum value was observed in treatment $T_3$ at 30°C (Figure 8). The results showed that temperature had a statistically significant ($p < 0.01$) effect on germination rates (Table 1).

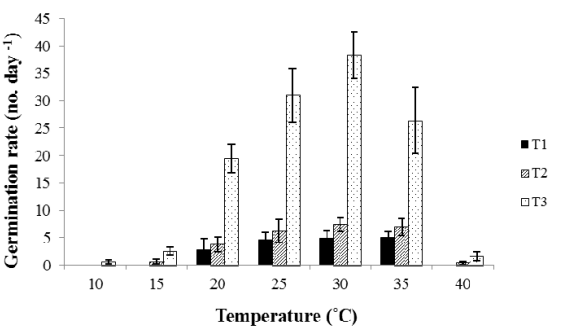


**Figure 8.** Effects of different temperatures on germination rate (no. day$^{-1}$) of *C. campestris* seeds ($T_1$- seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

The growth of weed seedlings could be affected by different factors, such as temperature, light, depth to which seeds are embedded in soil, seed storage conditions, soil bacteria and seed vigour (Haidar et al., 1997; Walters, 1998; Benvenuti et al., 2005; Li and Kremer, 2006). The growth *C. campestris* seedlings depends on seed vigour, which also determines whether a successful parasitic bond with the host plant will be established (Walters, 1998). Furthermore, Benvenuti et al. (2005) found that the growth of seedlings of this species was determined by the depth of seed embedment in soil, and by different ways of seed storage. There is no available data to our knowledge about the effect of temperature on seedling growth of *C. campestris*, but our research confirmed that this factor has a significant ($p < 0.01$) effect on seedling growth (Table 1). The length of seedlings after the tenth day of the experiment ranged from 0.31 to 9.08 cm (Figure 9). The seedlings were longest at the optimal temperature of 30°C (9.08 cm) in the treatment with seeds scarified with sulphuric acid, while seedling length was smaller in the other two treatments at the same temperature ($T_1$ = 4.99 cm, $T_2$ = 6.29 cm). Lower temperature (compared to the optimal) resulted in a decreased length of seedlings and it was lowest at 10°C in $T_3$ treatment (0.31 cm). Also, higher temperature (in relation to optimal) resulted in a reduction in seedling length, and the minimum length of seedlings in treatment $T_2$ (0.04cm) was measured at 40°C. Comparing treatments $T_1$, $T_2$ and $T_3$ (Table 2) based on seedling length, we detected highly significant statistical differences between $T_1$ and $T_2$, as well as $T_1$ and $T_3$ at 15°C, 20°C and 25°C, while significant statistical differences were found between $T_1$ and $T_2$ at 25°C, 35°C and 40°C ($0.01<p<0.05$). Highly significant differences

($p < 0.01$) were detected between treatments $T_2$ and $T_3$ at 10°C, 20°C, 25°C, 30°C and 35°C, while there were no significant differences at 15°C and 40°C.

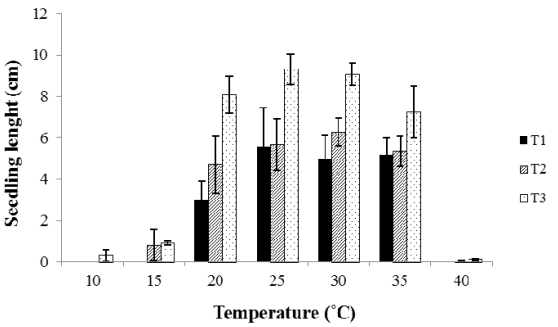


**Figure 9.** Effects of different temperatures on seedling length (cm) of *C. campestris* seeds ($T_1$ – seeds were stored at room temperature (approximately 22-25°C); $T_2$ – seeds were exposed to 4°C for 30 days; $T_3$ – seeds were scarified by concentrated sulphuric acid)

## CONCLUSIONS

Temperature had a significant effect on germination of *C. campestris* seeds. However, that effect depended on seed storage conditions. Seed exposure to low temperature (4°C) for 30 days increased seed germination, compared to seeds stored at room temperature. The best germination was reached after seed scarification by concentrated sulphuric acid. The effect of different temperatures and seed storage conditions on seed germination and seedling length had a similar trend.

In agricultural practice, the primary and constant source of new weed growth is the soil seed bank. Many biological characteristics of seeds and the processes normally occurring in them help plants to maintain a permanent reserve of seeds in soil, and an ensuing weediness of agricultural fields. A better understanding of seed ecology could be helpful for predicting the potential of weed species to spread, for predicting their invasiveness and for developing more effective weed management strategies. Seed germination is a key event for success of a weed in an agroecosystem and several environmental factors, such as temperature, light, pH and soil moisture, are known to affect seed germination. One of the most important factors that affect seed germination and seedling emergence is temperature. Our results contribute to better understanding of *C. campestris* germination and emergence, and can be useful in developing programs for prevention and control of this species.

## ACKNOWLEDGEMENT

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# Uticaj temperature na klijanje semena *Cuscuta campestris* Yunk.

**REZIME**

Izučavanje bioloških karakteristika semena i uslova u kojima klijaju ima veliki značaj za planiranje i realizaciju racionalnih mera za kontrolu korova. Cilj ovog istraživanja je bio da se ispita efekat različitih temperatura na klijanje semena *C. campestris*. U ogled su bila uključena tri tretmana ($T_1$ – semena čuvana u laboratorijskim uslovima na temperaturi 22-25°C, $T_2$ – semena koja su prethodno 30 dana izlagana niskoj temperaturi (4°C), $T_3$ – semena koja su skarifikovana koncentrovanom sumpornom kiselinom), pri čemu su svi tretmani ispitivani na sledećim temperaturama: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C. Svakodnevno, u periodu od deset dana, rađeno je prebrojavanje proklijalih semena, a poslednjeg dana su izmerene i dužine klijanaca. Dobijeni rezultati ukazuju da postoje značajne razlike u klijanju semena u odnosu na ispitivane temperature i tretmane. Semena nisu klijala na temperaturama od 5°C i 45°C ni u jednom od rađenih tretmana. Procenat klijanja se kretao od 6,25% do 96,88%, pri čemu je najveći procenat u sva tri tretmana zabeležen na temperaturi od 30°C.

**Ključne reči:** *Cuscuta campestris*; stopa klijanja; dužina klijanaca; temperatura