

The effect of mechanical stress on transplants of three ornamental Asteraceae species

Učinak mehaničkog stresa na presadnice tri ukrasne vrste iz porodice Asteraceae

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Abstract

Transplants of *Tagetes patula* L., *T. erecta* L. and *Calendula officinalis* L. were subjected to brushing treatment until appearance of visible inflorescence buds. At the end of treatment, the plant height was statistically significantly reduced only in *C. officinalis*, whereas, at the beginning of flowering, the height of treated plants was statistically equal to untreated ones, in all examined species. In *T. patula*, the treatment also did not influence other measured morphological parameters. However, in *T. erecta*, treated plants had higher numbers of lateral branches and leaves, and, in *C. officinalis*, the treatment resulted in more upright stems. Besides, treated plants in both *T. erecta* and *C. officinalis* had a higher number of inflorescence buds. The results of this study indicate that exposure to mechanical stress may improve some commercially important characteristics in *T. erecta* and *C. officinalis* transplants. However, some plants were damaged by brushing treatment, especially in *C. officinalis*. For these species, further research is needed to find the effectiveness of methods of applying mechanical stress in which physical contact with plants is reduced or avoided.

Keywords: brushing, *Calendula officinalis*, flowering time, leaf damage, morphological characteristics, *Tagetes erecta*, *Tagetes patula*

Sažetak

Presadnice niske kadifice (*Tagetes patula* L.), visoke kadifice (*T. erecta* L.) i nevena (*Calendula officinalis* L.) bile su izložene tretmanu četkanjem do pojave vidljivih cvatnih pupova. Nakon završetka tretmana, tretirane presadnice bile su statistički

značajno niže od netretiranih samo u nevena, dok je u vrijeme početka cvatnje visina tretiranih i netretiranih presadnica kod svih istraživanih vrsta bila statistički podjednaka. Kod niske kadifice tretman nije statistički značajno utjecao niti na ostale mjerene morfološke parametre. Međutim, tretirane presadnice visoke kadifice imale su veći broj postranih ogranaka i veći broj listova, dok su tretirane presadnice nevena imale uspravnije stabljike nego netretirane. Osim toga, u obje su vrste tretirane presadnice imale veći broj cvatnih pupova. Provedeno istraživanje pokazalo je da izlaganje mehaničkom stresu može poboljšati neke komercijalno važne karakteristike presadnica visoke kadifice i nevena. Međutim, tretman četkanjem je u nekih biljaka izazvao oštećenja listova, posebice kod nevena. Stoga je kod ovih vrsta potrebno istražiti učinkovitost metoda izlaganja mehaničkom stresu kod kojih je fizički kontakt s biljkama smanjen ili se u potpunosti izbjegava.

Ključne riječi: *Calendula officinalis*, četkanje, morfološke karakteristike, oštećenje lista, *Tagetes erecta*, *Tagetes patula*, vrijeme cvatnje

Introduction

Close spacing of plants common in commercial containerized transplant production triggers various morphological and physiological responses in plants, such as enhanced elongation of hypocotyl, internodes and petioles, upward bending of leaves (hyponasty), and increased apical dominance (Hamrick, 1996; Pierik and de Wit, 2014). As a result, plants become excessively elongated with weak and thin stems, which lowers their quality and commercial value.

Control of plant size in floricultural crops is commonly achieved by applying plant growth retardants. Most of these compounds act as inhibitors of gibberellin biosynthesis, which reduces extension growth and thus results in shorter and more compact plants (Rademacher, 2000; Bergstrand, 2017). However, alternative, non-chemical, strategies may also be effectively used for controlling plant height, such as manipulating the difference between day and night air temperatures, altering of light spectral quality, imposing a mild water stress or restricting fertilization (Rajapakse and Kelly, 1992; Berghage, 1998; Liptay et al. 1998; Rajapakse et al., 1999; Bergstrand, 2017).

Another, less common, non-chemical method for reducing stem elongation is by exposing plants to mechanical stress (mechanical conditioning). It has been shown that mechanical conditioning can reduce plant height from 20% to more than 50%, which is similar to reductions that could be achieved by growth retardants (Latimer and Beverly, 1993). Exposure to mechanical stress can also reduce petiole length and leaf area, which in addition to shorter stature, contributes to more compact appearance of plants (Biddington, 1986). Besides, mechanical conditioning can increase leaf chlorophyll content and improve stem and petiole strength (Latimer and Beverly, 1993).

Brushing is one of the most studied methods of mechanical conditioning of horticultural crops. It is performed by moving various materials, such as burlap, cardboard, folded paper, PVC pipe, etc., across the plants, aiming to cause bending of the stems, without breaking stems or leaves, or causing other types of injuries to

plants (Latimer, 1998). Mechanical stress applied by brushing can effectively reduce plant height in floricultural crops such as chrysanthemum [*Dendranthema x grandiflorum* (Ramat.) Kitam.], aster [*Callistephus chinensis* (L.) Nees], petunia (*Petunia* sp. Juss.) and dusty miller [*Senecio bicolor* (Willd.) Tod.] (Autio et al., 1994; Teixeira da Silva et al., 2006), while, in pansy (*Viola tricolor* L.), it reduces petiole length (Garner and Langton, 1997).

Although exposure to mechanical stress may be an effective strategy to achieve shorter and more compact transplants, so far it has been studied on relatively small number of floricultural crops. Besides, different species and cultivars may differ in their responses to mechanical stimuli as well as to applied method of mechanical conditioning. The plants may also show differences in the extent of damage that the treatments may cause (Latimer, 1991; Autio et al., 1994). In this work, the effects of mechanical stress applied by brushing on morphological characteristics and flowering time in transplants of *Tagetes patula* L., *T. erecta* L. and *Calendula officinalis* L. were examined.

Materials and methods

Experiments were carried out on transplants of *Tagetes patula* L. 'Safari Bolero' (Austrosaat), *Tagetes erecta* L. 'Discovery Orange' (Benary) and *Calendula officinalis* L. 'Ball's Orange' (Austrosaat), during spring 2015. Plants were grown in unheated greenhouse at the Faculty of Agriculture, University of Zagreb (latitude: 45°49' N, longitude: 16°01' E).

Seeds were sown one per cell in polystyrene trays with 84 cells, in standard potting soil (Substrat 2, Klasmann Deilmann), on April 22 (*T. patula* and *T. erecta*) and April 30 (*C. officinalis*). Trays with untreated (control) plants and plants subjected to brushing treatment were placed separately, on two adjacent greenhouse benches. Plants were grown under natural light and watered regularly. They were not additionally fertilized during the course of the experiment.

Transplanting plants from trays to polypropylene pots was carried out for *T. patula* on May 18, when inflorescence buds became visible on most of the plants, and, for *T. erecta* and *C. officinalis*, on May 24 and May 28, respectively, when the plants were at the stage of developing 5th and 6th true leaf. The plants were transplanted to 0.2-Liter round pots (9 cm in diameter) filled with commercial substrate (COMPO SANA® Universal Potting Soil). The pots were arranged in the rows, so that plants kept the same position as in the trays. On the day of transplanting and the day after transplanting the plants were not subjected to brushing treatment.

The brushing was performed manually, by dragging the cotton cloth across the canopy of plants. The cloth was hung from the thin wooden stick. Each back-and-forth dragging of cloth across the plants was considered as a single brush stroke. The treatment was aimed to achieve bending of plant stem at approximately 45°.

The treatment begun when seedlings were developing 1st and 2nd true leaf, in *T. patula* and *T. erecta*, and 3rd and 4th true leaf, in *C. officinalis*, and was continued until the first visible inflorescence buds appeared on brushed plants (Table 1). The plants were brushed each day between 11:00 and noon. Since, at the beginning of the treatment, the plants were still very young and easily damaged, the treatment

was started with 10 brush strokes and the number of strokes was gradually increased to 25 for *C. officinalis* and to 50 for both *T. patula* and *T. erecta*. Plants of *C. officinalis* were treated with lower number of strokes because cotton cloth was easily stuck to the hairy leaf surface, which often led to damage on leaves.

Morphometric measurements were carried out at the end of treatment (1st measurement) and at the day of the beginning of flowering (2nd measurement) (Table 1). At the end of treatment, the height of the plant (from the substrate level to the last node) and the length and width of the largest leaf were measured. At the day of the beginning of flowering, the height of the plant and dimensions of the largest leaf were determined along with the total number of leaves (> 3 cm in length), stem diameter (above 2nd node), number of lateral branches (> 3 cm long), peduncle length (of the 1st inflorescence) and number of visible inflorescence buds. The plant height, peduncle length and dimensions of the largest leaf were measured using a paper ruler. The diameter of the stem was measured approximately 1 cm above the midpoint of the node using a digital caliper. For *T. patula* and *T. erecta* the measurements were carried out on 35 plants and for *C. officinalis* on 25 plants per treatment. In addition, the date of beginning of flowering (the day when the first inflorescence opened) and the date of 50% flowering (the day when 50% of plants had first inflorescence opened) were recorded. Any damage on plants were also noted. Plants at the edges of trays were excluded in measurements and observations.

Table 1. Time (days after sowing) of brushing treatment, transplanting to pots, and measurements of *Tagetes patula*, *T. erecta* and *Calendula officinalis* transplants

Species	Days after sowing				
	Start of treatment	End of treatment	Trans-planting	1 st measurement (at the end of treatment)	2 nd measurement (at the beginning of flowering)
<i>Tagetes patula</i> 'Safari Bolero' ¹	11	26	27	28	41 - 59
<i>Tagetes erecta</i> 'Discovery Orange' ¹	12	42	35	43	63 - 78
<i>Calendula officinalis</i> 'Ball's Orange' ²	14	39	29	40	48 - 68

¹Sowing date: April 22, 2015; ²sowing date: April 30, 2015.

Statistical analysis was performed by testing the differences between the two sample means for independent samples using a t-test.

Results and discussion

At the end of brushing treatment, the plant height was statistically significantly lower (24%) only in *C. officinalis*, whereas, at the beginning of flowering, the height of treated plants was statistically equal to untreated ones in all examined species. In *T. patula*, the treatment also did not lead to differences in other measured morphological parameters, both at the end of treatment and at the beginning of flowering (Tables 2 and 3).

In treated *T. erecta* plants, stem diameter was slightly higher than in untreated plants (Table 2). When exposed to mechanical stress, plants may respond by increasing stem thickness, although reduction in stem diameter has been also reported in some studies (Biddington, 1986 and references therein; Samimy, 1993; Garner and Björkman, 1997; Anten et al., 2005). In *C. officinalis*, brushing treatment did not result in significant difference in stem diameter. However, treated plants had straighter stems and showed more upright growth. In treated group, bending of stems was observed in 36% of plants as compared to 76% of plants in untreated group. Also, untreated *C. officinalis* plants in many cases laid down under their own weight. Mechanical stress has been commonly reported to increase stem and petiole strength (Biddington, 1986; Latimer and Beverly, 1993; Mitchell and Myers, 1995; Latimer, 1998). This may result in plants less prone to breaking and more resistant to physical stress during handling and transport. Such sturdier plants also maintain more upright growth habit and show less stem deformations after planting outdoors (Latimer, 1998).

In *T. erecta* plants, brushing treatment resulted in 6% more lateral branches and 24% more leaves as compared to the untreated plants (Tables 2 and 3). This effect is particularly advantageous for transplants of ornamental bedding and pot plants as they appear more exuberant and vigorous. It has already been reported that mechanical perturbations may stimulate branching in plants. For example, mechanical stress enhanced stolon production in *Potentilla reptans* L. (Liu et al., 2007) and promoted basal branching in *Arabidopsis thaliana* (Pigliucci, 2002).

Treated *T. erecta* plants had significantly reduced length and width of the largest leaf (9% and 10%, respectively), but only when measured at the end of treatment (Table 3). In treated *C. officinalis* plants, the leaf length was in both measurements statistically lower than in untreated plants (11% and 7%, at the end of treatment and at the beginning of flowering, respectively) and the leaf width was higher (18%) as compared to untreated plants, but only at the beginning of flowering. Brushing treatment has been found to significantly reduce leaf area in *Petunia x hybrida* Vilm. and *Viola tricolor* L. (Garner et al., 1997). In *Callistephus chinensis* (L.) Ness and *Ocimum basilicum* L. brushing resulted in lower leaf length, and, in *Origanum majorana* L., reduced both leaf length and width (Autio et al., 1994; Horvat et al., 2010). Lower leaf length in mechanically stressed plants may considerably contribute to more compact shape of plants, especially in the cases in which mechanical stress also reduces plant height.

Table 2. The effect of brushing treatment on plant height, stem diameter, and number of lateral branches in *Tagetes patula*, *T. erecta* and *Calendula officinalis* transplants

Species	Number of brush strokes per day	At the end of treatment	At the beginning of flowering		
		Plant height (cm)	Plant height (cm)	Stem diameter (mm)	Number of lateral branches
<i>Tagetes patula</i> 'Safari Bolero'	0	7.7	12.2	4.6	4.5
	50	7.5	12.3	4.7	4.6
	t-test	n.s.	n.s.	n.s.	n.s.
<i>Tagetes erecta</i> 'Discovery Orange'	0	18.7	25	8.9	17
	50	19.1	25.4	9.1	18
	t-test	n.s.	n.s.	*	*
<i>Calendula officinalis</i> 'Ball's Orange'	0	20.2	46.3	6.8	9.5
	25	15.3	43.5	6.8	11
	t-test	**	n.s.	n.s.	n.s.

Statistically significant difference between mean values according to t-test: * $P \leq 0.05$ and ** $P \leq 0.01$; n.s. - not statistically significant.

One of the potential limitations to applying brushing as a method of mechanical conditioning is that some plants may be easily damaged by such treatment (Latimer, 1998). In all three species examined in this study, dragging of the cloth across the plant canopy caused damage to some plants, primarily to their leaves. This was most prominent in *C. officinalis*, in which as many as two-thirds of treated plants showed some kind of leaf injury. In both *T. patula* and *T. erecta* brushing caused leaf injuries to a much lesser extent, with higher percentage of damaged plants noted in *T. erecta* (Table 3). The injuries were mainly caused by sticking of cotton cloth to the leaf surface and this was particularly pronounced in *C. officinalis* due to its hairy leaves. In *C. officinalis* brushing treatment often resulted in partial or complete physical breakage of leaf blades. Treated *C. officinalis* transplants also frequently had curled leaves, mainly younger ones, and sometimes leaf tips of very young expanding leaves were damaged by brushing, becoming brown and necrotic. In both *T. patula* and *T. erecta*, injuries were mostly visible on leaf edges, which often became bleached, but the treatment sometimes also led to deformation of the leaf blades. All these types of leaf injuries considerably affected visual quality of transplants. Further research is thus needed to find effective but less damaging methods for mechanical

conditioning of these species, such as placing an obstacle on top of the plants (physical impedance) or treatments in which physical contact with plants is avoided (shaking, exposure to vibrations etc.). For example, Garner and Björkman (1997) found that mechanical stress imposed by impedance treatment can reduce elongation of tomato transplants as effectively as brushing.

Brushing treatment may not only result in direct damage to plant tissue, but might also facilitate the spread of pathogens. Besides, brushing material may have abrasive effect on plant tissue, which opens the way to pathogen entry into plants (Latimer and Beverly, 1993; Mitchell, 1996). In the present study, no pathogen-induced changes were visible in the examined species. However, injuries to leaves caused by pest feeding were observed in some *C. officinalis* transplants, but only in untreated group. This suggests that mechanical stress or damage to leaf tissue caused by brushing might have led to increased resistance to pest attack in treated *C. officinalis* plants. It has been shown that plant resistance to pathogens and pests can be activated by mechanical wounding (Chassot et al., 2008; Savatin et al., 2014), but also by exposing plants to mechanical stress without wounding tissue (Cipollini, 1997; Benikhlef et al., 2013). Brushing treatment has been found to significantly reduce thrips and mite populations in some floricultural crops (Latimer and Oetting, 1999), as well as, to reduce number of thrips and aphids in some vegetable transplants (Latimer i Oetting, 1994). Mechanical conditioning of horticultural crops might therefore be beneficial also as a part of integrated pest management program in greenhouses (Latimer, 1998).

Mechanical perturbation has been reported to affect reproductive development in some plants, such as increasing time to flowering (Biddington, 1986; Mitchell and Myers, 1995). For example, flowering in *Erysimum cheiri* (L.) Crantz can be delayed or inhibited by shaking treatment (Salehi and Salehi, 2009). In the present study, time of flowering onset was not markedly affected by brushing treatment. In *T. patula* and *C. officinalis*, treated plants started to flower nearly at the same time as untreated ones, and the number of days to the flowering of 50% of plants did not differ between the groups. In *T. erecta*, treated group reached flowering three days earlier than untreated one, but 50% of plants were in flower at nearly the same time in both groups (Table 4). In addition to shift in flowering time, mechanical stress can also lead to fewer and smaller flowers in treated plants (Akers and Mitchell, 1985; Biddington, 1986; Mitchell and Myers, 1995). In our study, treated *T. erecta* plants had 10% longer peduncles as compared to untreated ones, and treated plants of *T. erecta* and *C. officinalis* had significantly higher number of inflorescence buds (39% and 40%, respectively), at the beginning of flowering (Table 4). The latter effect of mechanical stress is of particular significance for commercial floricultural production, as number of flowers/inflorescences is one of the main criteria of visual quality of bedding and flowering pot plants.

Table 3. The effect of brushing treatment on leaves of *Tagetes patula*, *T. erecta* and *Calendula officinalis* transplants

Species	At the end of treatment			At the beginning of flowering				
	Number of brush strokes per day	Length of the largest leaf (cm)	Width of the largest leaf (cm)	Length of the largest leaf (cm)	Width of the largest leaf (cm)	Number of leaves per plant	Plants with injured leaves (%)	Injured leaves per plant (%) ¹
<i>Tagetes patula</i> 'Safari Bolero'	0	6.5	4.1	13.3	6.8	30.3	0	0
	50	6.4	4.1	10.9	6.6	31.8	16.7	10.5
	t-test	n.s.	n.s.	n.s.	n.s.	n.s.	-	-
<i>Tagetes erecta</i> 'Discovery Orange'	0	15.3	8.8	17.2	10.9	40.2	0	0
	50	14	8	16.9	11.1	50	23.3	3.8
	t-test	**	**	n.s.	n.s.	**	-	-
<i>Calendula officinalis</i> 'Ball's Orange'	0	17.5	4	16.7	4	56.2	0	0
	25	15.5	3.9	15.5	4.7	59	66.7	5.1
	t-test	**	n.s.	*	**	n.s.	-	-

Statistically significant difference between mean values according to t-test: * $P \leq 0.05$ and ** $P \leq 0.01$; n.s. - not statistically significant. ¹Percentage of injured leaves in total number of leaves >3 cm in length.

Table 4. The effect of brushing treatment on time of flowering, peduncle length, and number of inflorescence buds in *Tagetes patula*, *T. erecta* and *Calendula officinalis* transplants

Species	Number of brush strokes per day	Beginning of flowering (date)	50% flowering	Peduncle length (cm)	Number of inflorescence buds per plant
<i>Tagetes patula</i> 'Safari Bolero'	0	June 01	June 04	6	2.7
	50	June 01	June 04	6.3	2.7
	t-test			n.s.	n.s.
<i>Tagetes erecta</i> 'Discovery Orange'	0	June 29	July 04	4.1	6.2
	50	June 26	July 03	4.5	8.6
	t-test			**	**
<i>Calendula officinalis</i> 'Ball's Orange'	0	June 13	June 20	7.3	3
	25	June 14	June 20	6.3	4.2
	t-test			n.s.	**

Statistically significant difference between mean values according to t-test: ** $P \leq 0.01$; n.s. - not statistically significant.

Conclusions

In the species examined in this study, brushing treatment did not have statistically significant effect on plant height at the beginning of flowering. In *T. patula*, the treatment also did not influence other measured morphological parameters. However, treated *T. erecta* plants had higher numbers of lateral branches and leaves, while, in *C. officinalis*, the treatment resulted in more upright stems and shorter but wider leaves. Besides, brushed plants in both species had a higher number of inflorescence buds. The results of this study indicate that brushing treatment is not effective method for controlling plant height in the examined cultivars of *T. patula* and *T. erecta*, but can improve some commercially important characteristics in *T. erecta* and *C. officinalis* transplants. However, brushing also caused damage on some leaves, especially in *C. officinalis*. For these species further studies are needed to find the effectiveness of methods of mechanical conditioning in which physical contact with plants is reduced or avoided.

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