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Allelopathic potential of segetal and ruderal invasive alien plants

Alelopatski potencijal segetalnih i ruderalnih invazivnih alohtonih biljnih vrsta

Nenad NOVAK[1], Maja NOVAK[1*], Klara BARIĆ[2], Maja ŠĆEPANOVIĆ[2] and Dario IVIĆ[1]

[1] Croatian Centre for Agriculture, Food and Rural Affairs. Gorice 68b, 10000 Zagreb, Croatia, *correspondence: maja.novak@hcphs.hr

[2] University of Zagreb, Faculty of Agriculture, Department of Weed Science. Svetošimunska cesta 25, 10000 Zagreb, Croatia

Abstract

At the global level, the invasion of alien organisms is considered the second largest threat to biodiversity. The assumption is that the high allelopathic potential is one of the features that helps invasive plant species to spread to new areas. Allelopathic potential of 8 invasive plant species (donor species) and their impact on test-species was determined in the study. Donor species were velvetleaf (*Abutilon theophrasti* Med.), ragweed (*Ambrosia elatior* L.), jimsonweed (*Datura stramonium* L.), common cocklebur (*Xanthium strumarium* L.), tree of heaven (*Ailanthus altissima* (Mill.) Swingle), indigo bush (*Amorpha fruticosa* L.), Japanese knotweed (*Reynoutria japonica* Houtt.) and giant goldenrod (*Solidago gigantea* Aiton). Three cultivated plant species from 3 different plant families were used as test-species. Test-species were: oat (*Avena sativa* L.), oilseed rape (*Brassica napus* subsp. *oleifera*) and sunflower (*Helianthus annuus* L.). Water extracts made of whole plants of donor species were applied to the seeds of the test-species. High allelopathic potential was proven for all species included in experiment. Allelopathic effects were exclusively negative. Impact on germination for all species was much lower in relation to the impact on the radicle and shoot length of the test-species. Perennial donor species had a stronger allelopathic potential than annual donor species. Tree of heaven was the species with the strongest allelopathic potential. Its extracts inhibited germination, radicle and shoot length of oilseed rape by 19%, 94.88% and 98.91% respectively.

Keywords: allelopathy, germination, invasive plants, radicle length, shoot length, test-species

Sažetak

Na globalnoj razini, invazije alohtonih organizama predstavljaju drugu najveću prijetnju bioraznolikosti. Pretpostavka je da je visok alelopatski potencijal jedna od značajki koja pomaže invazivnim biljnim vrstama u širenju na nova područja. U radu je određen alelopatski potencijal 8 invazivnih biljnih vrsta (donor vrsta) i njihov učinak na test-vrste. Donor vrste su bile europski mračnjak (*Abutilon theophrasti* Med.), pelinolisni limundžik (*Ambrosia elatior* L.), bijeli kužnjak (*Datura stramonium* L.), dikica (*Xanthium strumarium* L.), pajasen (*Ailanthus altissima* (Mill.) Swingle), amorfa (*Amorpha fruticosa* L.), japanski dvornik (*Reynoutria japonica* Houtt.), velika zlatnica (*Solidago gigantea* Aiton). Kao test-biljke korištene su 3 kultivirane biljne vrste iz 3 različite biljne porodice: zob (*Avena sativa* L.), uljana repica (*Brassica napus* L.) i suncokret (*Helianthus annuus* L.). Vodeni ekstrakti napravljeni od cijelih biljaka donor vrsta aplicirani su na sjeme test-vrsta. Dokazan je visok alelopatski potencijal za sve vrste uključene u istraživanje. Alelopatski učinci bili su isključivo negativni. Učinak na klijavost svih vrsta mnogo je manji u odnosu na učinak na duljinu korjenčića i klica test-vrsta. Kod višegodišnjih donor vrsta zabilježen je jači alelopatski potencijal nego kod jednogodišnjih donor vrsta. Pajasen (*Ailanthus altissima* (Mill.) je vrsta s najjačim alelopatskim potencijalom. Njegovi ekstrakti inhibirali su klijanje, duljinu korjenčića i klice uljane repice za 19%, 94,88% i 98,91%.

Ključne riječi: alelopatija, duljina klice, duljina korjenčića, invazivne strane biljke, klijanje, test-vrsta

Introduction

Last few decades, with increasing public awareness of ecology, requirements for safer, more selective and more “natural” pesticides are increasingly growing i.e. for organic food produced without the use of chemical pesticides. The use of synthetic pesticides can be reduced by implementing allelopathy in agriculture. In weed science, allelochemicals can be used as natural herbicides, growth regulators or as the basis for the synthesis of new “environmental friendly” herbicides. An example for such herbicide is mesotrione which is one of the world leading herbicides in maize. Mesotrione was synthesized from an allelochemical leptospermone that was isolated from ornamental plant lemon bottlebrush (*Callistemon citrinus* (Curtis) Skeels) (Cornes, 2005).

To date, over 100 000 different secondary metabolites from plants and fungi have been identified. It is therefore logical to assume that those substances, if they were released from the plants, may have some effect on other plants (Wills, 2007). Weston (1996) states that allelochemicals can be extracted in an amount that can inhibit germination of the weeds. Duke et al. (2000 cited in Bhadoria, 2011) consider that biodegradable natural plant products can directly act as herbicides or can be used in herbicide synthesis.

Numerous examples of allelopathic effects between different plant species (cultivated and weed) suggest that allelopathy is directly or indirectly involved in weed control during agricultural production. The studies have shown that numerous crop species like sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.), barley (*Hordeum* *vulgare* L.), clover (*Trifolium pratense* L.), sesame (*Sesamum indicum* L.), tobacco (*Nicotiana tabacum* L.), sweet potatoes (*Ipomoea batatas* (L.) Lam.), rice (*Oriza sativa* L.), rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.) produce allelochemicals and can cause phytotoxic effect (Miller, 1996; Weston, 1996; Moradi et al., 2013). Weeds, unlike crop species, are evolutionally selected, and it is expected that in the most cases have greater allelopathic potential than the crop species. Further, emphasis of allelopathic studies was placed on invasive plant species that showed a high level of aggressiveness and spread faster than the other plant species. It is expected that high allelopathic potential, among other characteristics, helps them in spreading to new areas. Therefore, invasive plant species are a logical choice in searching for plants with high allelopathic potential. Baličević et al. (2015a and 2015b) investigated the allelopathic effect of invasive species giant goldenrod (*Solidago gigantea* Aiton) and 3 weed species on crops and weeds. Csiszár et al. (2013) have investigated the allelopathic effect of 14 woody and 20 herbaceous plant species. Their results confirmed that Japanese knotweed (*Reynoutria japonica* Houtt.), tree of heaven (*Ailanthus altissima* (Mill.) Swingle), ragweed (*Ambrosia elatior* L.), indigo bush (*Amorpha fruticosa* L.), silkweed (*Asclepias syriaca* L.), western hackberry (*Celtis occidentalis* L.), wild cucumber (*Echinocystis lobata* (Michaux) Torrey & A. Gray), Japanese maple (*Acer palmatum* T.), green ash (*Fraxinus pennsylvanica* M.), Canada potato (*Helianthus tuberosus* L.) and giant cow parsnip (*Heracleum mantegazzianum* Sommier & Levier) showed the effect of reducing percentage of germination and growth of white mustard (*Sinapis alba* L.). Many other authors have investigated and demonstrated the allelopathic effect of invasive alien species on native or other herbal and weed species (Saxena, 2002; Csiszár, 2009; Pisula and Meiners, 2010b).

The objective of this study was to evaluate the allelopathic potential of 8 invasive alien plant species (donor species) from 6 different plant families: velvetleaf (*Abutilon theophrasti* Med.), ragweed, jimsonweed (*Datura stramonium* L.), common cocklebur (*Xanthium strumarium* L.), tree of heaven, indigo bush, Japanese knotweed and giant goldenrod on the early growth of oat, oilseed rape (*Brassica napus* subsp. *oleifera*) and sunflower. All invasive alien plant species are included in the Preliminary list of invasive alien plants in Croatia (Boršić et al., 2008). Plant species with the highest allelopathic potential, the most sensitive test-species and the differences in allelopathic potential between annual agricultural and perennial ruderal donor species were determined.

Materials and methods

Collection of plants and preparation of extracts

To explore the allelopathic potential of different invasive alien plants on cultivated test-species, fresh plant material was collected in the typical habitat of each species (Table 1). The whole plants with roots were collected before flowering in late summer-early autumn. Agricultural weed species were collected in non-agricultural areas to avoid possible residues of herbicides and possible impact on the results. For the preparation of representative sample 10 individuals were taken from the same location. Collection of plant material, preparations of extracts and treatments were performed by following the adjusted method by Kazinczi et al. (2004b), Takács et al. (2004), Kazinczi et al. (2013).

Table 1. Sampling data for donor species

Donor species	Date of collecting	Location	Habitat	Plant height (cm) and density (number of plants/shoots/m2)
Abutilon theophrasti Med.	4.10.2011.	Sesvete (Šašinovec)	Along the path near the agricultural land	30-50; 5
Ambrosia elatior L.	10.9.2011.	Zagreb (Sigečica)	Green space between the buildings	20-40; 5
Datura stramonium L.	4.10.2011.	Sesvete (Šašinovec)	Along the path near the agricultural land	30-50; 3
Xanthium strumarium L.	4.10.2011.	Sesvete (Šašinovec)	Along the path near the agricultural land	30-50; 2
Ailanthus altissima (Mill.) Swingle	28.9.2011.	Zagreb (beside Faculty of Veterinary Medicine)	Green space between the buildings	30-70; 10
Amorpha fruticosa L.	23.9.2011.	Slavonski Brod	Next to the embankment of the Sava river	30-70; 15
Reynoutria japonica Houtt.	10.9.2011.	Zagreb (Savica)	Next to the embankment of the Sava river	20-40; 10
Solidago gigantea Aiton	10.9.2011.	Zagreb (Sigečica)	Green space between the buildings	40-50 (100); 10

Laboratory experiment

Freshly collected donor plants, along with roots and shoots, were cut into small pieces (size 0.5-1 cm). After mixing all pieces of the individual plant, 250 g of plant material was soaked into in 1 l of distilled water for 24 hours at room temperature. After 24 hours, plant material was removed and extracts were filtered through filter paper (wrinkled 21/N, Munktel & Filtrak). Eight water extracts were used in laboratory experiment.

Experiment was carried out in Petri dishes. In each sterilized Petri dish, by 25 seeds of oilseed rape, oats and sunflower were placed between two filter paper layers in 4 replicates. After placing seed, 8 ml of water extracts for oats and sunflower, and 4 ml of water extracts for oilseed rape water were added per Petri dish. For the control treatment, distilled water was used. Petri dishes were placed in darkness at 22 ±2 °C in a climate chamber.

Experiments were carried out in the Weed Science laboratory at the Croatian centre for agriculture, food and rural affairs - Institute for plant protection.

Data analysis

Percentage of germination, radicle length and shoot length were determined for each variant.

All investigated parameters were determined at the same time for each test-species. Oliseed rape germination, radicle length and shoot length were measured 4 days after sowing, while for oats and sunflower it was measured 5 days after sowing.

Data was analysed by analysing the variance (ANOVA) using XLSTAT (2014.1.01) software. For comparison of mean values, LSD test was used when the F-test was significant at the P≤0.05 level.

Results

The effects of all investigated plant species are presented separately for each test-species in Tables 2. - 4. It can be noted that the effect on germination was not significantly affected. Unlike the percentage of germination, radicle and shoot length of the test-species were significantly inhibited in all treatments. All reactions of test-species, a total of 48 measured reactions (8 extracts, 3 test-species, and radicle and shoot length) were negative.

The only statistically significant effect on germination among all plant species included in the study, was recorded on oilseed rape in the treatment with tree of heaven water extract (Table 2). Tree of haven extract inhibited oilseed rape germination to 21.8%.

Table 2. The effect of donor species extracts on *Brassica napus* subsp. *oleifera*

Donor species	Percentage of germination	Radicle length (mm)	Deviation of radicle length (%)	Shoot length (mm)	Deviation of shoot length (%)
Abutilon theophrasti Med.	92 a	9.71 b	-60.2	4.21 d	-75.86
Ambrosia elatior L.	85 a	6.04 c	-75.25	4.52 d	-74.08
Datura stramonium L.	83 a	2.1 e	-91.39	5.49 d	-68.52
Xanthium strumarium L.	89 a	8.76 b	-64.1	15.43 b	-11.53
Ailanthus altissima (Mill.) Swingle	68 b	1.25 e	-94.88	0.19 e	-98.91
Amorpha fruticosa L.	89 a	5.83 d	-76.11	5.44 d	-68.81
Reynoutria japonica Houtt.	86 a	7.48 c	-69.34	7.35 c	-57.86
Solidago gigantea Aiton	85 a	6.71 c	-72.5	5.14 d	-70.53
Control	87 a	24.4 a		17.44 a	

The results that differ significantly between each other at the P<0.05 level are indicated in different letters.

The lowest inhibition of oilseed rape radicle length (60.20%) was recorded in treatment with velvetleaf extracts, while the highest inhibition was determined in treatment with tree of heaven extracts (94.88%). The shoot length of oilseed rape was inhibited from 11.53% in treatment with common cocklebur extracts, up to 98.91% in treatment with tree of heaven extracts. The inhibitory effect on oilseed rape which has been recorded in treatment with tree of heaven water extracts was the highest in the whole study. The effect on radicle length which was determined in treatment with jimsonweed did not differ statistically from the effect caused by tree of heaven extracts. The study has shown that oilseed rape is the most sensitive among 3 test-species.

No significant effect on oat germination was recorded in treatments with donor species (Table 3). The differences in effect on radicle length and shoot length between the donor species extracts are presented in Table 3.

Table 3. The effect of donor species extracts on *Avena sativa* L.

Donor species	Percentage of germination	Radicle length (mm)	Deviation of radicle length (%)	Shoot length (mm)	Deviation of shoot length (%)
Abutilon theophrasti Med.	93 a	30.41 b	-25.7	1.1 d	-88.89
Ambrosia elatior L.	97 a	22.16 d	-45.86	1.31 d	-86.77
Datura stramonium L.	85 a	9.09 g	-77.79	2.68 c	-72.93
Xanthium strumarium L.	97 a	25.75 c	-37.09	4.74 b	-52.12
Ailanthus altissima (Mill.) Swingle	90 a	9.86 fg	-75.91	0.95 d	-90.4
Amorpha fruticosa L.	91 a	11.79 f	-71.19	0.63 d	-93.64
Reynoutria japonica Houtt.	95 a	20.37 d	-50.23	1.81 c	-81.72
Solidago gigantea Aiton	90 a	18.83 e	-53.99	0.71 d	-92.83
Control	94 a	40.93 a		9.9 a	

The results that differ significantly between each other at the $P<0.05$ level are indicated in different letters.

The second strongest inhibition in the study, effect on the shoot length, was determined in treatment with desert false indigo, giant goldenrod and tree of heaven. All 3 donor species caused inhibition higher than 90%. In treatments with other donor species on oat, the inhibition on the shoot development was higher than 80% and was recorded for velvetleaf, ragweed and Japanese knotweed (Table 3). The effect on radicle length was lower compared to the shoot length. The highest effect was recorded in the treatment with jimsonweed (77.79%), followed by tree of heaven (75.91%) and desert false indigo (71.19%).

No significant effect on sunflower germination was recorded in treatments with all donor species (Table 4). The differences in effect on radicle length and shoot length between the donor species extracts are presented in Table 4.

Table 4. The effect of donor species extracts on *Helianthus annuus* L.

Donor species	Percentage of germination	Radicle length (mm)	Deviation of radicle length (%)	Shoot length (mm)	Deviation of shoot length (%)
Abutilon theophrasti Med.	98 a	27.12 b	-16.94	4.57 c	-36.87
Ambrosia elatior L.	97 a	19.48 d	-40.34	4.12 c	-43.09
Datura stramonium L.	93 a	5.7 f	-82.54	4.54 c	-37.29
Xanthium strumarium L.	92 a	24.18 c	-25.94	5.1 b	-29.56
Ailanthus altissima (Mill.) Swingle	97 a	18 d	-44.87	4.25 c	-41.3
Amorpha fruticosa L.	94 a	22.91 c	-29.83	4.87 b	-32.73
Reynoutria japonica Houtt.	96 a	9.89 e	-69.71	4.83 b	-33.29
Solidago gigantea Aiton	98 a	17.4 d	-46.71	5.02 b	-30.66
Control	98 a	32.65 a		7.24 a	

The results that differ significantly between each other at the $P<0.05$ level are indicated in different letters.

The strongest inhibition of radicle length (82.54%) was measured in treatment with jimsonweed. It was the only reaction significantly different from all investigated donor species. The second strongest inhibition of radicle length (69.71%) was recorded in treatment with Japanese knotweed. The effect on sunflower shoot length was lower compared to the effect on radicle length. The most significant effect on shoot length was measured in the treatment with ragweed (43.09%). A similar result has been recorded in treatment with tree of heaven. The results of shoot length inhibition in treatments with ragweed, tree of heaven, jimsonweed and velvetleaf did not differ statistically. Results showed that sunflower was the most tolerant test-species among 3 species included in the study.

Results of the study showed that tree of heaven was the donor species which led to the strongest inhibitory effect on test-species. Along with tree of heaven, jimsonweed was also shown as a plant with high allelopathic potential. Jimsonweed caused high radicle inhibition on all test-species (77.79% oat, 82.54% sunflower and 91.39% oilseed rape) (Table 2. - 4). When the effect on the shoot length was measured, strongest reaction was observed in treatment with tree of heaven (98.91% oilseed rape) and indigo bush (93.64% oat) from perennial species, but also in the treatment with ragweed (86.77% on oat) and velvetleaf (88.89% on oat) from annual species (Table 2. - 4). It is evident that the inhibition of germination, radicle and shoot length was more pronounced among ruderal perennial plants (tree of heaven, indigo bush, Japanese knotweed and giant goldenrod).

Discussion

Results of the study confirmed the common allegation that allelopathic effect on the initial growth of seedlings is stronger in relation to the effect on germination of test-species. Norby and Kozlowski (1980), Đikić (2005), Lovett et al*.* (2006) and Kazinczi et al*.* (2013) confirmed that there was no significant effect on germination of test-species. However, Bruckner (1998) and Hodisan et al. (2009) reported a significant reduction in the germination percentage of test-species in treatment with water extract of ragweed. Differences in effect on germination in treatment with ragweed can be attributed to different concentrations and different parts of plant material used for the extracts preparation. The difference in test species influence the interpretation of the results. Pisula and Meiners (2010a) and Csiszár et al. (2012) reported the same effect in the treatment with water extract of giant goldenrod, but they also used only leaves for preparation of water extracts. Choi et al. (2010) determined the inhibition of germination for 68.3%, 74.6% and 87.3% on different test-species in treatments with leaf extract of ragweed. Šćepanović et al. (2007) confirmed the inhibitory effect of velvetleaf and jimsonweed extracts on germination of maize. Contrary to present study, the authors kept the plant material in the water for 48 hours longer when preparing the extracts, and the test-species were different. In research of Kazinczi et al. (2004a), water extract of jimsonweed reduced germination of sunflower seeds for 86%. Konstantinović et al. (2012) and Konstantinović et al. (2013) confirmed germination reduction of maize and soybean seeds after treatment with water extracts of velvetleaf. Inam et al. (1987), Kadioglu (2004) and Jalal et al. (2013) reported that an extract of common cocklebur inhibited germination of lettuce (*Lactuca sativa* L.), field mustard (*Brassica campestris* L.), maize, wheat, barley, perennial ryegrass (*Lolium perenne* L.) and sterile oat (*Avena sterilis* L.). The difference in the effect on germination in the reported studies can be attributed to the different concentrations of extracts, different plant parts used for preparation of extracts and seed size of the test-species.

The results related to the effect on radicle and shoot length of test-species in the present study are consistent with the results of De Feo et al. (2003) and Pisula and Meiners (2010b). The authors have also pointed tree of heaven as a plant with the strongest allelopathic potential, among 10 plant species tested. Results of the present study related to shoot inhibition in treatment with water extracts of desert false indigo, giant goldenrod and tree of heaven are comparable to those reported by Csiszár et al. (2013) and Pavićević (2013). The authors also demonstrated a significant inhibition of radicle and shoot length in treatments with the same donor species. Vrchota and Sera (2008) have also demonstrated negative allelopathic effects of Japanese knotweed on test-species. In the study of Csiszár (2009), early growth of white mustard was inhibited by the water extracts of desert false indigo and tree of heaven, which is also comparable to the results recorded in the present study. However, Csiszár (2009) reported that the extract of desert false indigo caused stronger inhibition compared to the tree of heaven extract. This is not in the line with obtained results, where tree of heaven was the most potent plant species. Such differences can be explained by the different test-species used in the studies. Results for velvetleaf in the present study are in the line with those reported by Bhowmik and Doll (1982), Simić and Uludag (2007) and Šćepanović et al. (2007). Those authors stated that velvetleaf provide high allelopathic potential and have negative effect on early growth of the test-species.

In treatment with giant goldenrod, it is interesting that many authors point out various allelopathic effects (inhibitory, neutral and stimulating) on parameters measured. Jezierska-Domaradzka et al. (2011) stated that the extracts of giant goldenrod had no effect on oilseed rape. Csiszár et al. (2012) reported that the water extract of giant goldenrod significantly inhibited radicle length of white mustard, while the impact on shoot length was not significant. Sekutowski et al. (2012) indicated that extracts of giant goldenrod had a stimulating effect on the radicle length of sunflower. All those results differ between themselves and from the results in this study, where the radicle length of sunflower and radicle and shoot length of oilseed rape were significantly inhibited. The differences between results on the effect on eary growth can be attributed, in the most cases, to different concentrations of extracts, different plant parts used for preparation of extracts and the difference in the sensitivity of the test species.

Chon and Nelson (2010) pointed out that many species of the Asteraceae family have a high allelopathic potential, but the authors do not consider *Ambrosia* genus significantly potent. Within the Asteraceae family, authors emphasized the genus *Xanthium*. The present study showed that ragweed was the species with the significantly stronger allelopathic effect compared to common cocklebur, which was not in the line with the Chon and Nelson (2010). However, it was comparable with the study performed by Shajie and Saffari (2007), Tanveer et al. (2008) and Konstantinović et al. (2013), who stated that common cocklebur has a high allelopathic potential. Results recorded for ragweed in the present study are consistent with those reported by Bruckner (1998), Csiszár et al. (2013) and Vidotto et al. (2013). Šimic and Uludag (2007) also considered ragweed as a species which reduces the yield of sunflower by negative allelopathic effect.

Except ragweed, the authors have also recorded jimsonweed as a plant of high allelopathic potential, which is in accordance with the results of this study. The results are also in line with those reported by Lovett et al. (1981), Shajie and Saffari (2009) and You and Wang (2011), who demonstrated radicle and shoot inhibition of several test-species, including maize, caused by jimsonweed extract. Contrary to that, Šćepanović et al. (2007) reported that the extract of jimsonweeds' above ground parts inhibited the shoot length, but stimulated the radicle length of the maize. Pacanoski et al. (2014) have also reported stimulating effect of jimsonweed extract on radicle and shoot length of sunflower, which is not in line with the results recorded in this study. It can be assumed that the differences in allelopathic effect of jimsonweed water extract were due to the differences in preparation of extracts and in different plant parts used for the extract preparation. Opposed to present study, Šćepanović et al. (2007) kept plant material for 96 hours. Again, the difference from Šćepanović et al. (2007) and Pacanoski et al. (2014) was also recorded for different parts of donor species (individual parts/whole plant). In studies where the same test-species were used, the differences in the effect could be attributed to the different cultivars of the test-species.

Conclusions

Extracts of all invasive alien plant species included in the study (velvetleaf, ragweed, jimsonweed, common cocklebur, tree of heaven, indigo bush, Japanese knotweed and giant goldenrod) showed inhibitory allelopathic effect on the initial development of test-species oat, oilseed rape and sunflower. Generally, the allelopathic effect on germination for all species is much lower than the effect on radicle and shoot length of the test-species. Tree of heaven was the only donor species which inhibited germination of one test-species, oilseed rape. The allelopathic effect varied depending on donor and test-species. Perennial donor species showed a stronger allelopathic potential than the annual species.

Tree of heaven showed the strongest allelopathic potential, followed by jimsonweed, indigo bush, giant goldenrod, ragweed and Japanese knotweed. The annual species velvetleaf and common cocklebur were the species with lower allelopathic potential. Oilseed rape was the most sensitive test-species, followed by oat and sunflower.

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