Effect of herbicide treatment on milk thistle (*Silybum marianum* (L.) Gaertn) germination

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Abstract: The effect of herbicide treatment on milk thistle has been observed in the years 2010 and 2011 in the experimental field station Žabčice. The milk thistle variety "Silyb" was sown. In the first year the seeds were obtained from the company IREL, Ltd.; in the second year the seed harvested in the year 2010 were used. The phytotoxicity of used herbicides was observed during the vegetation period in both years (Stomp 400 SC, Refine, Butisan Star, Afalon 45 SC, Fusilade Forte, Glean + Starane and untreated control plot. In 2010 the harvest was performed in two terms due to the gradual ripening; in 2011 only once due to excessive rainfall. The achenes harvested in 2010 were evaluated according to following characteristics: yield ($g.m^{-2}$), overflow of the sieve (%) (2.2, 2, and the rest), weight of thousand seeds (g), light and dark achenes germination in the year of harvest and light and dark achenes germination after storage (%). In 2011 following characteristics were evaluated: yield, germination ability and weight of thousand of seeds.

Key-Words: germination, milk thistle, yield, weight of thousand seeds

Introduction

Milk thistle, *Silybum marianum* (L.) Gaertn, belongs to the Asteraceae family; it is an annual herb of a height 150 – 200 cm. There are two varieties protected by the law in the Czech Republic - Silyb and Mirel [1]. Milk thistle is grown mostly for pharmaceutical use, for the flavonolignans found in fruits that protect liver cells, stabilise cell membranes and prevent toxins to enter hepatocytes [2]. The growing area varied in the range 500-5000 ha during last 5 years according to the need of processing companies [3, 4].

Milk thistle is suitable for potato-growing or beet-growing regions. Uneven maturation time of individual anthodia is typical and the achenes tend to fall out from ripe anthodia, so optimal time of harvest must be selected [5, 6].

Although the growing technology is known from 70s and 80s, it was never solved in complex including the plant protection; no plant protection products are registered for milk thistle [7].

Herbicides for milk thistle haven't been investigated yet in the framework on modern technologies. Milk thistle has good weed-removing qualities thanks to quick growth and high leaves cover, but decreased competitive ability during germination to extensive growth. It is an unpleasant weed itself. The seeds maintain their germination ability for a long time. According to Czyž [8] it is possible to sow milk thistle repeatedly in the same plot, but Andrzejewska and Lamparski [9] warn against the expansion of *Cleonis pigra*.

Most common weeds are *Sonchus arvensis*, *Elytrigia repens*, *Cirsium arvense, Chenopodium album*, *Raphanus raphanistrum, Sinapis arvensis, Galium aparine, Mentha crispa, Stachys arvensis, Convolvulus arvensis* and *Atriplex patula*. Milk thistle itself is often an unpleasant weed for next crops. To diminish the weeding, it is good to use a following crop that won't let germinated weed plants to spread seeds and weed the plot again, like grass or clover-grass mixtures where weed moving is used or monocotyledonous crops allowing the use of herbicides against dicotyledonous weeds. Weed removing should be based on mechanical cultivation. Herbicides can be given into the soil before sowing. However, the herbicides against dicotyledonous weeds and annual grasses must be applied not sooner than three days one after the other. There isn't any registered weed-regulation product for milk thistle growth at the moment in the Czech Republic, but this area is widely researched.

Material and methods

Experimental methodology

Milk thistle experiments were established in the field experimental station MENDELU in Žabčice, using the method of split parts. The position of field experimental station Žabčice is north latitude 49°0′ and east longitude 16°16′. The cadastral area is situated in corn-breeding region, subregion K_2, that belongs to the warmest in the Czech Republic. The locality altitude is 179 m above the sea level. This dry south-moravian region has typical inland climate with dryness caused by large evaporation of soil moisture due to stronger winds [10]. According to BPEJ classification it is very warm and dry climatic district. The value of Lang rain factor is ca. 57; that means one of the most dry localities. Average yearly temperature is 9.2°C, July is the warmest month with average daily air temperature 19.3°C, January is the coldest month with average temperature -2.0°C. As for the rainfall, the locality belongs to dry regions, 30-year average total rainfall is 480 mm. The rainfall is distributed very unevenly during the vegetation period. The length of sunlight varies in the range of 1800-2000 hours per year.

Table 1 Temperature and rainfall values in I.-XII. 2010 and 2011 at the experimental plot [10]

Year	Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	$\bar{x}$
2010	Average temper.	-3.9	-0.6	4.8	10.2	14.0	18.7	21.9	19.3	13.7	7.3	6.7	-3.9	**9.02**
	Total rainfall	46.8	22.8	9.8	53.1	102.4	79.8	87.9	75.8	57.8	10.4	32.8	11.1	**49.2**
2011	Average temper.	-0.4	-0.9	5.4	12.4	15.3	19.4	19.2	20.5	17.1	9.3	2.5	2.2	**10.2**
	Total rainfall	21.4	4.6	39.3	33.2	46.2	42.9	79.8	42.4	31.3	22.6	1.6	14.6	**31.6**

Source: The report of the experiment with milk thistle (Silybum marianum (L.) Gaertn.) in 2010 and 2011. The average temperature is given in °C, the total rainfall in mm

As shown in Table 1, average month temperatures and especially total rainfalls were very different in experimental years. In 2011 the average yearly total rainfall was 31.6 mm (per month), which is by 17.6 mm less than in 2010, but also less than the long-term average monthly rainfall (40 mm). As for the temperatures, in the year 2011 were found higher temperatures during the vegetation period compared to 2010, especially in June. These differences could affect both the effectiveness of herbicide treatment and the formation of yield components in milk thistle. The soil type in the locality is gleyic fluvisoil (FLq). Redoximorf characteristics are found from the depth of 70 cm. The level of underground water is in the depth of 100 cm. In terms of particle size distribution it is a heavy soil [10]. The experimental plot was prepared for sowing a common way and wasn't fertilized. The crop before milk thistle was winter wheat in 2010 and milk thistle in 2011.

The sowing was performed on April 1st 2010; treated milk thistle seeds were obtained from the company IREL, Ltd. The seed germination rate was 78.13 % (determined according to Methodologies of Seed Testing of the Ministry of Agriculture No. 34349/04-17220). For the sowing on March 28th 2011 the seeds gained from the cultivation in 2010 were used. The seeds were stored in laboratory conditions and the segmentation by the 2.2 mm sieve was performed. The seeds used for sowing had the germination rate 96.45%.

In both years the sowing was performed by the means of sowing machine KVERNELAND ACCORD PNEUMATIC DA-l into 0.25 m rows; with the seed rate 6 $kg.ha^{-1}$.

In the year 2010 the growth emerged on April 21st 2010. Total area of the experiment was 650 m^2. The area was divided to 18 plots of the size 24 m^2. In 2011 the growth emerged on April 18th 2011. Total area of the experiment was 730 m^2. The area was divided to 21 plots of the size 24 m^2.

Herbicides were applied on individual plots in below-given variants, always repeated triplicate.

1. Control,
2. STOMP 400 SC – 2.5 $l.ha^{-1}$
3. REFINE – 10 $g.ha^{-1}$
4. BUTISAN STAR – 1.5 $l.ha^{-1}$
5. AFALON 45 SC – 1.5 $l.ha^{-1}$
6. FUSILADE FORTE EC – 1.5 $l.ha^{-1}$
7. GLEAN + STARANE – 7 $g.ha^{-1}$ + 3 $l.ha^{-1}$

Evaluation of the experiment was performed in three terms according to the Methodology for the determination of preparation phytotoxicity [EPPO

No. 135/1988]. Part of the area was flooded in the end of May 2010 due to the cloudburst.

Because of consecutive ripening the harvest was performed in two terms in the year 2010: July 16th 2010 and July 23rd 2010. Hand cutted ripe anthodia were harvested (white pappus and brown achenes). In 2011 there was only one harvest term - July 20th 2011. Due to excessive rainfall the harvest was only performed with two repetitions and the rest of the growth was ploughed.

The anthodia with achenes were dried after the harvest at the temperature 20°C and the achenes were removed from anthodia by the trashing machines MOVIS and HALTRUB 20 in 2010 and 2011, resp., and manually cleaned. The weight of thousand kernel (TKW) was determined as well as the germination ratio from individual harvests and plots and for the harvest 2010 also the seed segmentation was performed according to the size by 2200 and 2000 µm sieves on Steinecker sieving machine.

Data were evaluated in the program STATISTICA CZ 10 by the means of one-factor and two-factor variance analysis and consequent testing.

Results and Discussion

Results from the harvest year 2010

Phytotoxicity

Toxicity evaluation, May 27th 2010

1. CONTROL - in the phase BBCH 14-18 the phytotoxicity was zero and a wide range of weeds were present, like emerging barnyardgrass, field penny-cress and shepherd's purse; the average amount of weeds in a control plot was 5 pcs.m^2.

2. STOMP 400 SC - in the phase BBCH 14-17 the phytotoxicity was up to 5%, edges of leaves where the preparation was applied were damaged, but newly grown leaves were unharmed. Necroses were observed in damaged leaves (15 – 30%).

3. REFINE - in the phase BBCH 14 – 18 the phytotoxicity was up to 5%; leaves where the preparation was applied were damaged, but newly grown leaves were unharmed. Necroses (15 – 25%) occurred on whole area where the preparation was applied.

4. BUTISAN STAR - in the phase BBCH 14 – 18 the phytotoxicity was 2 – 3%. Some leaves were harmed, the damage was 10 – 15%. Newly grown leaves were unharmed. Necroses occurred on whole area where the preparation was applied.

5. AFALON 45 SC - in the phase BBCH 14-16 the phytotoxicity was 15 – 20%. The growth delay was up to 40%. Necroses (30 – 40%) occurred on whole area where the preparation was applied. The leaves were drying from the edges in the direction to centre. Newly grown leaves were unharmed.

6. FUSILADE FORTE 150 EC - in the phase BBCH 14 – 16 the phytotoxicity was 4 – 5%. Necroses (30 – 40%) occurred on whole area where the preparation was applied. The leaves were drying from the edges in the direction to centre.

Toxicity evaluation, June 10th 2010

1. CONTROL - in the phase BBCH 18 – 51 the phytotoxicity was zero and a wide range of weeds were present, like emerging barnyardgrass, bedstraw, field penny-cress, shepherd's purse, doorweed, creeping thistle, wild radish, sorrel, earth smoke and common lambsquarters.

2. STOMP 400 SC - in the phase BBCH 18 – 32 the phytotoxicity faded without harm of the growth top, wide range of weeds occurred, the same as those in the control plot.

3. REFINE STAR - in the phase BBCH 18 – 32 the phytotoxicity also faded, part of the plot was flooded.

4. BUTISAN - in the phase BBCH 18 – 32 the phytotoxicity was 0%, without necroses.

5. AFALON 45 SC - in the phase BBCH 18 – 32 the phytotoxicity was 5%, without necroses and damage.

6. FUSILADE FORTE 150 EC - in the phase BBCH 18 – 32 the phytotoxicity was 0%, occurrence of dicotyledonous weeds.

Toxicity evaluation, July 15th 2010

The phase BBCH 63 – 87 is valid for all variants. There aren't visible differences among the beginning of flowering and ripening. The differences among plant height and density are given rather by the soil and moisture characteristics of the stand.

Measurement results

Table 2 Average values of the yield, TKW and size selection (overflow over a sieve) of the milk thistle achenes in two harvest terms and selected herbicide treatments in the harvest year 2010

Date	Variation	Yield ($g.m^{-2}$)	TKW	Grading %			Germination		Germination next year	
				2.2	2	remain	light achenes	dark achenes	light achenes	dark achenes
1. - 16. 7. 2010	1 – control	22.50 ^{abc}	28.70 ^{a}	4.96 ^{a}	80.29 ^{d}	14.75 ^{ab}	88.67 ^{ab}	92.67 ^{ab}	91.33 ^{abc}	92.67 ^{ab}
	2 - STOMP 400 SC	19.23 ^{ab}	28.06 ^{a}	13.63 ^{a}	75.77 ^{d}	10.60 ^{ab}	91.33 ^{b}	100.00 ^{b}	86.00 ^{a}	100.00 ^{b}
	3 – REFINE	20.49 ^{abc}	28.66 ^{a}	69.94 ^{bc}	23.05 ^{ab}	7.01 ^{ab}	94.00 ^{b}	96.67 ^{ab}	89.33 ^{ab}	96.67 ^{ab}
	4 - BUTISAN STAR	22.42 ^{abc}	28.12 ^{a}	50.38 ^{b}	42.11 ^{bc}	7.51 ^{ab}	74.67 ^{a}	96.00 ^{ab}	85.33 ^{a}	96.00 ^{ab}
	5 - AFALON 45 SC	7.43 ^{a}	28.88 ^{a}	19.63 ^{a}	67.13 ^{cd}	13.24 ^{ab}	93.33 ^{b}	90.67 ^{a}	90.00 ^{ab}	90.67 ^{a}
	6 - FUSILADE FORTE	13.89 ^{a}	28.79 ^{a}	12.55 ^{a}	73.48 ^{d}	13.97 ^{ab}	97.33 ^{b}	92.67 ^{ab}	94.67 ^{bcd}	92.67 ^{ab}
	Mean	**17.66**	**28.54**	**28.52**	**60.31**	**11.18**	**89.88**	**94.78**	**89.44**	**94.78**
2. - 23. 7. 2010	1 – control	39.43 ^{bcd}	26.56 ^{a}	63.27 ^{bc}	19.92 ^{ab}	16.80 ^{b}	85.33 ^{ab}	98.67 ^{b}	98.00 ^{cd}	98.67 ^{b}
	2 - STOMP 400 SC	43.40 ^{bcd}	26.63 ^{a}	91.33 ^{c}	4.25 ^{a}	4.42 ^{a}	92.67 ^{b}	100.00 ^{b}	97.33 ^{cd}	100.00 ^{b}
	3 – REFINE	44.86 ^{cd}	27.02 ^{a}	84.45 ^{c}	8.93 ^{a}	6.62 ^{ab}	85.33 ^{ab}	98.67 ^{b}	98.67 ^{d}	98.67 ^{b}
	4 - BUTISAN STAR	41.60 ^{bcd}	27.03 ^{a}	83.14 ^{c}	4.28 ^{a}	12.58 ^{ab}	87.33 ^{ab}	98.00 ^{ab}	98.00 ^{cd}	98.00 ^{ab}
	5 - AFALON 45 SC	31.04 ^{abc}	28.60 ^{a}	71.71 ^{bc}	16.04 ^{ab}	12.25 ^{ab}	86.00 ^{ab}	100.00 ^{b}	94.67 ^{bcd}	100.00 ^{b}
	6 - FUSILADE FORTE	52.27 ^{d}	28.38 ^{a}	75.79 ^{bc}	15.36 ^{ab}	8.85 ^{ab}	97.33 ^{b}	98.00 ^{ab}	98.67 ^{d}	98.00 ^{ab}
	Mean	**42.1**	**27.37**	**78.28**	**11.46**	**10.25**	**88.99**	**98.89**	**97.55**	**98.89**

Note: Average values in the same columns marked with different numbers varies statistically at p=0.05; germination ability is given in [%]

In the first term of harvest (July 16th 2010) the total average values were lower in comparison with the second term (July 23rd 2010, see Table 2). The highest yield was in the second term of harvest in the plot treated by Fusilade forte, but it didn't vary significantly from all other preparations in the second term of harvest. The grading made by the means of Steinecker sieving machine showed in the 2.2 mm sieve overflow highest values for the second harvest term and treatment with STOMP 400 SC (91.33%), but it didn't vary significantly from all other preparations in the second term of harvest.

The germination ability of milk thistle was evaluated for selected achenes according to sensory selection to light and dark colour of achenes. The colour of achenes was supposed to be the characteristics of their ripeness and thus could affect the germination ability.

The germination ability of light achenes was lower than the one of dark achenes both in the first harvest and second term comparison. The conclusion is that the germination ability of dark achenes is higher in general.

Germination ability of achenes was evaluated also after a year of storage. The lowest germination ability was found after the treatment by herbicide preparations Butisan Star (85.33%) and Stomp 400 SC in the first harvest term (July 16th 2010). In the second harvest term (July 23rd 2010) the lowest germination ability was found after the treatment by herbicide preparation Afalon 45 SC (94.67%), but it didn't vary significantly from all other values. The lowest germination ability of dark achenes was found in the first harvest term (July 16th 2010) after the treatment by herbicide preparation Afalon 45 SC (90.67%). As shown in the table, the germination ability was generally higher for both light and dark achenes compared to the first harvest term; it is also apparent that the germination ability of dark achenes is higher. The comparison of germination ability after the harvest and after one year storage showed that the germination ability didn't change much, the trend stayed the same even after a year of storage (see Table 3). However, it was found out that after one year of storage the average germination ability of light achenes was increased in the second harvest term by almost 8%.

Results from the harvest year 2011
Phytotoxicity

Toxicity evaluation, May 9th 2011

1. CONTROL - in the phase BBCH 14 – 16 the phytotoxicity was zero and a wide range of weeds were present, like field mustard, wild radish, barnyardgrass, bedstraw, common lambsquarters, field penny-cress, pigweed and creeping thistle.

2. STOMP 400 SC - in the phase BBCH 14 – 16 the phytotoxicity was 5 %, slightly delayed growth of leaves, older leaves damaged, necrosis from the edges of leaves.

3. REFINE - in the phase BBCH 14 – 16 the phytotoxicity was up to 5%, leaves without necrosis.

4. BUTISAN STAR - in the phase BBCH 14 – 16 the phytotoxicity was 0%, resistant weed - wild radish.
5. AFALON 45 SC - in the phase BBCH 14 – 16 the phytotoxicity was 85-100%, spots on plants after the application.
6. FUSILADE FORTE 150 EC - in the phase BBCH 14 – 16 the phytotoxicity was 5-7%, the same damage like in the case of STOMP 400 SC, including wild radish.
7. GLEAN + STARANE - in the phase BBCH 14 – 16 the phytotoxicity was 10-15%, chlorotic plants in comparison with the control, slower growth, plants without necrotic spots.

Toxicity evaluation, May 27th 2011
1. CONTROL - in the phase BBCH 29 – 32 the phytotoxicity was 0% and following weeds occurred: field mustard, wild radish, barnyardgrass, bedstraw, common lambsquarters, field penny-cress, pigweed, creeping thistle.
2. STOMP 400 SC - in the phase BBCH 29 – 32 the phytotoxicity was 0%, the plot was clean, total herbicide efficiency was 85 – 90%, with rare occurrence of wild radish, barnyardgrass and creeping thistle.
3. REFINE - in the phase BBCH 29 – 32 the phytotoxicity was 0%, total herbicide efficiency was 95-97%, just barnyardgrass and creeping thistle.
4. BUTISAN STAR - in the phase BBCH 29 – 32 the phytotoxicity was 0%, total herbicide efficiency was 90%, the plot looks weedy, contains grown cruciferous weeds.
5. AFALON 45 SC - in the phase BBCH 29 – 32 the phytotoxicity was 90 – 95%, total herbicide efficiency was up to 95%.
6. FUSILADE FORTE 150 EC - in the phase BBCH 29-32 the phytotoxicity was 0%, plant damage including wild radish.
7. GLEAN + STARANE - in the phase BBCH 29 – 32 the phytotoxicity was 0%, total herbicide efficiency was 95%, barnyardgrass and damaged creeping thistle.

Toxicity evaluation, June 6th 2011
The phytotoxicity faded for all treated plants except herbicide Afalon, where the phytotoxicity varied in the range 90 – 95%.

Measurement results
The analysis of variance for the selected qualitative characters of milk thistle achenes grown in 2011 shows a highly significant impact of varieties on germination and a highly significant effect on the yield.

Table 3 Average values of the yield, TKW and germination ability of milk thistle achenes treated by selected herbicides in the harvest year 2011

Variety	Yield ($g.m^{-2}$)	TKW (g)	Germination (%)
1-Control	50.71 ^{a}	28.55 ^{a}	97.0 ^{d}
2-STOMP 400 SC	166.46 ^{d}	27.15 ^{a}	91.0 ^{b}
3-REFINE	152.17 ^{cd}	26.41 ^{a}	93.0 ^{c}
4-BUTISAN STAR	140.67 ^{cd}	27.41 ^{a}	98.0 ^{d}
5-AFALON 45 SC	78.65 ^{ab}	28.97 ^{a}	92.5 ^{bc}
6-FUSILADE FORTE EC	118.77 ^{bc}	26.98 ^{a}	86.0 ^{a}
7-GLEAN+STARANE	149.40 ^{cd}	26.26 ^{a}	97.0 ^{d}
Average	**122.40**	**27.39**	**93.5**

Note: Average values in the same columns marked with different numbers varies statistically at p=0.05

As can be seen in Table 3, the lowest yield gave the control plot (50.71 g); the value varied significantly from the yields from plots treated with the preparations Stomp 400 SC (166.46 g), Refine (152.17 g), Butisan Star (140.67 g), Glean + Starane (149.40 g) and Fusilade Forte EC (118.77 g). The control didn't vary statistically significantly from the treatment with Afalon 45 SC (78.65 g). There weren't any statistically significant differences among the TKW values. The lowest germination ability was found for the treatment with herbicide preparation Fusilade Forte EC (86.00%) and it varies statistically significantly from the treatments with Butisan Star (98.00%), Glean + Starane (97.00%) and from the control plot (97.00%).

Conclusion

The effect of herbicide treatment to the milk thistle was studied in years 2010 a 2011 in the field experimental station in Žabčice. In both years the milk thistle variety Silyb was sown; the first year the seeds were gained from the company IREL, Ltd., and in the second year the seeds from previous harvest were sown. Phytotoxicity of used herbicides was observed during the vegetation for Stomp 400 SC, Refine, Butisan Star, Afalon 45 SC, Fusilade Forte, Glean + Starane and untreated control plot. In the year 2010 the harvest was performed in two terms due to consecutive ripening. In 2011 the harvest was performed only once due to excessive rainfall during the harvest period.

The achenes harvested in 2010 were evaluated for following characteristics: yield in $g.m^{-2}$, size separation in % (2.2; 2 and the rest), TKW in g, germination ability of light and dark achenes in the year of harvest and after a year of storage in %. In the year 2011 following characteristics were evaluated: yield in $g.m^{-2}$, TKW in g and germination ability in %.

The results were influenced by the weather course; because of that, the effect of herbicides wasn't unambiguous in the observed years. Following conclusions result from the findings:

2010

The yield of achenes in the first harvest was highest from the control plot (22.5 $g.m^{-2}$), in the second harvest the yield was highest from the plot treated with the herbicide Fusilade Forte EC (52.27 $g.m^{-2}$). Total average harvest yield in 2010 was 59.8 $g.m^{-2}$.

TKW was highest in the plot treated with the herbicide Afalon 45 SC in both harvests (28.88 g and 28.6 g, resp.). TKW is the characteristics that showed the lowest variability in all observations (in the range 26.6 g – 28.9 g).

Separation of the achenes according to the size. The highest ratio of achenes gained in the 2.2 mm sieve was in the first harvest from the plot treated with the herbicide Refine (69.94%) and in the second harvest from the plot treated with the herbicide Stomp 400 SC (91.33%).

Germination ability after the harvest. In both cases the dark-coloured achenes had higher germination ability than the lighter ones (94.78% and 98.89%, resp.). The difference between germination abilities of light and dark achenes was 7.4%.

Germination ability after one year of storage was also higher for dark achenes, but the difference compared to light achenes was only 3.3%.

2011

The yield of achenes was highest from the plot treated with the herbicide Stomp 400 SC (166.46 $g.m^{-2}$) and lowest from the control plot (50.71 $g.m^{-2}$). Total average harvest yield in 2011 was 122.4 $g.m^{-2}$.

TKW was highest in the plot treated with the herbicide Afalon 45 SC (28.97 g) and lowest in the plot treated with the herbicides Glean + Starane (26.26 g). The average TKW value is comparable with the results from the year 2010 (27.39 g).

The germination ability was highest from the plot treated with the herbicide Butisan Star (98.00%). The average germination ability in the year 2011 was 93.5%.

From all used herbicide preparation, highest values of observed characteristics were reached repeatedly for the herbicides Stomp 400 SC and Fusilade Forte. These herbicides were evaluated from the viewpoint of phytotoxicity in three terms in various BBCH phases. During the first evaluation in 2010 and 2011 the phytotoxicities of herbicide preparations Stomp 400 SC and Fusilade Forte were found to be 5% and 4 – 5%, resp. In both following controls the phytotoxicity faded. The results indicate that the herbicide preparations Stomp 400 SC and Fusilade Forte can be considered suitable for the milk thistle growing. However, further examination of these herbicides is necessary.

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