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Reducing Damage of Late Cold on Persian Maple (*Acer velutinum* Boiss.) Seedlings by Delaying Germination and Seedling Protection

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ABSTRACT In the last decade, frequency of late cold incidence has increased in the upland forest nurseries. The aim of present study was to reduce the damage caused by spring frost and to increase the efficiency of Persian maple seedlings production in mountain nurseries. This study was conducted as split plot design with two main treatments and three replications in two consecutive years (2009-2010) in Farim Wood Company nursery, Mazandaran Province, Iran. Maple seeds were collected from a site located near the nursery. The main treatment was seedlings protection in two levels of covering the seedlings during the cold days and without covering. Sowing date was sub-treated and applied at six levels (2nd week of Dec., mid-Jan., 1st week of Feb., 3rd week of Feb., 3rd week of Mar. and mid-April). The results showed that seedling covered and seed sowing dates had significant ($p<0.01$) effect on reducing frost damage. Besides, interaction effect of sowing date and seedling cover was significant ($p<0.01$) in both years. There was no significant ($p>0.05$) effect between the sowing dates in protected plots. Overall, mortality of seedlings gradually reduced with shifting sowing date to March in uncovered plots. The maximum mortality rate was related to the sowing in 3rd week of February. Losses of seedlings were the same in covered and uncovered plots in mid-April sowing dates. In the unprotected plots, the maximum losses of seedlings were recorded in the sowing dates before March. In fact, if seed sowing of *A. velutinum* was postponed until early spring, the seedlings mortality rates were not significantly different in both treatments.

Key words: *Acer velutinum, Germination, North of Iran, Seedling cold injury, Sowing date*

1 INTRODUCTION

Persian maple (*Acer velutinum* Boiss.), with about 8% of forest stock volume, is a most important species for forest industry in Iran (Resaneh *et al.*, 2001). This species is light demand which distributed from east to west of south edge of Caspian Sea (Kiani *et al*., 2103). Its suitable growing location is north, northeast and northwest direction with deep drained soil. This tree can grow as high as 35 meters with about 100 centimeters diameter in suitable conditions (Sabeti, 2001). Persian maple seedlings production and plantation is an Integral part of Iranian Forests and Rangelands Organization annual programs (Mosadegh, 2010). However, in upland nurseries, high percent of Persian maple seedlings are completely vanished or seriously damaged due to the early fall cold or late spring frost (Espahbodi and Khorankeh, 2013).

Industrial wood production has been one of the most objectives of more plantation programs (Wood, 1997). Until recently, commercial forest planting in most tropical and

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non-tropical countries has been growing. The increasing demand for wood will be the main factors influencing the rate of plantation investment (Meka, E.Z, 2009).

Using standard and healthy seedlings is the fundamental conditions for the success of any plantation program. Increasing productivity in reforestation using plantation is related to both genetic and environmental factors.

Spring late cold or autumn early cold causes damage to the leaves, buds, branches and even to the roots of the plants. It may be caused seedlings death and reducing growth of seedlings. Frequent spring frost along with the gradually global warming of the earth is important issues in agriculture and natural resources in the present century (Man *et al*., 2009). Totally, the various damages of cold resulted in reforestation in a 40 percent of plantation programs (Sarvas, 2002). For this reason, control of cold damages is economically important in forestation area (Stevenson *et al*., 1999).

Plants react typically in two ways against spring frost; resistance and avoidance. Resistance usually can be created with a change in chemical interaction or changes in the concentration of enzymes and hormones (Anderson *et al*., 1995; Ali Ahmad Korori, 1999). Supply some nutrients, such as nitrogen, phosphorus, potassium, or using Absisyk acid or Gibberellic acid, are effective in increasing plant resistance. It is reported that taking 100 to 130 mg of nitrogen (70 percent in growth phase and 30 percent at fall season) is effective in reducing cold damage (Andivia *et al*., 2011). In avoidance way, plants keeps itself away from cold and frost through delaying seed germination and/or bud bursting in spring or falling leaves in early autumn.

Delaying the start of the vital phenomenon in spring was reported for some Rocky Mountains plant species (Rehfheldt *et al*., 1989 and 1991), *Abies fraseri* (Emerson *et al*., 2006) and Douglas fire (Jones and Gregg, 2006). It was also reported for mountain ash in Iran (Espahbodi *et al*., 2013).

According to the studies of Kramer *et al*. (2000), phonological response of tree species has important role in countering the late spring frost and early fall frost. Tree damage caused by freezing is a sign of incompatibility of genotypes climate zone (Saenz-Romro *et al*., 2006). Seedlings defense mechanism against freezing in different zones may be controlled by genetic factors (Xin and Browse, 2000).

Weiser (1970) and Morin *et al*. (2007) reported that plant resistance against extreme environmental conditions is a kind of compatibility reactions. For some species, such as *Pinus devonuiana*, an inverse linear relationship between elevation seed sources and frost damage has been reported (Saenze-Romro and Tapia-Olivares, 2008). Timmis *et al*. (1994) noted that although early growth leaf may cause the more rapid growth for Duglas-fire seedlings, equally the seedlings will be exposed to spring frost. The same results were also reported by Townsend (1977) and Lechowicz (1984). If the growth of seedlings do not stop and lose of leaves take place later than usual time, despite long-term growth, the risk of early fall frost will be increased (Leinonen and Hanninen, 2002).

In recent decades, increasing the temperature due to rising greenhouse gases, have caused the imbalances of earth climate system (Goodarzi *et al.*, 2015). The probability of potentially damaging freezing temperatures during bud break has increased with the increase of mean temperatures in spring and the subsequent advance of bud break (Cannell and Smith 1986, Kramer 1994, Linkosalo *et al*. 2000).

Since the spring frost occur typically 2 to 3 times higher than early fall frost, breeders are trying to produce progenies began to grow later and nursery managers effort to change the sowing date for delaying the begin of vital phenomenon (Stevenson *et al.,* 1999). Sudden and continuingly drop in temperature in the growing season, cause heavy damage on seedlings. Physical protection of seedlings against all types of cold is common in many countries, which doubled the production efficiency of the seedlings. For example Tinus *et al*. (2002) used the black nylon sheet to protect the roots of longleaf pine against the late autumn frost in Brooklyn. They reported that the tiny threshold temperature of longleaf pine seedling is -4 °C.

Effects of sowing date (February 24, March 17, April 21 and May 19), herbicides and covering of seedbed on germination of ash seeds were investigated by Jinks *et al.* (2006), in a UK's nursery. The results showed that seed germination increased gradually until April 21, and then decreased suddenly, which caused by reduction in soil moisture. But the effect of seedbed covering was not significant on seed germination. The effect of plant covering and seed sowing date on reducing of mountain ash seedlings due to spring cold was reported by Espahbodi and Khorankeh (2013). Mortality of seedlings was 80% and 20% in plots without and with covers sheet, respectively. In bare plots, mortality rate gradually decreased with approaching to the 4^{th} week of April.

There is no doubt, maple seedlings are high sensitive to spring late cold. Spring late frost injuries decrease the seedlings quality and also efficiency of seedling production. This raises the cost of seedling production. On the other hand, transmission of defective seedling is the main reasons for the lack of economic success of reforestation programs. For this reason, reducing the cold injuries in Persian maple seedlings is important. Therefore the main objectives of present study were to reduce the damage caused by spring frost and to increase the efficiency of Persian maple seedlings production in mountain nurseries.

2 MATERIALS AND METHODS

2.1 Research area

This research was conducted in the Darzikola nursery of Farim Wood Company (53° 16 '10" E and 35° 04' 32" N), located in southeast of Polesefid, Mazandaran Province, Iran. It is 1250 meters above sea level and its direction is southwest. The soil of nursery is brown forest, heavy texture and so deep with pH about 7. Based on the last 10-year weather information, the average annual rainfall of the nursery was between 630 and 834 mm.The average monthly temperature was recorded from 10.8 to 12.2 °C with absolute minimum and maximum temperature ranged from -11°C to -17.5°C and 33.5 to 37.5 °C, respectively. The minimum and maximum relative humidity was 68% and 89%, respectively. Climate of nursery based on DeMartenne index by a factor of I=30 was wet (Jazirehei, 2001).

2.2. Methods

Persian maple seeds were collected during October of 2009 from four healthy trees in a site located near the nursery and was repeated following year.

Covering of the seedlings with nylon sheet was the main treatment, which were adjusted at two levels (i.e., with and without nylon sheet). Sowing date was sub-treatment, determined at 6 levels (2^{nd} week of December, mid-January, 1^{st} week of February, 3^{rd} week of February, 3^{rd} week of March and mid-April). It was carried out in a split-plot design with three replications and repeated for two consecutive years.

Plot size was 150 ×120 cm with 5 rows of 20 cm intervals, and 125 healthy seeds were planted on each raw. To set the correct date for using nylon cover, weather data of three years before plan implementation (for March and April) was evaluated which showed that the temperature was dropped below zero degrees in the three years prior to this study. This information was used to determining the appropriate time for covering the seedlings. The arc-shaped thin woods were used for nylon cover which was 50 cm in the middle, but decreased gradually toward the periphery of plots until touched the ground. From February 24 to the end of May, prior planted plots were examined for recording seed germination. From the third week of March to the end of May, cold damage in seedling was recorded. The absolute minimum temperature during the plan implementation and sudden fall of temperature in March, April and May were evaluated.

2.3 Data analysis

According to repeated in two consecutive years, combined analysis with repeated in year was used (Yazdi-Samadi *et al*., 2002). Normality of the variables was checked by Kolmogrov-Smirnov test and Levene test was used to examine the equality of the variances. A Two-Way ANOVA was used to examine a significant difference between effects of various treatments on seed germination and seedlings mortalities. Duncan test apply to separate the averages of the dependent variables which were significantly affected by treatments. All analyses were performed using MSTATC and SPSS 21.

3 RESULTS

3.1 Weather temperature reduction during the research implementation

The year before this study (2008), temperature was suddenly dropped from +3 to -3°C and resume to +8, and then dropped to -4°C in Mar. 10 and 24, respectively (Figure 1). The same temperature fall was occurredin12 Mar. 2009 and 17 Mar. 2010 (Figures 2 and 3). In both years, the Persian maple seeds planted in 2nd week of December, mid-Jan. and early February germinated about 80%, 40% and 30%, respectively. All of these seedlings were exposed a sudden spring late cold in March. In that time, in the plots without protected cover, nearly 50% of Persian maple seedlings lost, however the seeds sown in 3rd week of February not germinated and remained intact.

The 2nd frost shock in two consecutive years occurred at the 4th week of March 2009 and 3rd week of Mar. 2010 (Figures 2 and 3). Persian maple seedlings planted until the 3rd week of February faced this long cold shock. The 3rd frost shock (temperature dropped from +5.5 to -5°C) occurred at mid-April 2009 (Figure 2) and 3rd week of April 2010 (Figure 3).

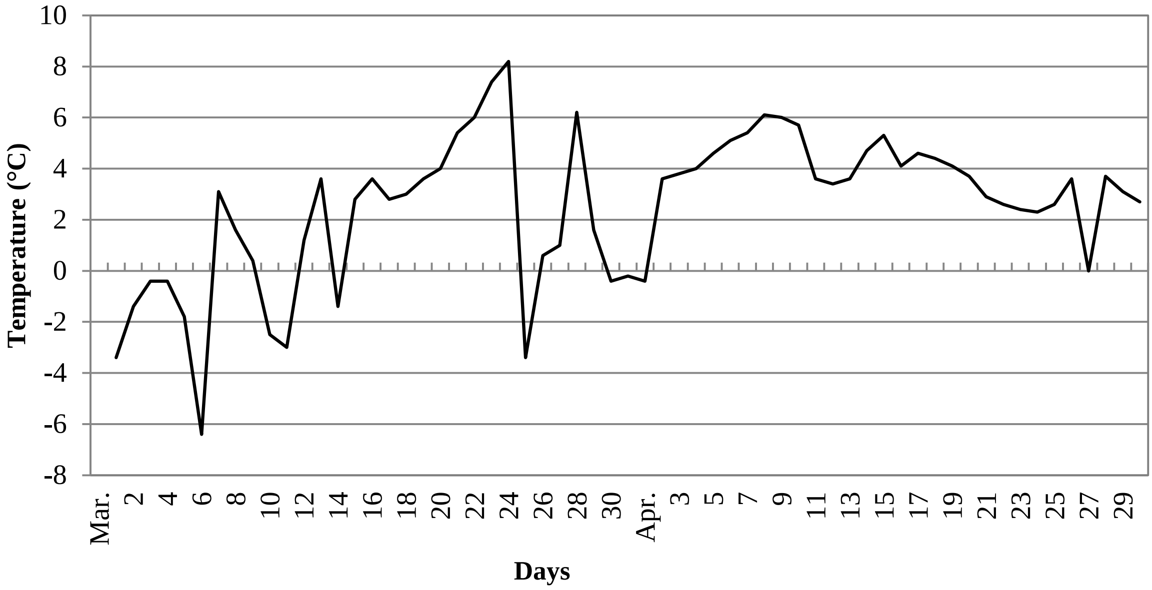

Figure 1 Absolute minimum temperature changes in March and April, 2008

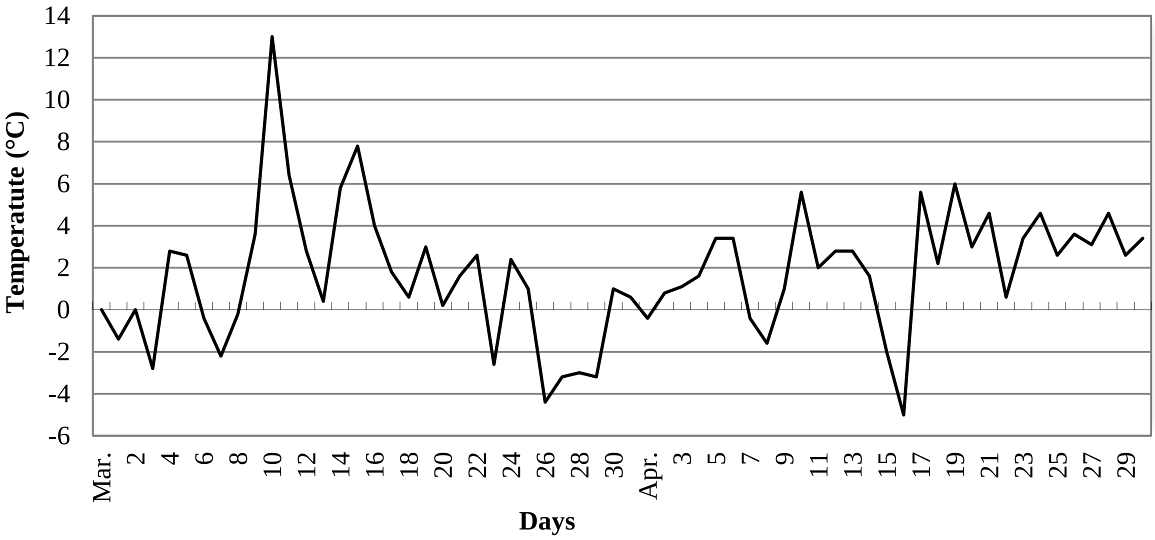

Figure 2 Absolute minimum temperature changes in March and April, 2009

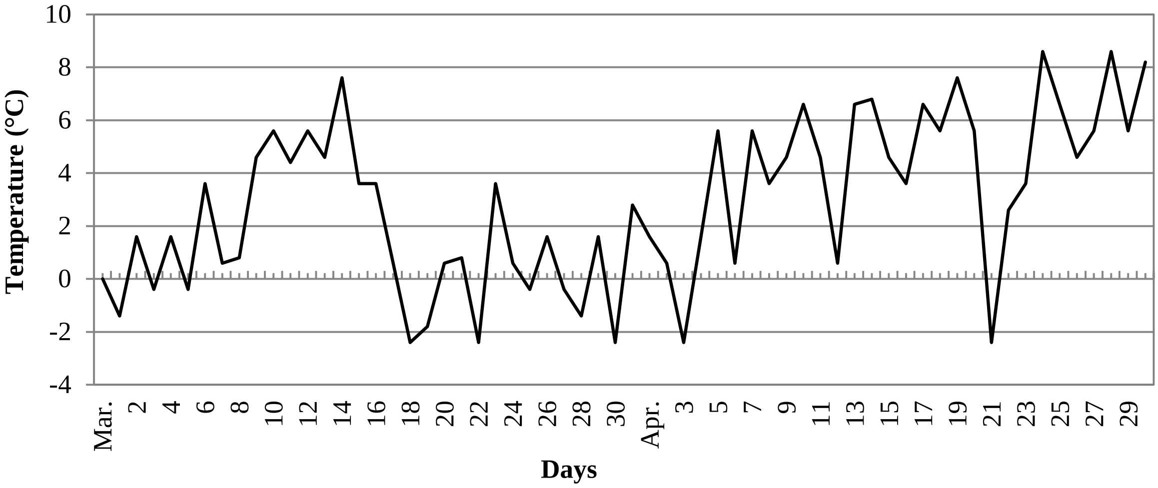

Figure 3 Absolute minimum temperature changes in March and April, 2010

The temperature fluctuation in the beginning of March was equivalent to 2 °C, and slowly warmed toward the end of March, temperature suddenly dropped about 5 °C in mid-April and again about 10 °C at the 4th week of April (Figure3). At this time except seeds which sown in the end of April, the other plots were full of seedlings. Therefore, from March 2009 until May 2010, spring late frost has occurred at least 3 times a year.

3.2 Seed germination

The effect of year and covering on *A. velutinum* seed germination was not significant (Table 1). But seed germination was significantly affected of sowing date ($p<0.01$). The interaction effect between seed sowing date and year and also between sowing date and covering of plot was not significant (Table 1).

The average seed germination during two years was ranged from 19.47% to 48.32% (Table 2). Seed germination was extended approaching to third week of February. But gradually trend downward, finally reached the lowest level in mid-April (Figure 5). The highest and the lowest rate of germination were related to seeds planted during 1st till 3rd week of February and the 3rd week of March until mid-April, respectively (Table 2).

Table 1 Variance effect of combined analysis of treatments on Persian maple seed germination

Source of change	Sum of Squares	Df	Mean of squares	f
Year	84.63	1	84.63	2.83^{ns}
Replication ×year	119.32	4	29.83	
Covering	252.9	1	252.9	2.36^{ns}
Covering ×year	129.49	1	129.49	1.21^{ns}
Error of covering	426.16	4	106.54	
Sowing date	6026.7	5	1207.34	25.58^{**}
Year × sowing date	464.70	5	92.94	1.97^{ns}
Sowing date ×covering	162.90	5	32.58	0.69^{ns}
Yea × date ×covering	179.95	5	39.59	0.84^{ns}
Error	1888.4	40	47.21	

**,* and ns refer to 1%, 5% and no significant, respectively

Table 2 Two years range of seed germination percent in different sowing date

Seed sowing date	Minimum germination	Maximum germination	mean	Standard error
2nd week of Dec.	24.29	47.50	37.97 [bc]	2.10
Mid Jan.	15.11	56.67	39.52 [bc]	3.45
1st week of Feb.	33.32	51.67	44.87 [ab]	1.46
3rd week of Feb.	37.62	62.50	48.42 [a]	1.84
3rd week of Mar.	18.10	46.10	36.71 [c]	2.17
Mid Apr.	10.83	24.29	19.47 [c]	1.22

Dissimilar letters in rows indicate significant differences between the groups.

Table 3 Variance effect of combined analysis of treatments on seedlings mortality

Source of change	**Sum of square**	**Df**	**Mean of square**	**f**
Year	78.18	1	78.18	0.73^{ns}
Replication ×year	428.52	4	107.13	
Covering	18147.93	1	18147.93	29.71**
Covering ×year	4638.93	1	4638.93	7.59^{ns}
Errors of covering	2442.6	4	610.65	
Sowing date	8790.9	5	1757.18	12.16**
Year × sowing date	1568.15	5	313.63	2.16^{ns}
Sowing date ×covering	7645.8	5	1529.16	10.57**
Year ×Sowing date ×covering	1562.9	5	312.58	2.16^{ns}
Error	5782.4	40	5782.4	

**,* and ns refer to 1%, 5% and no significant, respectively

3.3 Seedlings mortality

Due to late frost, seedlings mortality was 33% and 30.74% in the first and second year, respectively (Table 4). There was no significant difference between mortality rates between two years (Table 3). Seedling cover reduced the death of seedlings significantly ($p<0.01$) (Table 3). Interaction effect of seedling cover and years as well as seedling cover and sowing date on death of seedlings was significant ($p<0.01$) but those of sowing date and years was not significant (Table 3).

In both two years of the study, the most mortality rates were pertained to planting seeds in December, January and 1st week of February (Table 4), then gradually reduced with approaching planting date to March in uncovered plots. Most mortality rate related to the all sowing dates by 3rd week of February (Figure 4). In mid-April, the mortality was equal in both uncovered and covered plots. In fact, if sowing of *A. velutinum* seeds is delayed until early spring, mortality rate of seedlings were not different in both uncovered and covered plots. However seed germination strongly reduced in April planting due to soil drought (Figure 5).

Table 4 Two years range of seedling mortality percent in different sowing date in non-protected plots

Seed sowing date	First year planted	Second year planted
2nd week of Dec.	51.82[a]	37.43[a]
Mid Jan.	44.88[a]	37.06[a]
1st week of Feb.	41.69[a]	38.70[a]
3rd week of Feb.	28.28[b]	29.17[b]
3rd week of Mar.	24.92[bc]	20.18[c]
Mid Apr.	16.02[c]	21.87[c]
2nd week of Dec.	33.00	30.74

Dissimilar letters in rows indicate significant differences between the groups

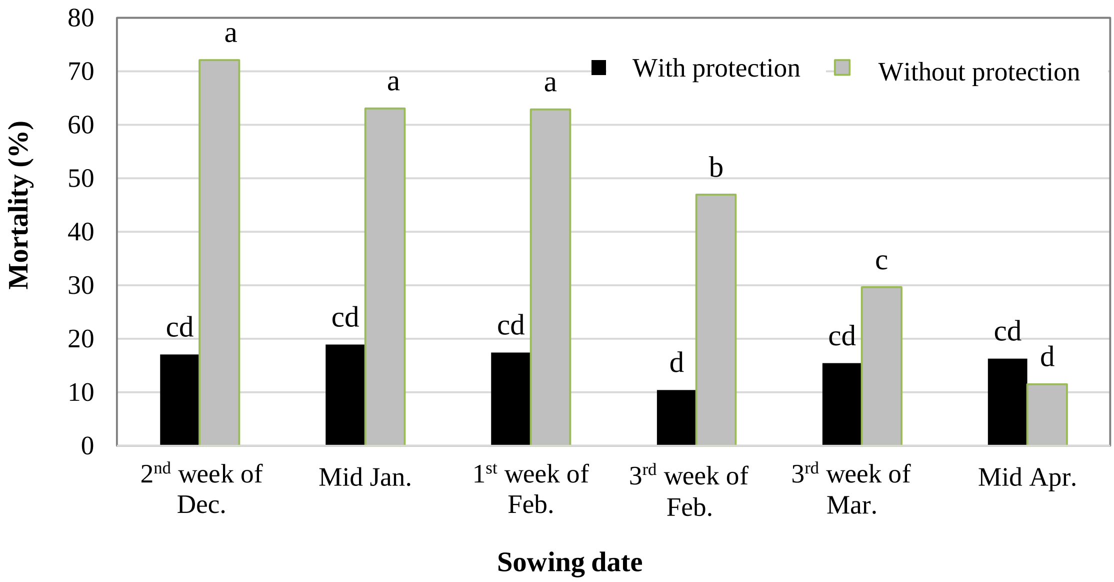

Figure 4 Two-year average mortality of Persian maple seedlings in different sowing date (Dissimilar letters in rows indicate significant differences between the groups at $p<0.05$)

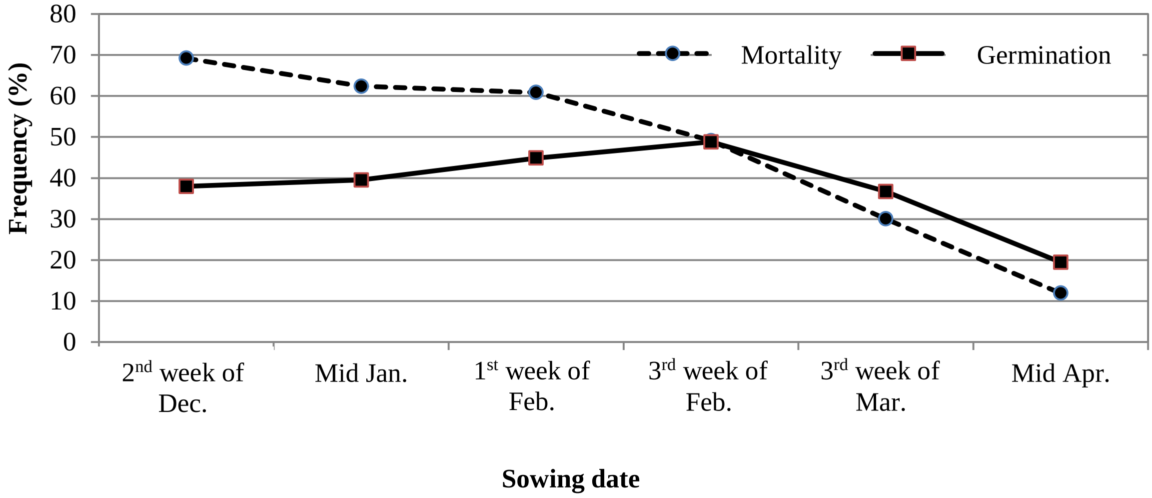

Figure 5 Comparison of germination and mortality of Persian maple seedlings in different sowing date

4 DISCUSSION

Based on the results of this study, due to planting date, average seed germination gradually increased from late fall to mid-winter, and reached its peak in the 3rd week of February and then declined (Figure 5). Thus, postponement of sowing date to mid-Feb. will be suitable for seed germination. Decreasing the seed germination in early planting may cause by a long term exposure to soil seed predators (Espahbodi *et al.*, 2013). There are two reasons in connection with the reduction of seed germination in the 3rd week of March until mid-April sowing dates as follows; 1)The seeds which sown in the mid-April, are usually faced with drought, relate to the lack of soil moisture in early spring. 2) These seeds with a longer root meristem compare with those of planted in early March are highly sensitive to soil drought (Jensen, 2001).

According to climatic data, during the 5-year study, the temperature drastically reduced at least once in March and once in April (Figures 1, 2 and 3). In some cases, the temperature reduction was between 8 ˚C- 10 ˚C. In the last century, the probability of potentially damaging freezing temperatures during bud break has increased with the increase of mean temperatures in spring and the subsequent advance of bud break (Cannell and Smith 1986, Kramer 1994, Linkosalo *et al*. 2000). This study also showed that within recent five years, seed germination of many species such as *A. velutinum* started in early March in Farim Wood Company nursery, where still a month remain to beginning the spring (Espahbodi and Khorankeh., 2013). This may be related to global warming. Increasing of temperature due of rising greenhouse gases which have caused the imbalances of earth climate system in recent decades (Goodarzi *et al.*, 2015). This phenomenon raises the risk of exposure the seedlings to spring late cold. In the recent century, climate is changing slowly towards the warming (Man *et al*., 2009). They reported that a heavy damage was entered to the under 20 year old reforestation and natural population due to the occurrence of unusually cold weather in May in Ontario. They also reported that bud bursting of many species of hardwood has gradually retreated to mid-March since 1918, due to global warming. In contrast, the incidence of late cold has increased.

Based on our results, the frost damage reduced with the delay in seed sowing date (Table 4). Variations the absolute minimum of temperature in March and April in both two years of the study, occurred in the 3rd week of March, 1st and 3rd weeks of April (Figure 1 and 2). The seeds were planted in 3rd week of February, which became to seedlings in early March, encountered with frost in 3rd week of March and then after. Seeds planted in mid March, early April and 3rd week of April became seedlings in early April, mid-April and mid October, respectively. Thus, delaying in planting date, seedling emergence will escape at least a case of frost occurrence. However, delaying the planting date until mid-April decreased the cold damage, but also strongly decreased seed germination, because of soil moisture reduction (Jinks *et al.*, 2006). On the other hand, for achieving the peak of the Persian maple seed germination, conserving soil moisture by irrigation application will be necessary in March until April sowing. The seedling resulted from the seeds planted before March is required to be protected by covers, because of high risk of spring frost. Application of protective coatings against spring frost and even winter frost is common. It was reported by Jinks *et al.*, (2006) for sycamore and *Pinus longifolia* (Tinus *et al.*, 2002). The absolute minimum temperature in 3rd February was about -2°C to -5°C, in which it caused frost damage to the seedlings (Townsend, 1977; Lechowicz, 1984). Therefore, early germination causes seedlings more susceptible to spring frost. For this reason, postpone the start of germination would be a great help to reduce spring frost damage. It has been reported in conjunction with the many Rocky Mountain species (Rehfheldt, 1989 and 1991;Emerson *et al.*, 2006). Delaying the start of the vital signs can be available through the accomplished provenance and provenance-progeny tests. According to our results, to reduce the damage caused by spring frost, Persian maple seeds should be held in cold and dry conditions, for suitable time of sowing which start from early March till early April. In these circumstances, irrigation the seeds planted in April to reduce the drought stress, will effectively help to increase seedling production.

5 CONCLUSION

Analysis of climatic data showed that during the 5-yearstudy, the temperature drastically dropped 8 to 10 °Cat least once in March, April and early May (Figure1, 2 and 3) which coincidence with seed germination of many species such as Persian maple in early March (Espahbodi and Khorankeh, 2013).This phenomenon raises the risk of exposure to late cold damage. Average seed germination gradually increased from late fall to mid-winter, and reached its peak in the third week of February and then declined. With delaying the sowing date, the frost damage has been reduced. Thus, the delay in planting date, seedling emergence escapes at least a case of frost. In conclusion, to achieve the peak of the Persian maple seed germination, covering seedlings grown before March and irrigation of those emerged after March is an integral part of seedling protection management in counter with climate changes.

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کاهش خسارت سرمای دیررس بر نهال افرا از طریق تاخیر در جوانه‌زنی بذر و محافظت نهال

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چکیده در ده سال اخیر فراوانی وقوع سرمای دیررس بهاره در نهالستان‌های کوهستانی روند افزایشی داشته است. هدف این تحقیق کاهش اثر سرمای دیررس بهاره روی نهال‌های افراپلت در نهالستان کوهستانی درزیکلا شرکت چوب فریم می‌باشد. این تحقیق به‌صورت طرح اسپلیت پلات با دو تیمار اصلی در سه تکرار و در دو سال متوالی (۱۳۸۸ و ۱۳۸۹) انجام شد. بذر افراپلت از رویشگاهی نزدیک به نهالستان جمع‌آوری شد. تیمار اصلی شامل محافظت نهال‌ها در دو سطح با پوشش محافظ و بدون پوشش محافظ در طول ایام سرما و تاریخ کاشت در شش سطح (اواسط آذر، هفته آخر دی، اواسط بهمن، هفته اول اسفند، هفته اول فروردین و هفته آخر فروردین) بوده است. نتایج نشان داد که اثر تیمارهای پوشش نهال و تاریخ کاشت روی کاهش خسارت سرمای دیررس در سطح (p< ۰/۰۱) معنی‌دار شد. علاوه بر این اثر متقابل تاریخ‌کاشت و پوشش کرت روی خسارت سرما در هر دو سال در سطح (p< ۰/۰۱) معنی‌دار شد. در کرت‌های محافظت شده بین تاریخ‌های کاشت از نظر خسارت سرمای دیررس تفاوت معنی‌دار (p> ۰/۰۵) دیده نشد. در مجموع در هر دو سال متوالی با نزدیک شدن تاریخ کاشت به اسفند و اوایل فروردین از میزان مرگ و میر نهال‌ها کاسته شد. بیش‌ترین مقدار تلفات نهال‌ها به تاریخ کاشت هفته اول اسفند مربوط شد. تلفات نهال‌ها در تاریخ کاشت هفته سوم فروردین در کرت‌های محافظت شده و نشده با هم برابر بوده است. در کرت‌های محافظت نشده بیش‌ترین مقدار تلفات نهال در کاشت‌های قبل از اسفند دیده شد. در حقیقت اگر کاشت بذر افراپلت به نزدیک بهار موکول شود، میزان تلفات در کرت‌های محافظت شده و محافظت نشده تفاوت چندانی با هم نخواهند داشت.

کلمات کلیدی: افراپلت، تاریخ کاشت، جوانه‌زنی، سرمازدگی نهال، شمال ایران