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After-ripening and germination of crab apple (*Malus sylvestris* Mill.) and common pear (*Pyrus communis* L.) seeds

Abstract

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Seeds of crab apple (*Malus sylvestris* Mill.) from Lower Silesia (elevation of 500 m) needed for overcoming dormancy and up to the start of germination a cool stratification at 3°C lasting 14 weeks for freshly collected undried seeds, and 15 weeks for seeds dried to 9-10% m.c. and stored without decrease of germinability at −3°C in a sealed container to early January without decrease of germinability. When stratification at 3°C was continued more than 90% germinated within the next 6 weeks, regardless whether at the start of the stratification treatment they were fresh or already stored. When after pretreatment they were transferred to stratification conditions at a daily alternating temperature of 3°~20°C (16 + 8 hours/day) germinability remained unchanged but the extent of the germination period was reduced to 1-2 weeks. Seeds of wild growing common pear (*Pyrus communis* L.) collected in the region of Poznań (elevation 65 m) treated like crab apple seeds needed 12 weeks of stratification at 3°C both when fresh undried or dry stored seeds were used. Germinability exceeded 90% but at 3°C the duration of germination period was 6 weeks, and at 3°~20°C the duration was 1-2 weeks.

Additional key words: alternating temperature, seed dormancy, germination tests, seed stratification, seed storage.

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INTRODUCTION

Information concerning behaviour of seeds of the wild growing crab apple (*Malus sylvestris* Mill.) and the common pear (*Pyrus communis* L.) is very scarce. Seeds of both species were rarely taken for investigations. More common are data originating from practical observations of nurserymen. It should be pointed out that European apple cultivars originate from *Malus pumila* Mill. or from its hybrids with other wild species of apple, but only very rarely with *Malus sylvestris* (Brown 1975). European pear varieties originate from *Pyrus communis*, but because of selection by man continued since antiquity they differ seriously, especially in the shape and taste of their fruits and now in the very differentiated period of fruit ripening, from their wild ancestors. According to Rubzov (1944) also European pears belong to a hybrid complex.

Data on storage and on after-ripening of seeds of cultivated forms cannot therefore be utilized directly for seeds of the wild growing species still existing in Europe, also in Poland. On the other hand they can be taken into consideration when planning investigations on seeds of *Malus sylvestris* and *Pyrus communis*. It is well known that both genera produce „orthodox" seeds i.e. seeds which can be dehydrated to a reduced (9-10%) moisture content (Soloveva, Kocjubinskaja 1955) and can be stored for longer periods. The duration of storage without loss of viability estimated in earlier work for apple and pear seeds (Crocker, Barton 1931) for 2.5 years is now extended to 7 years (Soloveva 1966). Temperature of storage should be low (− 3° to − 5°C). When the relative moisture content of the atmosphere within the sealed container is held by $CaCl_2$ at a level of 50-55% the temperature of storage can be higher, even in the range of 2-10°C (Soloveva 1950). Grześkowiak et al. (1983), have stored seeds of the apple cultivar 'Antonovka' and of *Pyrus caucasica* Fed., closely related to the common pear another species, up to the fourth winter without loss of viablity and field seedling emergence when the containers were sealed, the initial moisture content of seeds was 9-10%, and temperature was held constant in the range between −1° and −18°C. Much earlier Karnatz (1949) has stored pome fruit seeds for 12-17 months more successfully when temperature was −16°C than at −5° or 15°C. When seeds of the apple cvs. 'Antonovka' and 'Grušovka Moskovskaja' were stored at a moisture content of 8-10% at 10°C their high initial viability remained unchanged after 25 months of storage in polythene bags but decreased seriously when jute sacs were utilized (Karaseva et al. 1981). The same was demonstrated by Grześkowiak et al. (1983), who have proven the supremacy of sealed storage over all methods of storage in non-tightly sealed containers or packings.

Seeds of European apple and pear cultivars are deep dormant and this dormancy can be broken by a cold only stratification in the temperature range 0-10°C lasting 10-14 weeks (Dolmatowa 1970, Sladen 1973). After-ripening of apple seeds was less effective, when stratification periods at 4-8°C were interrupted by those at −1°C, because of insufficient duration of the action of temperature in the useful range 0-10°C (Kask 1964). At 4°C after-ripening of seeds of cv. „Antonovka" run energetically, but after reduction of stratification temperature to −1°C germinative capacity became much lower and germination lasted longer (Kallio 1962, 1963). Stratification of seeds of apple cultivars on melting ice was similarily long as during stratification in the sand medium at 3-7°C, about 60-65 days, until the majority of seeds started to germinate (Bolotskij 1954). De Haas (1954/55) has found that a temperature of 3°C is optimal for stratification of seeds of apple cultivars. Evenari et al. (1947) knew that stratification of such seeds should last about 90 days. Informations concerning shorter stratification periods can be understood when realizing that seed after-ripening occurs within the fruits stored at a temperature stimulating this process i.e. in cool conditions (above freezing point). Such seeds need a shorter duration of stratification and this is a frequent reason for

discrepancies between data on stratification of seeds of pome fruit cultivars. Tylkowski (1978) has replaced the normal stratification of 'Antonovka' seeds by sowing them in boxes, which were afterwards placed at 3°C. Most intensive seedling emergence (82%) was obtained when the boxes were transferred to 15° or 20°C after 80 days of the cool treatment. At that time already 13-18% of seeds started to germinate under the 1 cm (optimal sowing depth) cover of the sand/peat sowing medium.

These findings do not differ seriously from data presentend by earlier authors: Crocker 1927, Piskarev 1937a, Chadwick after Piskarev 1937b, Belochonov 1938, Belochonov, Piskarev 1939, Jefimov 1939, Ślaski 1950, who report that seeds of wild *Malus sylvestris* and of *Pyrus communis* stratified in different media need about 84-90 days of stratification at 3-6°C, while in peat only 60-70 days (Belochonov 1938).

Seeds of the wild growing *Malus sylvestris* and *Pyrus communis* have never been investigated in Poland in controlled conditions. The aim of this study was to find the necessary duration of cool stratification at 3°C and to investigate the germination behaviour of freshly collected and dry stored seeds at a daily alternating temperature 3° ~ 20°C.

MATERIAL AND METHODS

Seed origin and quality:

Malus sylvestris – Fruits were collected under 2 trees on September 8th 1987, in Witków near Kamienna Góra (region of Wałbrzych in Lower Silesia) at an elevation of 500 m, shortly after falling off to the ground. The fruits were stored at room temperature until September 18th, when the seeds were extracted, cleaned and stored at 3°C until September 21st. A part of the seeds was immediately used without drying for stratification test (fresh seeds), another part was dried at room temperature until October 10th when storage in a sealed bottle at −3°C was started (stored seeds). Storage lasted until January 11th, 1988, when the seeds were used up for a new stratification. Moisture content of fresh seeds was 40.5%, that of dried seeds 9%. Initial viability of fresh seeds was 97.0% (indigo carmine embryo viability test).

Pyrus communis – Fruits were collected on October 12th, 1987 from the ground under a single tree, growing on the bank of the Kórnik Lake in Kórnik in the region of Poznań (65 m elevation), about 2 weeks after the mass fruit fall. Seeds were extracted from fruits on October 14th, to be used after cleaning for immediate stratification. A part of the seeds, however, was dried at room temperature until October 23rd, when storage was started in the same conditions as in the case of crab apple seeds. It lasted until January 11th, 1988 when a new stratification was initiated. Moisture content of fresh seeds was 45.2%, that of the dried seeds 9.7%. Initial viability of fresh seeds was 92.0% (indigo carmine embryo viability test).

Designs of the experiments: Designs of experiment 1 (*Malus sylvestris*, lab. file 683) and of experiment 2 (*Pyrus communis*, lab. file 687) are presented in figs. 1 and 2.

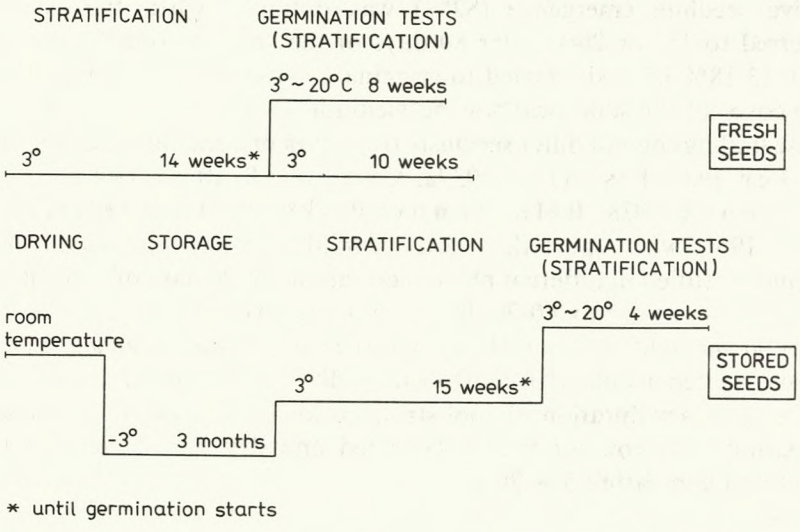

Fig. 1. *Malus sylvestris*. L. Design of the experiment 1 (lab. file 683)

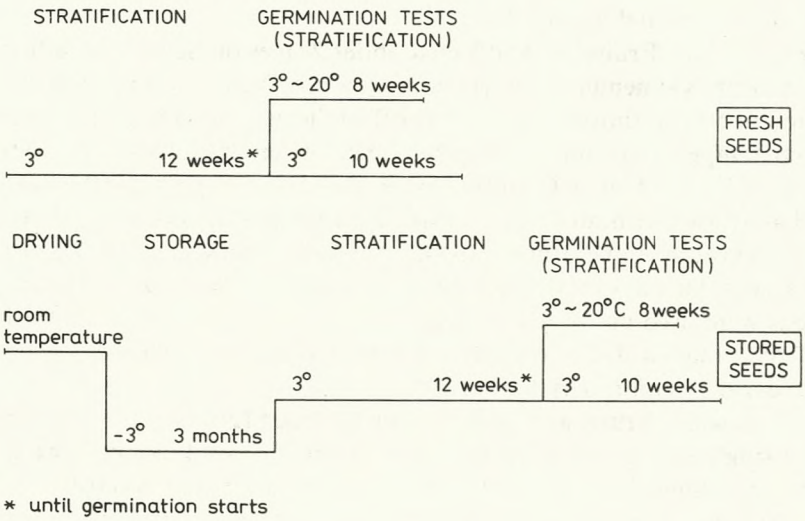

Fig. 2. *Pyrus communis* L. Design of experiment 2 (lab. file 687)

Stratification of seeds: Seeds of both species were stratified in a moist sand/peat (1:1, by vol.) medium. Moisture of the seed-medium mixture was checked at first every 2 weeks, but when approaching the expected germination period it was repeated at weekly intervals. On every checking day the losses of

moisture were replaced, germinating (with radicle 3 mm or longer) and decaying seeds were counted and discarded. The medium was aerated by unloading and mixing. Temperature of cool stratification was 3°C ± 0.5°C. When seeds started to germinate a part of them was left at the same temperature of 3°C and in the same stratification conditions (germination test at low temperature) for 10 weeks, and the other part was placed at a daily alternating temperature of 3° ~ 20°C (16+8 hours/day) for the next 8 (apple) or 10 (pear) weeks (germination test at alternating temperature). The duration of both tests should permit all seeds capable to germinate to complete germination.

Replications: All variants of the experiments were replicated 3 times with 50 seeds in each replicate.

Estimation of moisture content: The oven method was utilized (105°C, 24 hours, 3 × 10 seeds).

Viability test: The indigo carmine embryo staining test (1:2000, 20°C, 2 hours, 3 × 25 seeds) was applied.

Testing viability after termination of germination tests: All non-germinating seeds were examined by the cutting test, to recognize the reason of their behaviour.

Presentation of results: All data were calculated as percent values, the course of germination is presented in the form of cumulative germination curves.

RESULTS

VIABILITY AND HEALTH CONDITION OF SEEDS

Initial data concerning viability, insect infestation and seed decay are collected in table 1 for seeds freshly extracted from fruits and cleaned, in the case of wild pear also for stored seeds. The obtained levels of germinative capacity are presented for both species in table 2. Germinability was high, its lowest level was 89.3%, the highest one 97.3%. It was never reduced by the alternating germination temperature. The duration of cool stratification preceding the germination tests was determined by appearance of the first germinating seeds and was 14-15 weeks for *Malus sylvestris* and 12 weeks for *Pyrus communis*. The percentage of seeds starting to germinate was at this time low, this being assured by the short, weekly checking intervals:

Malus sylvestris: fresh seeds 5.3%, stored seeds 7.5%,
Pyrus communis: fresh seeds 4.7%, stored seeds 4.6%.

The high germinability reflects high level of viability of seeds at the start of the experiments and during all treatmens i.e. during the cool stratification pretreatment and during the germination test in both conditions: at 3°C and 3° ~ 20°C. Conditions of these tests did not harm the viability of seeds, though the total duration of being placed in stratification conditions was 25 weeks for the longest treated apple seeds and 22 weeks for pear seeds. Percentage of seeds decaying

Table 1

Results of viability test (indigo carmine embryo viability tests) of seeds of wild growing *Malus sylvestris* Mill. and *Pyrus communis* L. Seeds were tested immediately after extraction from ripe fruits and cleaning (fresh seeds), and after cold storage at –3°C at a decreased moisture content (stored seeds). (Experiments 1 and 2, lab. files 683 and 687)

Species	Seed lot no. of file	Category of seeds	Viable seeds %	Insect infested seeds %	Empty seeds %	Mechanically damaged and decayed seeds %
Malus sylvestris	683	fresh	97.0	2.0	0.0	1.0
Pyrus communis	687	fresh	92.0	5.3	0.0	2.6
		stored	90.0	1.7	0.0	8.3

Table 2

Germinative capacity of seeds of wild growing *Malus sylvestris* Mill. and *Pyrus communis* L. obtained in germination tests in stratification conditions at 3°C and at a daily alternating temperature 3°~20°C (16+8 hours/day). Germination tests were preceded by cool stratification at 3°C until the first seeds started to germinate. Stratification pretreatment was started either immediately after extraction of seeds from ripe fruits and cleaning (fresh seeds) or after storage at –3°C of seeds dried to 10–11% of moisture content (stored seeds) until mid-winter (experiments 1 and 2, lab. files 683 and 687)

Species	Germinative capacity at 3°C		Germinative capacity at 3°~20°C	
	fresh seeds %	stored seeds %	fresh seeds %	stored seeds %
Malus sylvestris	97.3	–	96.0	95.0
Pyrus communis	89.3	94.2	91.3	91.3

during the treatment was also low. A part of the seed decay was caused by damage by insect larvae. A part of decayed seeds could be detected only during the final cutting tests. The total number of decayed seeds at termination of all tests is as follows:

after germination tests at 3°C

Malus sylvestris: stratified when fresh 1.3%,
Pyrus communis: stratified when fresh 9.9%,
stratified after storage 5.4%,

after germination tests at 3°~20°C

Malus sylvestris: stratified when fresh 3.9%,
stratified after storage 5.0%,
Pyrus communis: stratified when fresh 8.0%,
stratified after storage 8.6%.

From these data the following conclusion can be drawn: Drying and storage does not decrease markedly the health condition of both apple and pear seeds. Conditions of germination tests at 3°~20°C seem to contribute to only a very slight decrease of seed viability during the tests.

COURSE OF GERMINATION AFTER SEED STRATIFICATION

When after initial stratification pretreatment the seeds were placed again either at 3°C (germination test at low temperature) or at 3° ~ 20°C (germination test at alternating temperature) both these conditions did not effect germinability of seeds, which was always very high. On the other hand the rate of germination depended strongly on the thermal conditions of the germination tests, these differences are presentend in figs. 3 and 4. At the constant low temperature the

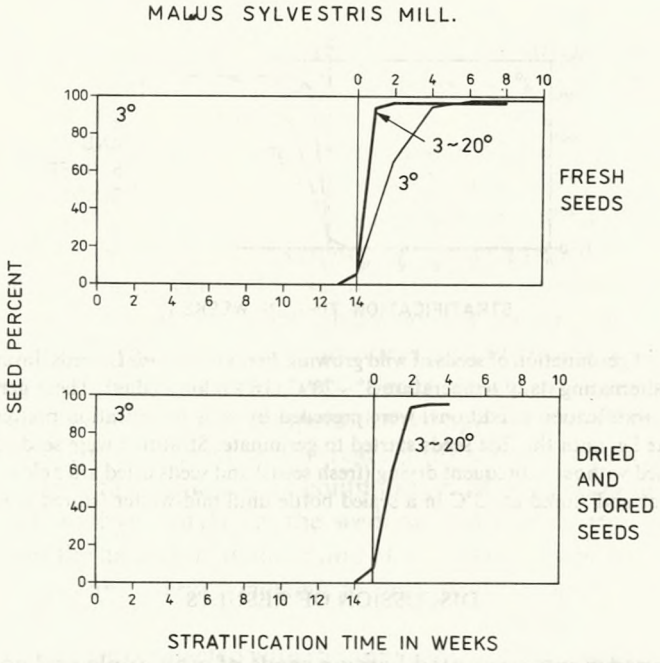

Fig. 3. Course of germination of seeds of wild growing *Malus sylvestris* L. seeds during germination tests at 3°C and at an alternating daily temperature 3 ~ 20°C (16 + 8 hours/day). These germination tests (performed in stratification conditions) were preceded by cool stratification pretreatment at 3°C lasting 14–15 weeks i.e. until the first seeds started to germinate. Stratified were seeds extracted from fruits and cleaned without subsequent drying (fresh seeds) and seeds dried after cleaning to 9.0% of moisture content and stored at −3°C in a sealed bottle until mid-winter (stored seeds)

extension of the germination period was 7 weeks for seeds of *Malus sylvestris* and 6 weeks for those of *Pyrus communis*. At the alternating temperature the germination period was extremely short, lasting for seeds of both species 2 weeks only. Most seeds germinated within the first week of the tests, only the stored apple seeds germinated in 51.7% in the first week, and in 35.0% in the second one, expressing in this way a more extended germination behaviour.

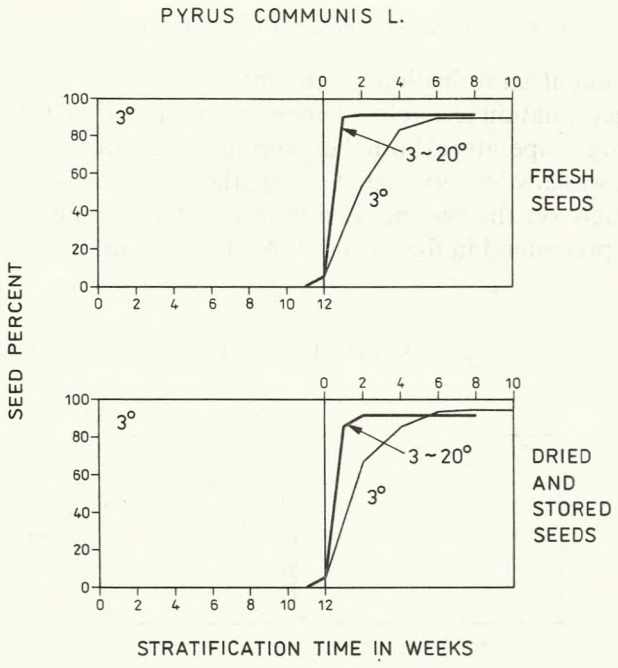

Fig. 4. Course of germination of seeds of wild growing *Pyrus communis* L. seeds during germination at 3°C and at alternating daily temperature 3°~20°C (16+8 hours/day). These germination tests (performed in stratification conditions) were preceded by cool stratification pretreatment at 3°C lasting 12 weeks i.e. until the first seeds started to germinate. Stratified were seeds extracted from fruits and cleaned without subsequent drying (fresh seeds) and seeds dried after cleaning to 9.7% of moisture content and stored at –3°C in a sealed bottle until mid-winter (stored seeds)

DISCUSSION OF RESULTS

For the experiments presented here on seeds of crab apple and common pear only single seed lots could be utilized. The applied storage period was also short and lasted 103 days for apple seeds and 80 days for the pear seeds. It was however of a similar duration and was started and terminated on dates comparable with those applied for seeds used in the first winter after collection for production of seedlings in the standard nursery work.

These results cannot, however, be generalized for seeds of the same species of other provenances or collected even on the same site but in other years. We cannot also foresee how would seeds used in the experiments presented here germinate if they were stored at the same conditions (−3°C, 9-10% m.c., sealed container) but for a longer period e.g. for 3 or 5 years. Data already available from the few experiments conducted so far on seeds of both species, and much more results obtained for seeds of European cultivars of apple and pear justify the assumption that we can expect similar reactions of much broader seed material, though seeds collected in various years could perhaps differ in

germinability. Eventual differences in the duration of the necessary stratification period can be easily overcome by starting this treatment somewhat, (e.g. 4 weeks) earlier, and to freeze the seed-medium mixture at −3°C temporarily, when the start of germination during stratification precedes the possibility of sowing the seeds in the nursery. This technique has been demonstrated recently on the example of seeds of ash (S u s z k a 1987). It is also very probable that storage of seeds of both wild species could be extended even for several years as was demonstrated for seeds of the cultivated apple and pear varieties. With the same probability it can be assumed that stratification and storage conditions applied here could be used successfully also for broader seed material of both species.

Completely new in this study is the adaptation for already stratified apple and pear seeds, of alternating temperature with a broad amplitude (3° ~ 20°C), applied for the first time successfully by the author (S u s z k a 1967) for *Prunus avium* L. (mazzard cherry) seeds. Each 24-hour day consist in this thermal cycle of two parts: the 16 hours of low temperature, and of 8 hours of the elevated one. A reverse arrangement of both temperature levels could cause a strong decline of germinative capacity, because of a high possibility to induce secondary dormancy in those seeds which are still not fully after-ripened by the foregoing cool stratification. The longer cold phase repeated daily permits a completion of the necessary cold period for seeds which need a longer action of the low temperature, on the other hand the short warm part of the cycle is too short to induce secondary dormancy effectively. Seeds which are completely after-ripened when the alternating temperature is applied (whether the visible germination has already started or not) react easily even to the short warm part of the cycles with a fast initiation or continuation of elongation growth of the radicles and of the hypocotyls i.e. the seedlings start to emerge. It has to be proven, whether the danger of an induction of secondary dormancy is in the case of *Malus sylvestris* and *Pyrus communis* as high as in the case of *Prunus avium* seeds.

A special advantage connected with the application of alternating temperature for the already pretreated seeds consists in the extraordinary shortening of the germination period to 1-2 weeks. For the practical nursery work this means that stratification of *Malus sylvestris* and *Pyrus communis* seeds should be started at such a date that sowing (whether following a period of freezing or not) could be performed early, i.e. in a period when fluctuations of the soil temperature at the sowing depth between night and day are still big. In western Poland such situation occurs in March and early April. In such cases sowing should be performed early and the surface of the seedbeds should be covered with an insulating layer of a mulching material e.g. with straw. The effectiveness of this technique for seeds of 'Antonovka' apple and for seeds of other species used for rootstock production, among them for *Pyrus caucasica,* was demonstrated already in an earlier paper (G r z e ś k o w i a k et al. 1983). In laboratory testing of seed germinability the germination tests at the alternating temperature can be

used with high efficiency. When only one refrigerated room is available there exist still the possibility to perform germination tests at low temperature only (3°C) with the same degree of effectiveness but lasting at least four times longer.

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Ustępowanie spoczynku i kiełkowanie nasion jabłoni dzikiej (*Malus sylvestris* Mill.) i gruszy pospolitej (*Pyrus communis* L.)

Streszczenie

1. Świeże, niepodsuszone nasiona jabłoni dzikiej (*Malus sylvestris* Mill.) pochodzące z naturalnego stanowiska (Witków koło Kamiennej Góry, wysokość 500 m n.p.m.) na Dolnym Śląsku wymagały dla ustąpienia spoczynku i zainicjowania kiełkowania chłodnej 14-tygodniowej stratyfikacji w 3°C w wilgotnym podłożu piaskowo-torfowym. Nasiona podsuszone do wilgotności 9-10% i przechowywane w −3°C w szczelnie zamkniętej butli szklanej, wymagały 15 tygodni stratyfikacji chłodnej. Przechowywanie w takich warunkach do początku stycznia nie obniżało w żadnym stopniu zdolności kiełkowania nasion. Nasiona już przysposobione, pozostawione w warunkach stratyfikacji w 3°C, kiełkowały w ponad 90% w ciągu 6 tygodni, bez względu na to, czy użyto ich do stratyfikacji w stanie świeżym, czy po przechowaniu. Gdy po przysposobieniu przeniesiono je, nadal w warunkach stratyfikacji, do temperatury zmiennej w cyklu dobowym 3°~20°C (16+8 godz./dobę) zdolność kiełkowania pozostała nie zmieniona, lecz rozciągłość okresu kiełkowania została zredukowana do 1-2 tygodni.

2. Nasiona dziko rosnącej gruszy pospolitej (*Pyrus communis* L.) pochodzące z Kórnika w rejonie Poznania (65 m n.p.m.), traktowane tak samo jak nasiona jabłoni dzikiej, potrzebowały 12 tygodni przysposabiania przez stratyfikację aż do pojawienia się pierwszych kiełków, bez względu na to, czy korzystano z nasion świeżych, czy przechowywanych w stanie podsuszonym. Zdolność kiełkowania przewyższała 90% bez względu na przeszłość nasion i warunki próby kiełkowania, lecz w 3°C rozciągłość okresu kiełkowania wynosiła 6 tygodni, w 3°~20°C znowu 1-2 tygodnie.

Уступание состояния покоя семян яблони дикой (*Malus sylvestris* Mill.) и груши обыкновенной (*Pyrus communis* L.)*

Резюме

1. Свежне, неподсушенные семена яблони дикой (*Malus sylvestris* Mill.) происходяшие с натурального местообитания (Виткув около Каменной Гуры на высоте 500 м над уровнем моря) на Нижнем Шленские, требовали для уступания состояния покоя и готовности прорастания холодной 14-недельной стратификации в 3° С во влажном песочно–топфяном субстрате. Подсушенные до 9-10% влажности и хранимые в –3°С в герметический закрытой бутылке семена требовалии 15-недельной холодной стратификации. Хранение в таких

условиях от начала января не понижало ни в какой мере способности прорастания семян. Уже приспособленные семена, оставленные в условиях стратификации в 3°C, прорастали в более 90% в течение 6 недель, несмотря на то были ли они стратифицированы в свежем состоянии или после хранения. Когда после приспособления перенесено их, по прежнему в условиях стратификации, в температуру изменяющуюся по суточному циклу 3°~20°C (16+8 час/сутки), способность прорастания была сокращена до 1-2 недель.

2. Семена растущей дико груши обыкновенной (*Pyrus communis* L.) происходящие из Куринка в районе Познаня (65 м над уровнем моря), обработанные так же как и семена яблони дикой, требовали 12 недель холодной стратификации до появления первых пропостков, невзирая на то применяли ли свежие семена или хранящиеся в нодсушенном состоянии. Способность прорастания превышала 90% несмотря на прошлое семян и условия пробы прорастания, но в 3°C протяженность периода прорастания выносила 6 недель, в 3°~20°C снова 1-2 недели.

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