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Requirements for seed germination in North American pawpaw (*Asimina triloba* (L.) Dunal)

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Summary

North American pawpaw is a temperate species in the mostly tropical Annonaceae. A small proportion (12%) of the seed population used in this study germinated after removal from the fruit. The remaining seed required 8 weeks of chilling stratification to satisfy dormancy. In addition, pawpaw seeds displayed a moderate form of recalcitrance. Seeds lost 50% viability when seeds were dried from their initial 37 to 25% moisture. Critical value for total loss in viability was between 15 and 5% moisture. Also, following stratification, pawpaw seeds germinated at 45% without additional water in the germination medium. There was no significant effect of light or temperature (25, 30 or 20/30°C) on standard germination. Seeds showed the best germination with 1ml of water added to the germination medium in petri dishes. For standard germination testing, pawpaw seeds should be stratified for 100 days at 5°C followed by germination in rolled towels at constant 25 or alternating 20/30°C. Light was not required and final germination counts should be after 5 weeks.

Introduction

The North American pawpaw is a temperate member of the mostly tropical Annonaceae or Custard Apple family. The pawpaw is a deciduous woody tree (12–15 m) with a pyramidal growth habit and attractive foliage, flower and fruit characteristics. Pawpaw has commercial value as a small landscape tree and as an orchard fruit crop (Layne, 1996). It is also the source of several novel botanical and medicinal extracts (Zhao, Miesbauer, Smith and McLaughlin, 1994). Nurseries commonly propagate pawpaw from seed. Trees are often sold commercially as unnamed seedlings, or seedlings are used as rootstocks for grafting or budding named cultivars. Although seed propagation of pawpaw is important to the nursery industry, there are no systematic studies describing seed handling and germination in pawpaw seeds.

Pawpaw seeds have a small rudimentary embryo imbedded in a large ruminant endosperm (Finneseth, 1997). Observations by several authors suggest that pawpaw seeds lose viability if allowed to dry and that a period of chilling stratification is required to relieve dormancy (Bonner and Halls 1974; Dirr and Heuser 1987). However, no critical value for desiccation tolerance has been established for pawpaw and only general guidelines for stratification requirements have been suggested. Most recommendations range between 60 and 120 days of stratification, but several authors indicate that pawpaw will germinate without stratification (Bonner and Halls, 1974; Dirr and Heuser 1987).

Current recommendations for handling pawpaw seeds include mixing seeds with moist paper towels, sawdust or spaghnum moss for 60 to 120 days at 5 °C prior to germination (Young and Young, 1992). However, it is not clear how long seeds can be stored under these conditions without loss of viability (Callaway, 1993; Darrow, 1975).

Standard germination tests for pawpaw are not included among the tree and shrub species listed in the International Rules for Seed Testing (ISTA, 1993) or the Rules for Testing Seeds (AOSA, 1995). Environmental considerations for germination, including light, temperature, water and germination substrate, have not been studied and a seed testing protocol for pawpaw has not yet been developed.

The objectives of this study were to: (1) describe the stratification requirement for pawpaw seeds; (2) describe the tolerance of pawpaw seeds to desiccation; (3) provide evidence for the longevity of stored seeds; and (4) develop a protocol for laboratory seed testing.

Materials and Methods

Seed extraction and germination

Fresh seeds were removed from ripe fruit, cleaned of adhering pulp using water and surface-sterilized using a 10% bleach (NaOC1) solution for 10 min followed by three rinses with sterile deionized water. After rinsing, seeds were surface treated with dry Captan™ fungicide. Three sheets of germination paper (30 × 38 cm, Anchor Paper Co., St. Paul, MN) were moistened with approximately 250 ml of deionized water. Ten seeds were placed between the second and third sheet of rolled paper. Rolls of seeds were placed in 0.5 mil polyethylene bags (20 × 7.5 × 38 cm, Mobil Chemical, Covington, GA). Unless otherwise specified, germination conditions were in the previously described moistened germination paper rolls in a 25 °C growth chamber in darkness. For all experiments, germination was visually assessed after seeds were exposed to the germination conditions for 4 weeks and germination was considered successful when radicle length exceeded 2 mm.

Effect of stratification

Fruits were collected from six sites in Kentucky during the fall of 1996. Seeds were cleaned as previously described, combined into one seed lot and mixed. Stratification of seeds occurred in rolls of moistened germination paper at 5 °C in darkness. At 7 day intervals for 14 weeks, one set of 50 seeds was moved from stratification to germination conditions and percentage germination determined.

Effect of seed moisture content on germination

Seeds were extracted in the fall of 1995 from ripe fruit gathered from pawpaw trees planted in the pawpaw orchard at the Western Maryland Research Experiment Station, Keedysville, MD. Fifty fresh seeds were either air-dried in an open plastic tray (8 × 8 ×

2.5 cm), placed in a glass jar (13 cm height × 6 cm diameter) sealed with a screw-on lid or rolled between three sheets of moistened germination paper in polyethylene bags as previously described. All vessels were kept on a laboratory bench at room temperature (approximately 25 °C). At 3 day intervals, seeds were weighed to calculate the approximate change in seed moisture content for each storage environment based on the initial dry weight of a random set of 50 seeds dried for 5 days at 50 °C. After 12 days, seeds were stratified for 120 days at 5 °C as previously described.

In addition, 10 seeds were placed in glass jars (9.5 cm height × 6.5 cm diameter) with a 25 ml Erlenmeyer flask containing 25 ml of a saturated KC1 solution (approximately 87% RH).

Fifteen jars were prepared and sealed with screw-on lids. Fifty seeds (5 jars of 10 seeds each) were dried to moisture percentages of 25, 20 or 15%. When the seeds reached the desired moisture percentages, seeds were removed from the glass jars, stratified for 120 days, germinated and evaluated as previously described.

Effect of storage time and temperature on germination

Seeds were dried to a moisture content of approximately 25% and then exposed to various storage conditions described below. Seed treatments included moistened germination paper at 5 °C and 25 °C or sealed jars without additional water at 5 °C and 25 °C. Seeds stored in moistened germination paper were prepared as previously described and seeds stored in sealed jars were placed in glass jars (13 cm height × 6 cm diameter) sealed with screw-on lids. At 4 week intervals from 28 through 52 weeks, 10 seeds were used to determine moisture content and 30 seeds were stratified at 5 °C for 60 days then germinated and evaluated as previously described. In addition, germination was evaluated in seeds stored in moist peat moss or moist paper towels in polyethylene bags at 5 °C for up to 3 years. Fifty seeds were evaluated for each interval.

Effect of substrate moisture, light and temperature on seed germination

Fruits were collected from trees in the pawpaw orchard at the Western Maryland Research Experiment Station in Keedysville, MD. The trees were grown from seed collected from native stands along the C&O Canal, MD and Prince Georges County, MD. In the fall, fruit was harvested and seeds extracted after macerating the pulp and floating off the fruit flesh. Seeds were washed, surface sterilized using a bleach solution, rinsed and stored in hydrated Terrasorb™ (hydrophyllic polymer) at 5 °C for approximately 9 months until used for germination studies.

Stratified seeds were surface-sterilized with 10% bleach solution and rinsed three times with sterile deionized water. Germination paper was cut to fit the bottom of 100 × 15 mm polystyrene petri dishes (Becton Dickinson and Co., Franklin Lakes, NJ). Germination paper was autoclaved and one piece placed in the bottom of each petri dish. Five seeds were placed on germination paper and 0 to 5 ml of sterile deionized water added to each plate. Petri plates were sealed using a 10 × 2.5 cm strip of Parafilm 'M' (American National Can, Greenwich, CT).

Seeds were placed in germination chambers set at 25 °C constant, 30 °C constant and an alternating temperature of 30 °C for 16 h and 20 °C for 8 h. Photoperiod in all germination chambers was 16 h of 20–35 µmol·sec^{-1}·–m^{-2} light and 8 h of darkness. Germination was visually assessed as previously described and germination percentage recorded following 2, 3, 4 and 5 weeks.

In a separate experiment, the effects of light and water on seed germination of pawpaw was studied. Light treated seeds in petri dishes were placed randomly in a germination chamber and exposed to continuous light at 30 µmol·sec^{-1}·–m^{-2}. Seeds receiving dark treatment were in petri dishes wrapped in aluminum foil to provide complete darkness and placed randomly in the same germination chamber. At each light treatment, petri dishes were wetted with either 0, 0.5, 1 or 5 ml of autoclaved deionized water. Germination temperature was maintained at 25 ± 1 °C. Germination was visually assessed as previously described and germination percentage recorded after 5 weeks. Germination of dark-treated seeds was evaluated in a dark room using a safelight to prevent exposure to light. A reference set of plates were never exposed to the safelight as a control for light exposure. Five plates containing 5 seeds each were assigned to each treatment, prepared as described in the preceding experiment. This experiment was conducted twice using seeds from the 1995 harvest.

Results and discussion

Effect of stratification

A small percentage of the seed population (12%) germinated without stratification (Figure 1). However, to obtain germination percentages above 50%, seeds required 7

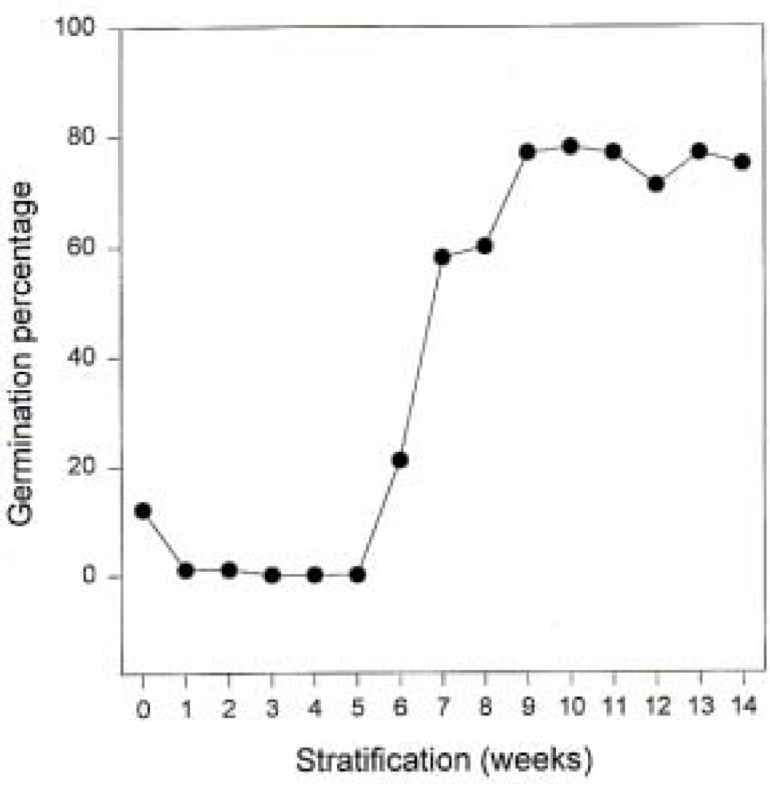

Figure 1. Germination percentage of North American pawpaw seeds after 0 to 13 weeks of stratification at 5 °C. Standard error for all data points was between 0.02 and 0.08.

weeks of stratification. Maximum germination of 78% was obtained after 10 weeks of stratification. Additional stratification did not improve germination.

Many sources have indicated that chilling stratification was necessary for germination of pawpaw seeds (Bonner and Halls, 1974; Darrow, 1975; Layne, 1996). Dirr and Heuser (1987) observed germination percentages of 50, 62 and 82% from three pawpaw seed lots after 2 months of chilling at 4 °C. Bonner and Halls (1974) recommended 100 days of stratification and reported 65% germination of a seed sample following a 60 day stratification treatment at 5 °C.

In the current study, a small proportion of the seed population germinated immediately when exposed to germination conditions, but the majority of the population required at least 7 weeks of stratification before germination occurred. Seeds for this experiment were pooled from six different Kentucky populations. This represents populations approximately in the middle of its natural geographic range. It has been suggested that southern pawpaw seed sources can germinate at high percentages without any stratification treatment, but other seed provenances require stratification (Dirr and Heuser 1987). Evert and Payne (1991) found that germination percentage was independent of stratification for pawpaw seeds from a Georgia source. Southern seed sources not requiring a chilling treatment indicate that provenance may affect the stratification time required to overcome seed dormancy in pawpaw.

The dormancy condition that requires chilling stratification observed in some *Asimina* populations appears to be unique within the Annonaceae. This may be an ecological adaptation related to the temperate distribution of this genera. Although this type of dormancy is unique in its family, there are numerous examples of large seeded, recalcitrant species with temperate distributions that require chilling stratification. These include sycamore maple (*Acer pseudoplatanus*), common horsechestnut (*Aesculus hippocastanum*), European filbert (*Corylus avellana*) and American chestnut (*Castanea dentata*) (ISTA, 1993; Young and Young, 1992).

Effect of seed moisture content on germination

Fresh seed had a moisture content of approximately 37% which did not increase significantly when seeds were imbibed (Figure 2). Seeds stored in an open container at room temperature dried significantly within the first 3 days. After 12 days, seed moisture percentages were 5, 35 and 37% for open air, sealed jar and moist storage conditions, respectively. After stratification, seeds stored in open air did not germinate, but seeds stored in sealed jars and moistened germination paper germinated at 92 and 95%, respectively.

Seeds used in the second experiment had an initial moisture content of 35% and germination percentage of 84% after 120 days of stratification (Table 1). Seeds that were dried in sealed glass jars to moisture percentages of 25, 20 and 15% prior to stratification germinated at 42, 40 and 18%, respectively, after 120 days of stratification at 5 °C. These data indicated that pawpaw seeds were recalcitrant and that viability was significantly reduced at seed moisture percentages below 35%. The critical value for total viability loss was between 15 and 5% moisture (Table 1; Figure 2).

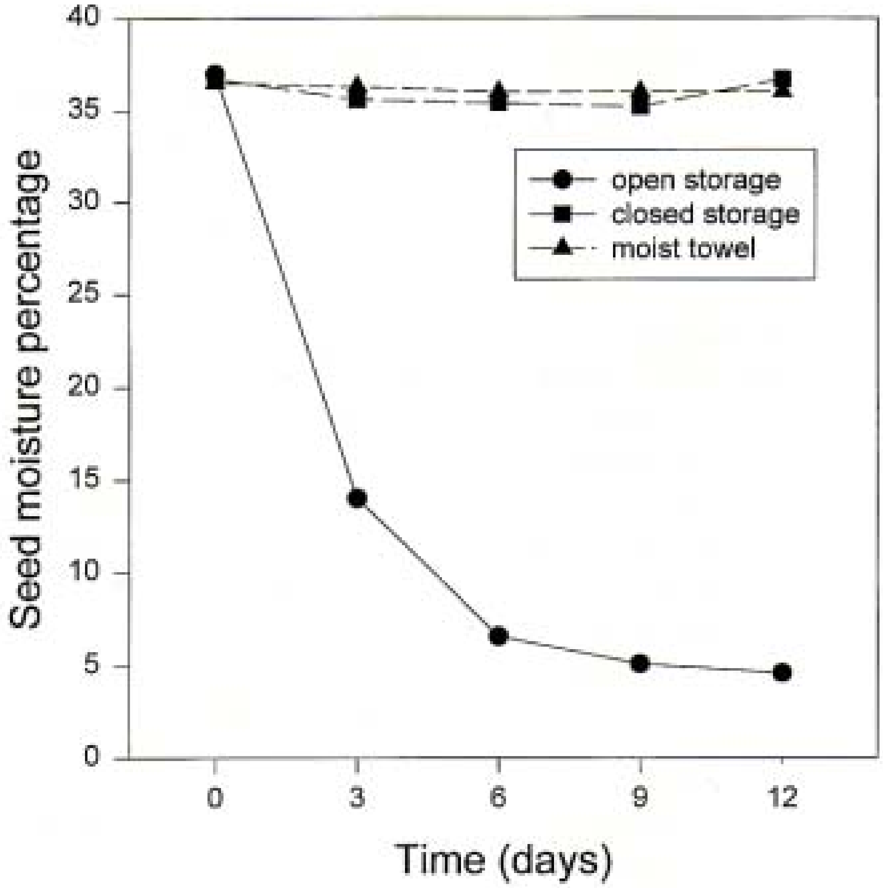

Figure 2. Changes in seed moisture content of North American pawpaw seeds stored at 25 °C in an open plastic container, rolled in moistened germination paper in a fastened plastic bag or in a sealed glass jar up to 12 days. Standard error for all data points was between 0.2–0.4, 0.1–0.2 and 0.1–0.2 for seeds stored open, in moistened paper and in jars, respectively.

Recalcitrance can be classified by the degree of desiccation that seeds tolerate. Farrant, Pammenter and Berjak (1988) proposed a classification system for recalcitrant seeds that includes highly, moderately and minimally recalcitrant categories. Highly recalcitrant species tolerate very little desiccation, germinate rapidly even when additional water is not available, usually are sensitive to low temperatures and are found in tropical forests and wetlands. Moderately recalcitrant species tolerate a moderate amount of

Table 1. The influence of seed moisture content on germination percentage of North American pawpaw seeds dried in sealed glass jars to moisture contents of 25,20 or 15% using KCl as a desiccant.

Seed moisture (%)	Germination (%)[1]
35	84 a
25	42 b
20	40 b
15	18 c

[1] Mean germination percentage of 50 seeds after stratification treatment of 120 days at 5 °C. Means are separated within the column using LSD test (P=0.05).

desiccation and germinate at an intermediate rate in the absence of additional water. Most moderately recalcitrant species are temperature-sensitive and are also of tropical origin. Minimally recalcitrant species are relatively desiccation tolerant and germinate slowly without additional water. Most minimally recalcitrant species tolerate lower temperatures and many have temperate or sub-tropical geographical distributions (Farrant *et al.*, 1988). In this study, pawpaw seeds lost over 50% viability when dried below 25% moisture. This suggests that pawpaw seeds should be classified as moderately recalcitrant, although they not only tolerate low temperature, but in most cases require low temperature to break dormancy and allow germination. Other members of the Annonaceae have also been reported to be intolerant to desiccation including *Annona crassiflora* (Rizzini 1973).

Effect of storage time and temperature on germination

Pawpaw seeds can be stored moist at 5 °C for an extended period (Table 2). Viability remained relatively high (52% in the 1994 seed lot and 74% for the 1995 seed lot) for up to two years. By three years, some seeds remained viable, but germination percentage for the 1994 seed lot was reduced significantly to only 22%.

Moisture content of seeds prior to and during storage affected germination percentages and storage life of pawpaw seeds (Figure 3). Initial moisture content of seeds prior to storage was reduced from approximately 35 to 25%. Seeds stored in moist towels rehydrated to approximately 35% moisture and remained at this percentage throughout the study (Figure 3). Seeds stored in glass jars had moisture contents between 20 and 25%. Seeds stored at 5 °C in wet towels showed germination percentages above 50% through 48 weeks of storage. Germination was only 15% after 52 weeks of storage; however, the same seed lot not exposed to an initial reduction in moisture content still showed greater that 70% germination after two years (Table 2).

Germination of seeds stored at 5 °C and approximately 25% moisture was more variable compared to seeds stored in wet towels and averaged only 45% germination over the 48 week period compared to 68% for seeds stored in wet towels (Figure 3).

A portion of seeds stored at 25 °C remained viable and responded to chilling stratification (Figure 3). However, germination percentages were generally below 25% and seeds lost all viability after 48 weeks. Seeds stored at 25 °C and approximately 25% moisture germinated better than seeds at 35% moisture at 28 and 32 weeks. This could

Table 2. Percentage germination in two seed lots of North American pawpaw after one, two and three years storage under stratification conditions at 5 °C.

Years of storage	1994 seed lot	1995 seed lot
	---------------%	---------------
one	90a[1]	84a
two	52b	74a
three	22 c	–

[1] Means are separated within the column using LSD test (P=0.05).

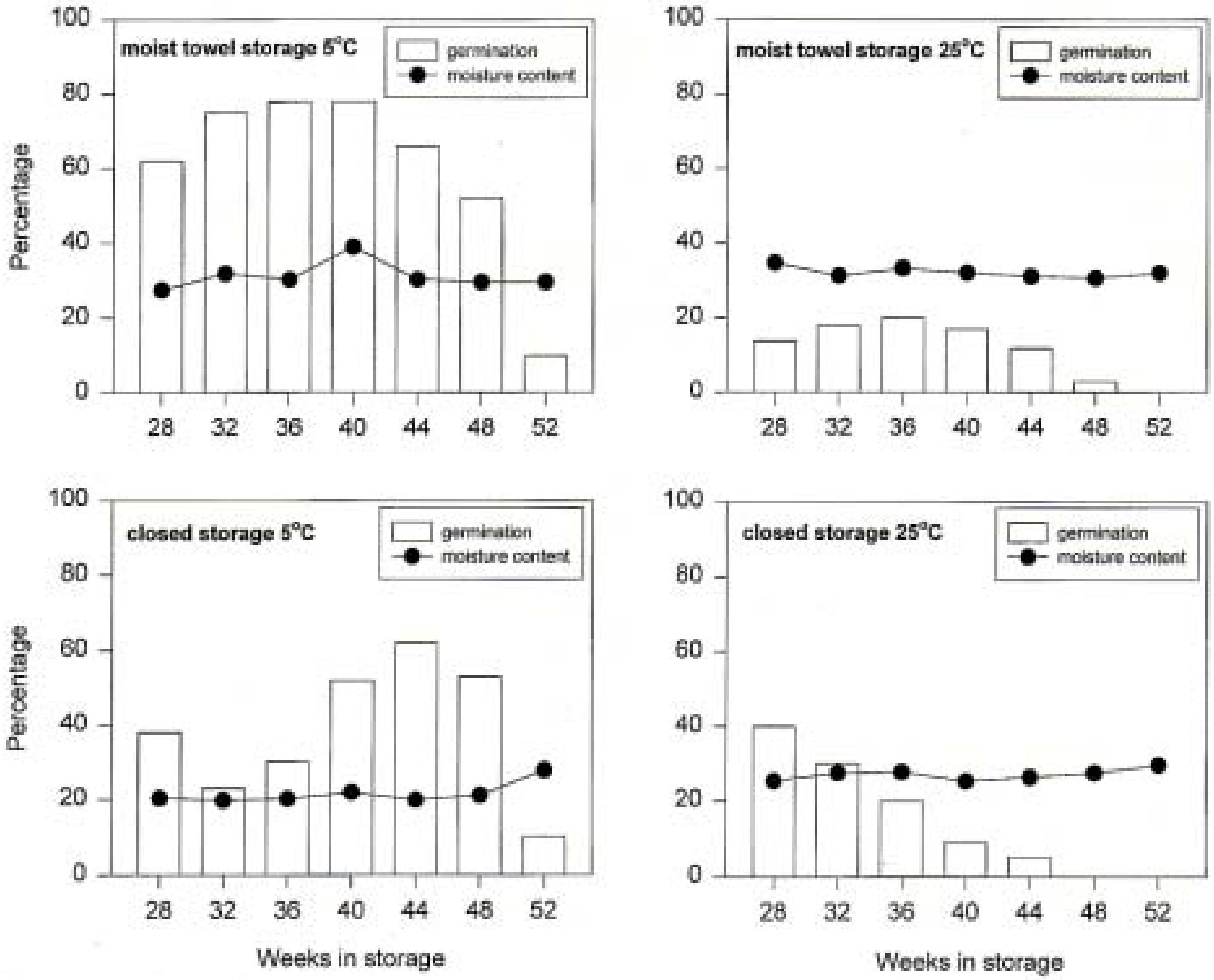

Figure 3. Moisture and germination percentage of North American pawpaw seeds stored in moistened germination paper or in sealed glass jars at 5 or 25°C for 28 to 52 weeks. Standard error of mean percentages ranged from 0.001 to 0.11.

be related to low seed moisture effects on aging or from reduced pathogen activity at lower seed moisture content.

Suggestions for seed storage include removing seeds from the fruit and storing at 5°C in a plastic bag with moistened spaghnum, sawdust or paper towels (DalTow, 1975). Pawpaw seeds have also been stored by treating seeds with a fungicide and storage in gas-permeable Ziplock™ bags containing a hydrated superabsorbent polymer gel (Terrasorb Ag™, Bradenton, Fl). The cross-linked polyacrylamide copolymer creates a storage substrate unfavorable to fungi, but provides a consistently moist environment for the pawpaw seeds (Boland, personal communication). The current study indicates that seeds can be stored for one year at chilling temperatures with only minimal loss in viability, but care should be taken not to allow seeds to dry during removal from the fruit.

Effect of substrate moisture, light and temperature on seed germination

Temperature did not significantly affect seed germination in stratified pawpaw seeds

Table 3. Influence of light and water on germination percentage of stratified North American pawpaw seed germinated at 25 °C exposed to continuous light or dark in petri dishes with 0, 0.5, 1 or S ml of water after 5 weeks.

Treatments	Water (ml)	Germination Percentage
Light	0	12
	0.5	36
	1.0	80
	5.0	8
Dark	0	28
	0.5	44
	1.0	72
	5.0	20
ANOVA	Pr>F	
Light	0.4410 ns	
Water	0.0001 **	
Light × Water	0.7939 ns	

(P=0.78; data not shown). The amount of water in the germination medium significantly affected germination percentage (Table 3). Maximum germination occurred with 1 ml of water in the petri dish (80% in light and 72% in dark) or in rolled towels (84%). Germination of seeds without additional water added to the medium was another indication that pawpaw seeds have a moderate form of recalcitrance (Farrant *et al.* 1988). Germination percentage was lowest at all evaluation dates for seeds with 5 ml of water added to the petri dish. Exposure of seeds to continuous light did not significantly effect germination (Table 3).

Conclusion

These data demonstrate that pawpaw produces recalcitrant seeds. For the seedlots examined, pawpaw also produces seeds with combinational dormancy (Nikolaeva 1997). First, pawpaw shows an intermediate physiological endogenous dormancy requiring at least 60 days of chilling stratification before germination commences. Pawpaw also has a form of morphological dormancy producing only a rudimentary embryo that does not increase in size during stratification (data not shown). Before germination is completed, the cotyledons of the embryo must elongate into the endosperm (Finneseth 1997). This explains the relatively long duration required to observe complete radicle emergence in germination studies. For standard germination testing, pawpaw seeds should be stratified for 100 days at 5 °C followed by germination in rolled towels at constant 25 or alternating 20/30 °C. Light was not required and final counts should be after 5 weeks.

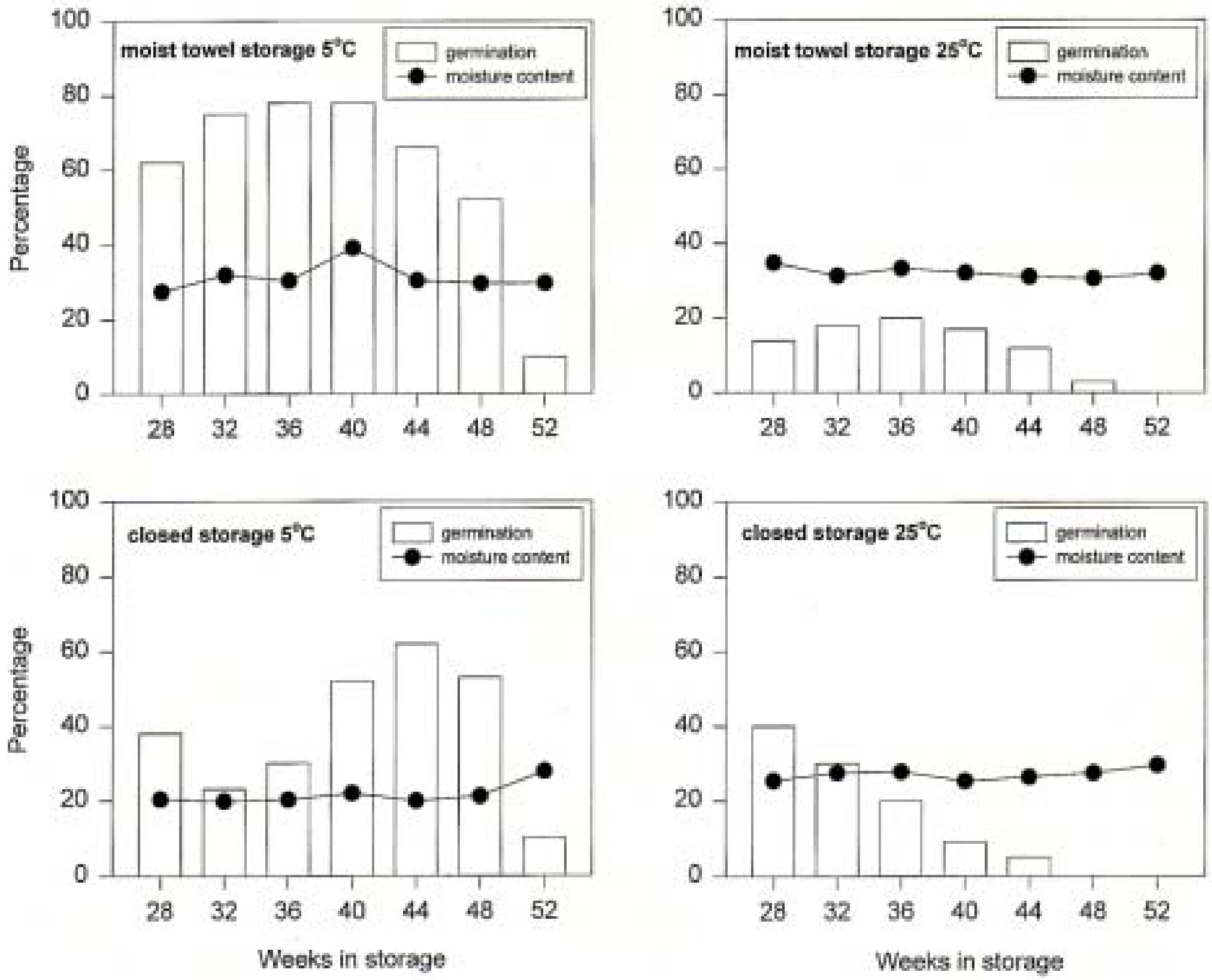

Figure 3. Moisture and germination percentage of North American pawpaw seeds stored in moistened germination paper or in sealed glass jars at 5 or 25°C for 28 to 52 weeks. Standard error of mean percentages ranged from 0.001 to 0.11.

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Effect of substrate moisture, light and temperature on seed germination

Temperature did not significantly affect seed germination in stratified pawpaw seeds

Table 3. Influence of light and water on germination percentage of stratified North American pawpaw sei germinated at 25°C exposed to continuous light or dark in petri dishes with 0, 0.5, 1 or 5 ml of water after weeks.

Treatments	Water (ml)	Germination Percentage
Light	0	12
	0.5	36
	1.0	80
	5.0	8
Dark	0	28
	0.5	44
	1.0	72
	5.0	20

ANOVA	Pr>F
Light	0.4410 ns
Water	0.0001 **
Light × Water	0.7939 ns

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Acknowledgements

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