Watermelon seed germination. 2. Osmomanipulation of photosensitivity

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Abstract

Seed germination of watermelon (*Citrullus lanatus* cv. Sugar Baby) was fully suppressed by intermittent far-red (FR) irradiation (1 min every 30 min). When the intervening dark period was increased, a linearly increasing final germination percentage was obtained. However, a 4-day intermittent FR treatment induced phytochrome-controlled dormancy and the longer the dark interval the deeper was the dormancy of the non-germinated seeds. When seeds were soaked in a fully inhibitory osmotic solution, no dormancy was imposed. However, a single FR pulse at the time of transfer induced partial, secondary dormancy; the kinetics of the imposition of dormancy followed a negative exponential curve (half-life 1.5 days; 3 days for the cultivar Crimson Sweet). Seeds osmotreated for 10 days in darkness and subsequently dehydrated (with and without a final FR pulse) acquired germination characteristics similar to those in light-requiring and dark-germinating achenes, respectively, of the lettuce cultivar Grand Rapids. In the light-requiring osmomanipulated seed population, the induction of germination was brought about by the low-energy reaction of phytochrome, chilling, dry storage and decoating. The transformation through osmomanipulation of the dark-germinating watermelon seeds (the inhibition of which required prolonged exposure to light) to positively or negatively photosensitive seeds (that responded to brief light pulses), might be attributed to the slow relaxation of existing meta-Fa and meta-Rb phytochrome intermediates to P_{fr} upon hydration.

Keywords: *Citrullus lanatus*, osmomanipulation, phytochrome, dormancy, germination, watermelon.

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Introduction

Watermelon seeds are dark germinators (Loy and Evensen, 1979; Botha *et al.*, 1982a,b); inhibition of their germination requires prolonged exposure to light, FR being the most effective. Only continuous or intermittent FR can suppress germination; a single short pulse is ineffective (Thanos and Mitrakos, 1992).

Phytochrome has been measured in numerous members of the Cucurbitaceae, in imbibed seeds and in 'dry' seeds. This is because such seeds are large and readily available and because their testas are transparent or easily removed. Phytochrome has been detected in seeds of *Cucumis melo* (Malcoste, 1969), *C. prophetarum* (93.5% P_{fr} in 'wet' seeds, Gutterman and Porath, 1975) *C. sativus* (75% P_{fr} in dry seeds, Spruit and Mancinelli, 1969; 66% P_{fr} in dry seeds, Malcoste *et al.*, 1970) *Cucurbita maxima* (Malcoste *et al.*, 1970), *C. pepo* (100% P_{fr} in dry seeds, Malcoste *et al.*, 1970 and Zouaghi *et al.*, 1972) and *Citrullus colocynthis* (75% P_{fr} in dry seeds, Malcoste *et al.*, 1970). The appearance of considerable quantities of phytochrome during imbibition led to the postulation of a new reaction, the 'inverse dark reversion' of phytochrome (Boisard *et al.*, 1968). Kendrick and Spruit (1974) explained this paradox in terms of phytochrome molecules trapped as intermediates (between P_r and P_{fr}) during desiccation of mature seeds. These intermediates relax to either P_r or P_{fr} during the early phases of imbibition, resulting in an apparent increase of spectrophotometrically identifiable P_{fr} and P_{tot}.

Although there are no published data on phytochrome measurements in watermelon seeds, the fact that germination is optimal in the dark (Thanos and Mitrakos, 1992) suggests that most of the phytochrome occurs in the P_{fr} form, at least in imbibed watermelon seeds. Since phytochrome intermediates might be present in considerable quantities in 'dry' watermelon seeds, it was decided in the present work to incubate the seeds in an osmoticum and subsequently to monitor the photosensitivity of germination in these osmomanipulated seeds. The role of

an osmotic solution in inhibiting germination is thought to take place by restricting the elongation of the radicle (e.g. Welbaum and Bradford, 1990). Osmotic solutions have been used extensively for seed 'priming' (e.g. Sachs, 1977; Thanos and Georghiou, 1988); in another type of application, an osmotic restraint has 'sensitized' germination with respect to light or dark (e.g. Thanos and Mitrakos, 1979).

A third use of osmotica has been to dissociate the final event of germination (radicle emergence) from the pregerminative processes, which are allowed to take place during imbibition in the osmoticum (e.g. Thanos, 1984). In the present study, this last application has been adopted to investigate the possible relaxation of phytochrome intermediates prior to radicle elongation and its effect on eventual germination.

Materials and methods

Plant material

Seeds of the watermelon (*Citrullus lanatus* [Thunb.] Matsu. et Nakai, cultivars Sugar Baby and Crimson Sweet, the latter used only for the experiment shown in Fig. 3B) were purchased from KYDEP, Greece. Seed batches were stored in moisture- and light-proof containers at room temperature (20 ± 5°C). Throughout the experiment, no changes in germination characteristics were observed. 'Opening' of seeds was performed manually, by applying lateral pressure, which resulted in cracking across the suture of the seed coat, at the radicle end.

Germination conditions

Imbibition and germination tests were carried out with at least eight samples of 25 seeds sown in glass Petri dishes 9 cm in diameter, as described previously (Thanos and Mitrakos, 1992). The osmotic pretreatment was performed in dishes containing a 0.6M mannitol solution ($\Psi_s = -1.49$ MPa) that had been shown to be fully inhibitory for germination, at 25°C. For Crimson Sweet seeds, a 0.5M mannitol solution ($\Psi_s = -1.24$ MPa) was used. The osmotreated seeds were rinsed thoroughly with distilled water and resown immediately or left to desiccate over filter paper in a dark room, at 25°C and RH of approx. 60%. The dehydration rate was slow, and after about 24 h the seeds had returned to their initial air-dry weight. All manipulations of hydrated seeds took place under a dim green safelight; this and the other light sources used have been described (Thanos and Mitrakos, 1992).

Results

Suppression of watermelon germination had been shown to be induced by continuous and intermittent (but not by a brief pulse of) far-red (FR) light. The more frequent the FR pulses the greater the suppression of germination, and the escape kinetics seem to follow a straight line (Fig. 1).

When seeds were given intermittent FR irradiation, germination of that part of the population escaping FR inhibition was completed in 2–3 days. Seeds that were unable to germinate after 4 days of intermittent FR were transferred to darkness and allowed to germinate (Fig. 2). The resulting time courses are sigmoid with the exception of two nearly linear curves (4 and 5 in Fig. 2). Their rates were considerably slower than that of untreated seeds; T_{50} was approx. 3.5 days in all curves and germination was completed in 8 days from the time of transfer to darkness. Seeds that had been irradiated more frequently eventually showed higher germination percentages; partial dormancy had been imposed on 12, 16, 56, 79 and 86% of the ungerminated seeds for the five FR pretreatments (curves 1–5, respectively in Fig. 2). However, an additional experiment showed that, when the 1 min per 30 min intermittent FR pretreatment (curve 1 in Fig. 2) was extended to 8 or

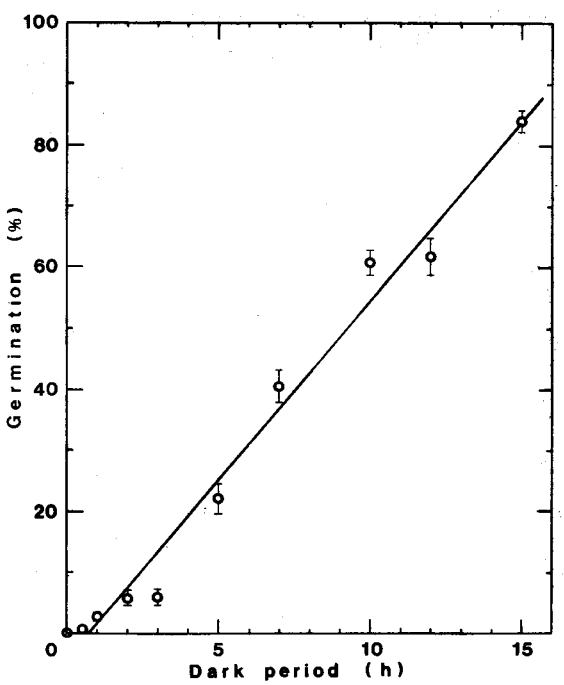

Figure 1. Final germinability of watermelon seeds, at 25°C, as a function of the length of the dark period between consecutive, brief (1 min) irradiations of far-red light given throughout the experiment. Vertical bars represent 2 SE. The linear regression curve is given by the equation:

$$Y = -4.022 + 5.846\,X$$

$$(r^2 = 0.982,\ \text{d.f.} = 78,\ P \ll 0.001),$$

where X is the length of dark period (in h) and Y is the germination percentage.

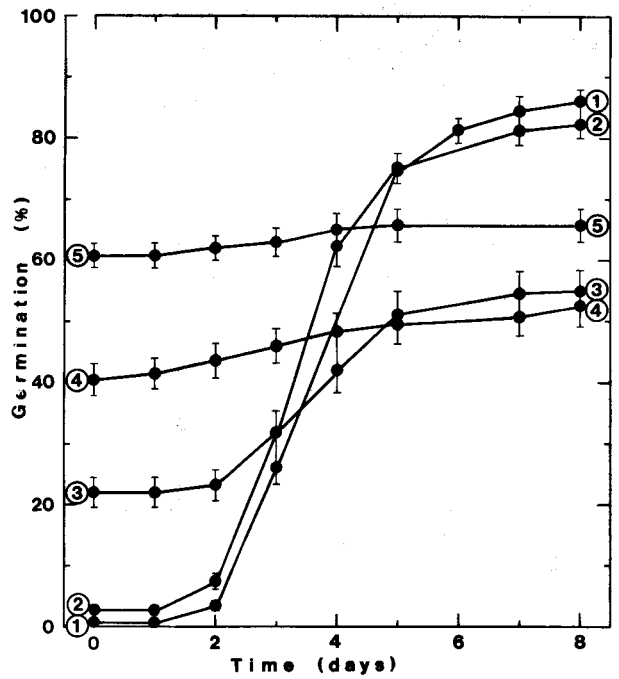

Figure 2. Time course of watermelon seed germination in darkness, after 4 days of intermittent far-red (iFR) irradiation. Time 0 is at the transfer of seeds to darkness; germination percentages at time 0 represent final germination during the pretreatment period. Curves 1–5 correspond to iFR irradiation pretreatments: 1 min FR given every 0.5, 1, 5, 7 and 10 h, respectively. Temperature was kept throughout at 25°C. Vertical bars represent 2 SE.

10 days, the subsequent dormancy imposed became maximal (95 and 100%).

Seeds of the cultivar Sugar Baby left to imbibe in darkness for up to 15 days (data not shown), in an inhibitory osmoticum (0.6M mannitol) germinated fully when transferred to water. On the other hand, an FR pulse at the time of transfer to water induced dormancy; the longer the osmotic pretreatment the deeper the FR dormancy imposed (Fig. 3A). Similar results were obtained with the cultivar Crimson Sweet (Fig. 3B). In both cultivars, the kinetics of the FR-induced dormancy followed a negatively exponential curve.

Seeds osmotically pretreated for 10 days (Fig. 3A), and (i) kept in darkness throughout or (ii) illuminated

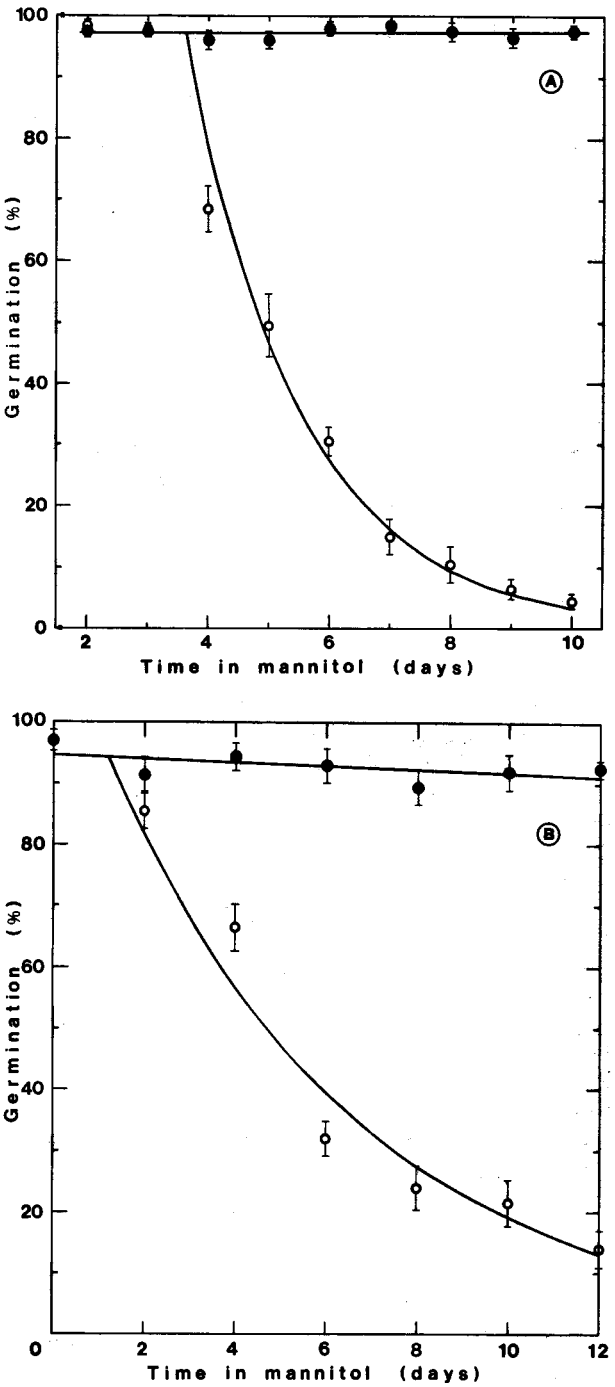

Figure 3. Effect of the duration of osmotic pretreatment on the subsequent final germination of seeds of two watermelon cultivars in water; (●) continuously in darkness, (○) illuminated with 30 min far-red immediately before transfer to water. Temperature was kept throughout at 25°C. Vertical bars represent 2 SE. (A) Sugar Baby in 0.6M mannitol solution; (B) Crimson Sweet in 0.5M mannitol solution.

The equations of the two curves are for (A) and (B), respectively:

$$Y = 658.098\, e^{-0.532\, X}$$

$$(r^2 = 0.994,\ \text{d.f.} = 5,\ P < 0.001);$$

$$Y = 118.117\, e^{-0.182\, X}$$

$$(r^2 = 0.956,\ \text{d.f.} = 4,\ P < 0.001);$$

where X is time (days) in the osmoticum and Y is final germination (%).

with a final, brief (30 min) FR pulse, were eventually left to dehydrate in darkness. Thus, two new seed populations (i and ii) were produced by osmomanipulation: (i) dark-osmotreated and (ii) dark-osmotreated, but with a final brief FR pulse. Even as early as 2 h after the onset of reimbibition, FR was considerably effective in inhibiting germination (Fig. 4); maximal suppression was obtained between 4 and 8 h, while full escape was observed 16 h after rehydration. When seeds were illuminated with brief and consecutive FR (30 min) and red (R) light (10 min) pulses, full R/FR reversibility was observed and germination was determined by the final pulse. The germination-potential curve in dark-osmotreated seeds (i), i.e. the germinability as a function of the osmolarity of the imbibition solution, was typically sigmoid with a G_{50} (inhibition of 50% of seed population) at 0.28M or −0.69 MPa (Fig. 5). An R pulse promoted considerably the final germination percentage in seeds imbibing in 0.25M mannitol. On the other hand, an FR pulse given immediately before the onset of imbibition resulted in significant inhibition.

The dark-osmotreated seeds given a final brief FR pulse (population ii) could not germinate in darkness; a short R pulse was sufficient to induce full germination and its action was reversed by an immediate FR pulse. With consecutive R and FR pulses, germination was determined by the final irradiation. In addition to the effect of brief R illumination, the opening of seeds promoted full induction of germination in darkness. Nevertheless, the germination of opened seeds was once more suppressed by a 0.3M mannitol solution; an R pulse restored germination and its effect was, in turn, reversed by a short FR illumination (Table 1). In addition, a 36-h chilling treatment promoted germination in nearly all the seeds in darkness, while shorter chilling periods resulted in partial promotion (Table 2).

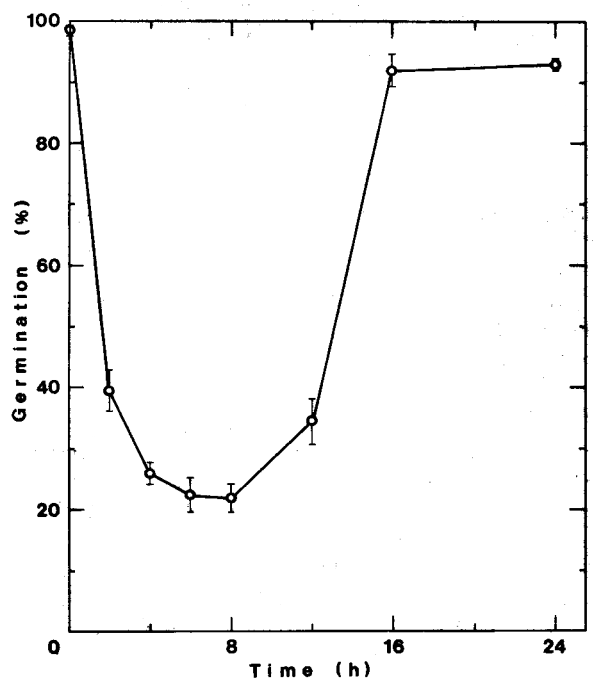

Figure 4. Far-red (FR) sensitivity curve of germination in osmotically pretreated watermelon seed as a function of timing of FR irradiation during re-imbibition in water. The pretreatment consisted of imbibition for 10 days in the dark in 0.6M mannitol solution, followed by rinsing and desiccation. An FR pulse of 10 min was given. Temperature was kept throughout at 25°C. Vertical bars represent 2 SE.

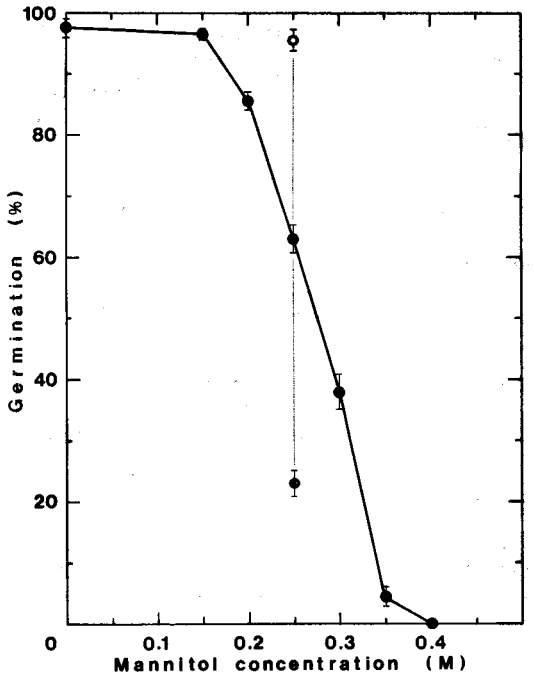

Figure 5. Germination-potential curve (final germination as a function of osmolarity in the imbibition medium), at 25°C in the dark, for osmotically pretreated watermelon seeds. The pretreatment consisted of imbibition for 10 days in the dark in 0.6M mannitol solution, followed by rinsing and desiccation; pretreated seeds were subsequently transferred to various mannitol solutions and allowed to germinate (●); (⊙) seeds illuminated with 20 min far-red immediately prior to rehydration; (○) seeds illuminated with 10 min red, 4 h after onset of imbibition. Vertical bars represent 2 SE.

Discussion

Besides inhibiting germination in watermelon, continuous (Thanos and Mitrakos, 1992) and intermittent FR (Fig. 1) can also impose secondary dormancy. The germination of these FR-dormant seeds is under classical, R/FR reversible phytochrome control.

On the other hand, the inhibitory effect of osmoticum on germination is not followed by dor-

Table 1. Effect of various treatments on the final germination percentage of watermelon seeds that were dark-osmotreated but given a final brief far-red (FR) pulse

Seed treatment		Germination (% ± SE)
Intact in H_2O, dark		12.5 ± 2.5
Opened in H_2O, dark		96.0 ± 1.6
in 0.3M mannitol,	dark	6.5 ± 2.7
	red light	94.5 ± 1.3
	red + far red	3.0 ± 1.3

The pretreatment consisted of imbibing for 10 days in the dark in 0.6M mannitol solution followed by a final FR illumination (30 min) immediately before rinsing and desiccation. The osmotica used were mannitol solutions and the illuminations (red, 10 min; FR, 30 min) were given 6 h after the onset of rehydration. Temperature was kept throughout at 25°C.

Table 2. Promotive effect of chilling (3°C) on the final germination percentage of watermelon seeds that were dark-osmotreated but given a final brief far-red (FR) pulse

Duration of chilling (h)	Germination (% ± SE)
0	9.0 ± 2.7
12	26.0 ± 3.2
18	77.5 ± 1.6
24	87.0 ± 3.2
36	99.0 ± 0.7

The pretreatment consisted of imbibing for 10 days in the dark in 0.6M mannitol solution followed by a final FR illumination (30 min) immediately before rinsing and desiccation. The seeds were left to reimbibe (in water) for various periods at 3°C and then were transferred for germination to 25°C (darkness throughout).

mancy, since full germinability was restored in subsequent transfer to water. Osmotreated seeds were, however, under the low-energy reaction, mediated by phytochrome. Moreover, when osmotreated seeds were dehydrated, either throughout in darkness or with a final FR pulse, two new seed populations, (i) and (ii), respectively, were produced. Both these populations were different from the untreated one in their ability to respond to brief R and FR irradiation; the control of their germination was shown to be a low-energy reaction of phytochrome. The germination characteristics of seeds in population (ii) closely resembled those exhibited by the achenes of the lettuce cultivar Grand Rapids, which are well known for requiring light for germination (Bewley and Black, 1982). In these and in the osmotreated watermelon seeds that had been turned dormant by a final FR pulse (population ii), promotion of germination was eventually achieved by (i) a brief R irradiation (low-energy reaction of phytochrome), (ii) a short chilling treatment, (iii) dry storage (at 25°C) and (iv) removal or opening of the seed coat (light was required when seeds were imbibed in an osmoticum).

Therefore, it might be concluded that, during dark osmotic pretreatment, although germination of watermelon seeds was completely suppressed, slow and gradual dark transformation of phytochrome intermediates took place. Thus, after a 10-day pretreatment, the whole pool of intermediates, possibly trapped during the late stages of seed maturation, may be almost depleted. Since inhibition of dark germination requires prolonged exposures to FR, it is reasonable to suppose that most of the pool consists of meta-Fa and meta-Rb that convert to P_{fr} upon hydration in darkness (Kendrick and Spruit, 1977). The kinetics of the imposition of dormancy (Fig. 3) suggest a very slow relaxation rate (half-life approx. 1.5 and 3 days, respectively) for phytochrome intermediates; although both meta-Fa and meta-Rb are known to be particularly stable among phytochrome intermediates (Kendrick and Spruit, 1977), such a long half-life cannot be explained readily.

Besides the relaxation process, during osmotreatment of the seeds a certain amount of P_{fr} seems to have been destroyed and/or to have thermally reverted to P_r. This is deduced from the germination-potential curve (Fig. 5), which should have been shifted to much higher values as a result of P_{fr} accumulation. Instead, a 0.10 MPa shift to lower values was observed (compare with the germination-potential curve of the untreated seeds; Thanos and Mitrakos, 1992, Fig. 3). The germination potential was promoted considerably by an R pulse and this should be attributed to the presence of a significant amount of P_r in the dark-osmotreated seeds. However, the latter osmomanipulated seed population closely resembled those of seeds of high-P_{fr} (red-light-pretreated and then dried) Grand Rapids lettuce (Kendrick and Russell, 1975) and *Sinapis arvensis* (Frankland, 1976) that have been produced by photomanipulation. Moreover, when 'dry' dark-

osmotreated seeds were irradiated with an FR pulse immediately before resowing, germination was inhibited. This effect has also been reported for the previously mentioned photomanipulated seeds and has been attributed to the phototransformation of P_{fr}, in the dry state, to a complex of phytochrome intermediates (namely meta-Fa), which eventually relax to P_r upon immediate imbibition (Bartley and Frankland, 1984, 1985).

The major conclusion of this study is that dark-germinating watermelon seeds (in which inhibition of germination requires prolonged irradiation) can be transformed, through osmomanipulation, to either positively or negatively photosensitive seeds that respond to brief light pulses (like the well-studied light-requiring or dark-germinating achenes of Grand Rapids lettuce).

Acknowledgments

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