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Aspects of germination of *Impatiens capensis* Meerb., formae *capensis* and *immaculata*, and *I. pallida* Nutt.

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NOZZOLILLO, C. AND I. THIE. (Dept. Biol., Univ. Ottawa, Ottawa, Canada K1N 6N5). Aspects of germination of *Impatiens capensis* Meerb., formae *capensis* and *immaculata*, and *I. pallida* Nutt. Bull. Torrey Bot. Club 110:335-344. 1983.—Dormancy of seeds of *Impatiens capensis* Meerb. forma *capensis*, collected locally (Ottawa, Canada) and stored at 5C in a fully imbibed condition, was broken after a storage period of about 4.5 months. Seeds of *I. capensis* Meerb. f. *immaculata* (Weath.) Fern. and Schub. seemed to require an additional week under similar conditions before showing evidence of breaking dormancy, while seeds of *I. pallida* Nutt. required 5 months stratification at 5C before germination was possible. Seeds of *I. capensis* f. *capensis* were stored for 5 and 6 months at 5C at relative humidities ranging from 0 to 100%. Seeds that had been stored at 100% RH had only a 40% increase in weight (fully imbibed seeds increased by 75%), were 95% viable, but 0% germinable. Seeds stored at 5 to 83% RH lost about 16% of their weight, retained only 30 to 70% viability, and were 0% germinable. Seeds that had been stored over silica gel (0% RH) lost about 35% of their weight, retained 40% viability, but were 0% germinable. The results are interpreted as meaning that a) free standing water around the seeds is necessary during stratification to remove inhibitory substances responsible for the dormant condition and b) a loss of 16% of their water content represents a critical value for survival of these desiccation-intolerant seeds. Seedlings of *I. capensis* usually formed an average of 12 adventitious roots at the base of the hypocotyl, while those of *I. pallida* formed 16. One seed of *I. capensis* f. *capensis* contained twin embryos, the first time twins have been reported in this genus.

Key words: *Impatiens capensis* Meerb., formae *capensis* and *immaculata*, *I. pallida*, Nutt., seed germination, stratification, seedling development

Touch-me-not or jewelweed, *I. capensis* Meerb., and the less common pale jewelweed, *I. pallida* Nutt., are the only species of the genus *Impatiens* native to eastern North America (Gleason 1963; Russell 1976). In both species germination is vernal, seedlings appearing more or less simultaneously and forming a dense carpet over the surface of the ground. A requirement of several months of cold exposure before germination of seeds can occur has been demonstrated for *I. capensis* (Barton 1939; Jouret 1976; Leck 1979) but the stratification requirements of *I. pallida* have not yet been reported in the literature. In the present study, seeds of both species were stored fully imbibed at 5C (Leck 1979) to determine the after-ripening period for seeds adapted to the climatic conditions of Ontario. Leck (1979) demonstrated that seeds of *I. capensis* must be kept moist, although Jouret (1976) indicated that some drying was permissible without marked loss of viability. In order to settle this difference, seeds of *I. capensis* were stored at various humidities to determine how low the moisture level in the seed could fall without affecting viability. Photographs were taken at various stages of the early development of seedlings of both species.

Materials and Methods. DETERMINATION OF THE STRATIFICATION PERIOD. Seeds of *I. capensis* Meerb. were collected throughout September of 1980 and 1981 and on October 7, 1982 from plants of both forma *capensis* (spotted orange flowers) and forma *immaculata* (Weath.) Fern. and Schub. (unspotted orange flowers) growing in a mixed population in the parkland bordering the Rideau River within the city limits of Ottawa, Ontario. Twelve flats of soil (containing seeds) were collected from the mixed population site in mid-November, 1980 and stored out-of-doors. Start-

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ing in December, one flat was brought into the greenhouse at weekly intervals. A garden where *I. capensis* f. *capensis* was growing as a weed, and to which 4 plants of *I. capensis* f. *immaculata* were transplanted in tubs served as a second site of seed collection in 1981. About 100 seeds of *I. pallida* Nutt. were collected in early September (1981) in St. James Park, Toronto, Ontario. Several plants grown from these seeds matured in the greenhouse and 25 seeds were stratified in early July, 1982. In all cases, seeds were harvested only from capsules that dehisced readily upon touching. Each harvest was cleaned to remove ovary tissue and only mature plump seeds with dark seed coats were used experimentally.

STRATIFICATION AND GERMINATION CONDITIONS. All seeds were stored at 5C in dim light as soon as possible after collection. Seeds of both *I. capensis* and *I. pallida* were placed on moist filter paper in 9 cm Petri plates, no more than 125 seeds per plate, and sealed in plastic bags (Leck, 1979). The plates were examined at three monthly, then at weekly, intervals. Appearance of one or more germinated seeds was taken as evidence that dormancy had been broken (after-ripened seeds of both species germinate at 5C, although more slowly than at 15C), and the plates were then transferred to a Conviron growth cabinet at 15C under a 14 hour day (5 Klux intensity) for further germination and development.

DETERMINATION OF HUMIDITY REQUIREMENT. Several thousand seeds of *I. capensis* f. *capensis* were collected in the fall of 1980 from another nearby site in the same parkland area. Controlled humidity conditions were prepared as follows: A 1 cm-deep layer of silica gel or various concentrations of sulfuric acid in water (0, 20, 30, 40, 45, 50, 65, 80, 85, 90%, v/v) were used to control relative humidity (RH) in sealed jars 9 cm in diameter by 8 cm high. Twelve 2 dram vials, uncapped, each containing 25 weighed seeds in a single layer on the bottom, were set in each jar, one jar for each RH, and the jars were placed at 5C. After 5 and 6 months, 6 of the vials were taken out. All seed lots were weighed upon removal; from each sampling time 4 lots were tested for germinability, 1 lot was tested for viability, and 1 lot was dried to constant weight at 50C. The response of radish (10 seeds), lentils (10 seeds) and alfalfa (50 seeds) to similar conditions of storage was tested with 45% and 90% sulfuric acid.

DETERMINATION OF % RELATIVE HUMIDITY. Each of the solutions used in the humidity experiment was sealed in a container large enough to hold an Airguide Relative Humidity Indicator and kept at 5C for several days to equilibrate. The reading on the gauge was then recorded. Accuracy of the gauge was assessed by taking the reading in a cold storage room (high humidity) and in the laboratory (low humidity), both with the gauge and with a standard wet bulb-dry bulb thermometer. Readings were 100, 83, 62, 42, 31, 25, 18, 10, and 5% respectively for 0, 20, 30, 40, 50, 65, 80, 85% and 90% sulfuric acid.

VIABILITY TEST. Two lots of 25 seeds, one stored for 5 months and one for 6 months under controlled humidity conditions were tested with a 1% aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC). After immersion at 37C for 34 hours, embryos stained red were scored as viable. All seeds ungerminated after 1 month under germination conditions were also tested with TTC.

SEEDLING DEVELOPMENT. Seedlings were allowed to grow in the same Petri plates in which they had germinated. Development was observed until the cotyledons were fully expanded.

Results. STRATIFICATION PERIOD for *I. capensis* seeds. There was no consistent effect of time of collection on time required for dormancy break (Table 1). Breaking of dormancy as indicated by the presence of germinated seeds on wet filter paper in Petri plates stored at 5C was usually a few days earlier for *I. capensis* f. *capensis* than for *I. capensis* f. *immaculata* (Table 1). Germination at 15C was equally rapid for seeds of both formae once initiated (Fig. 1).

A variety of plant species germinated in the soil from the mixed population collection site within a week of transfer to the greenhouse in early December, but *Impatiens* seedlings appeared first in flats brought in on January 18, and then only after four weeks in the greenhouse. Development was

Table 1. Stratification period (days to germination of the first seed at 5C) of seeds of *Impatiens capensis* f. *capensis* (SF) and of *Impatiens capensis* f. *immaculata* (UF) collected in the wild from a mixed population (a), from self-sown SF plants in the garden (b) or from UF plants transplanted to tubs (c).

Date of collection		# seeds SF	# seeds UF	Days to germinate at 5C SF	Days to germinate at 5C UF
Aug. 26, 1981	c		38		154
Sept. 1, 1981	c		99		150
Sept. 3, 1980	a	24	22	(153)*	155
Sept. 10, 1980	a	100	100	(142)*	(144)*
Sept. 10, 1981	b,c	102	90	(139)*	164
Sept. 14, 1981	a	100	70	(136)*	(148)*
Sept. 14, 1981	a	90	236	151	(150)*
Sept. 14, 1981	b	100		(145)*	
Sept. 17, 1981	c		120		147
Sept. 25, 1981	a	74	56	151	160
Sept. 26, 1980	a	100	100	(137)*	140
Sept. 26, 1981	b	70		132	
Sept. 26, 1981	c		104		130
Oct. 7, 1982	a	46	100	140	148
(from 9	a	62	100	142	146
individual	a	50	85	144	148
plants)	a		30		145
	a		50		142
	a		57		145

* By extrapolation of germination curve (e.g., Fig. 1). Mean ± S.E. of days to germinate of SF seeds (143 ± 2) significantly different from that of UF seeds (148 ± 2) by Student t test (P = .0548).

SF = spotted flowers; UF = unspotted flowers.

progressively accelerated as the period of cold exposure continued (data not presented) so that seedlings appeared after 3 days in a flat brought in on February 18.

STRATIFICATION PERIOD FOR *I. pallida* SEEDS. Seeds collected in early September (1981) began to germinate 159 days after being placed in cold storage, about two weeks later than *I. capensis* (Table 1). Seeds from greenhouse-grown plants also required about 159 days stratification. Germination was rapid and virtually 100% thereafter.

EFFECT OF RH DURING STRATIFICATION ON VIABILITY, GERMINATION AND FRESH WEIGHT OF SEEDS OF *I. capensis*. None of the seeds stored under controlled humidity conditions germinated when kept in the germination condition for 1 month, although a positive TTC test indicated that at least some were still viable at the time of removal from cold storage. Seeds stored at RH of 25% or higher showed more viability than those stored at RH lower than 25% (Fig. 2, open circles). Only a small fraction of the seeds that had been stored at RH less than 65% were still viable after one month under germination conditions, however, (about 28% of seeds kept at 62% RH declining to only 3% viability for seeds stored over silica gel) but seeds that had been stored at RH of 83% or 100% retained viability (Fig. 2, closed circles). There was no significant difference in the responses of seeds stored for 5 or 6 months and results for the two groups are combined in Fig. 2. Seeds of radish, lentils, and alfalfa germinated over 90% when tested after a 6 month period at 4.5% and 31% RH under similar conditions.

Seeds stored in a water-saturated atmosphere (100% RH) gained in weight, to 140% over initial, but not as much as seeds stored in contact with water-saturated filter paper, to 175% (Fig. 3). Seeds stored in atmospheres less than saturated (with water) lost about 16% of their moisture content regardless of the RH. Seeds stored over silica gel dried to the same extent as oven-dried seeds, to about 65% of initial wt.

SEEDLING DEVELOPMENT. Early development of *I. capensis* (Fig. 4) and *I. pallida* (Fig. 5) was similar except for the number of adventitious roots formed at the base of the hypocotyl, 8 for *I. capensis* (Fig. 4b) and 12 for *I. pallida* (Fig. 5b). Some variability in numbers of these adventitious roots was noted: rarely 4 or as many as 10 were seen on *I. capensis* seedlings, and no less than 8, or as many as 16, on seedlings of *I. pallida*. A dense mat of hairs was usually, but not invariably, produced on the swollen end of the hypocotyl (Fig. 4b, 5b). Four more adventitious roots appeared by the fifth day to give the seedlings the appearance of having a fibrous root system (Fig. 4c, 5c). By one week, some of the roots were no longer elongating as quickly as others (Fig. 4d, e), and the cotyledons were green and ready for photosynthesis. Most seedlings of *I. capensis* f. *capensis* and all seedlings of *I. pallida* had red-tipped roots (e.g., Fig. 4a, 5b), and anthocyanin pigmentation appeared in mature roots, hypocotyls, and cotyledons (e.g., Fig. 5c) as development progressed.

In the course of observations on seedling development of *I. capensis* f. *capensis*, a twinned embryo was noted. Two separate

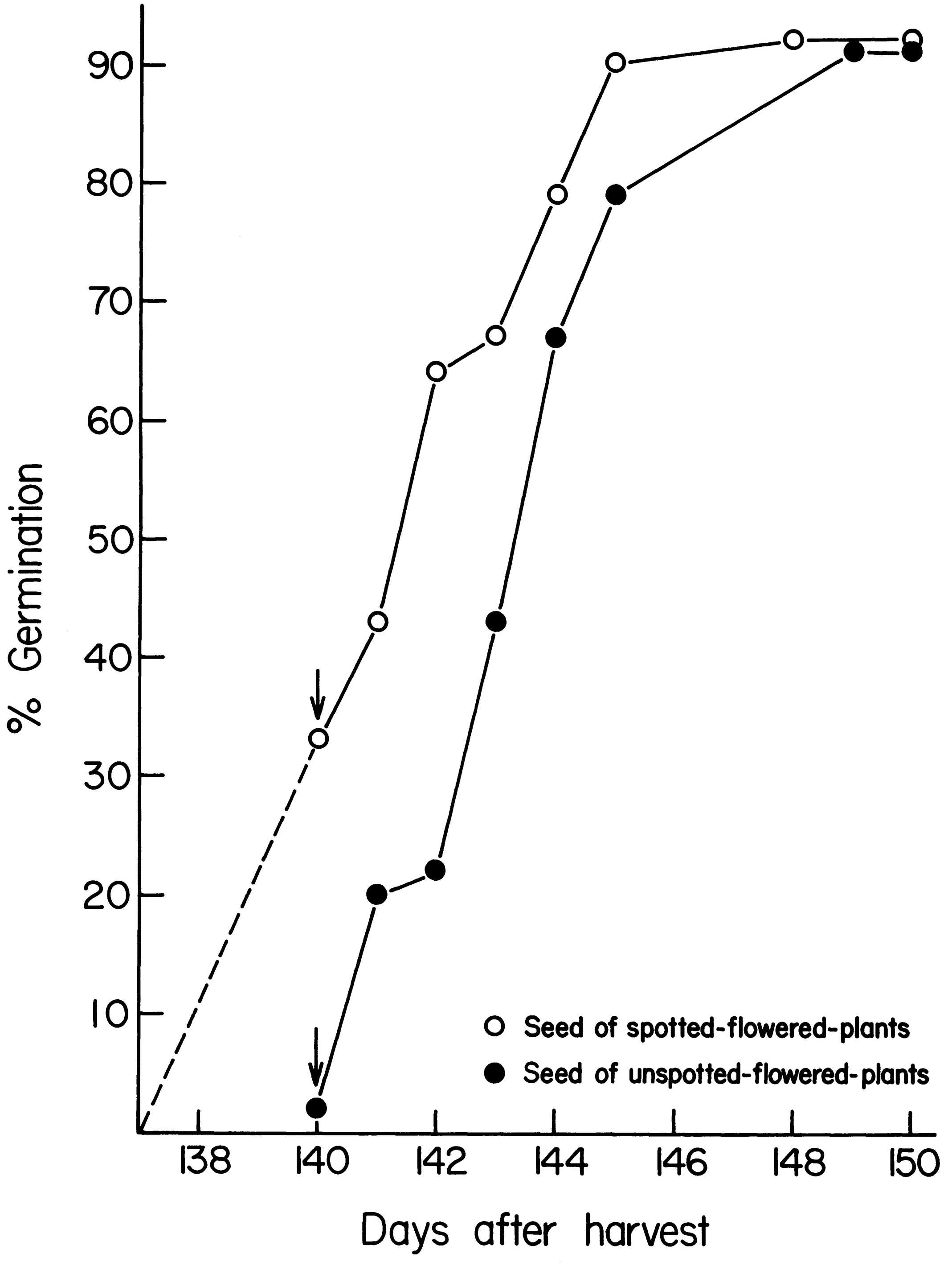


Fig. 1. Germination curves obtained for the September 26, 1980, collection of seeds of *I. capensis*. Arrow indicates time of transferral from cold storage to a 15C growth cabinet. Time of initiation of germination of seeds of spotted flowers (*I. capensis* f. *capensis*) indicated by extrapolation of the germination curve (dotted line).

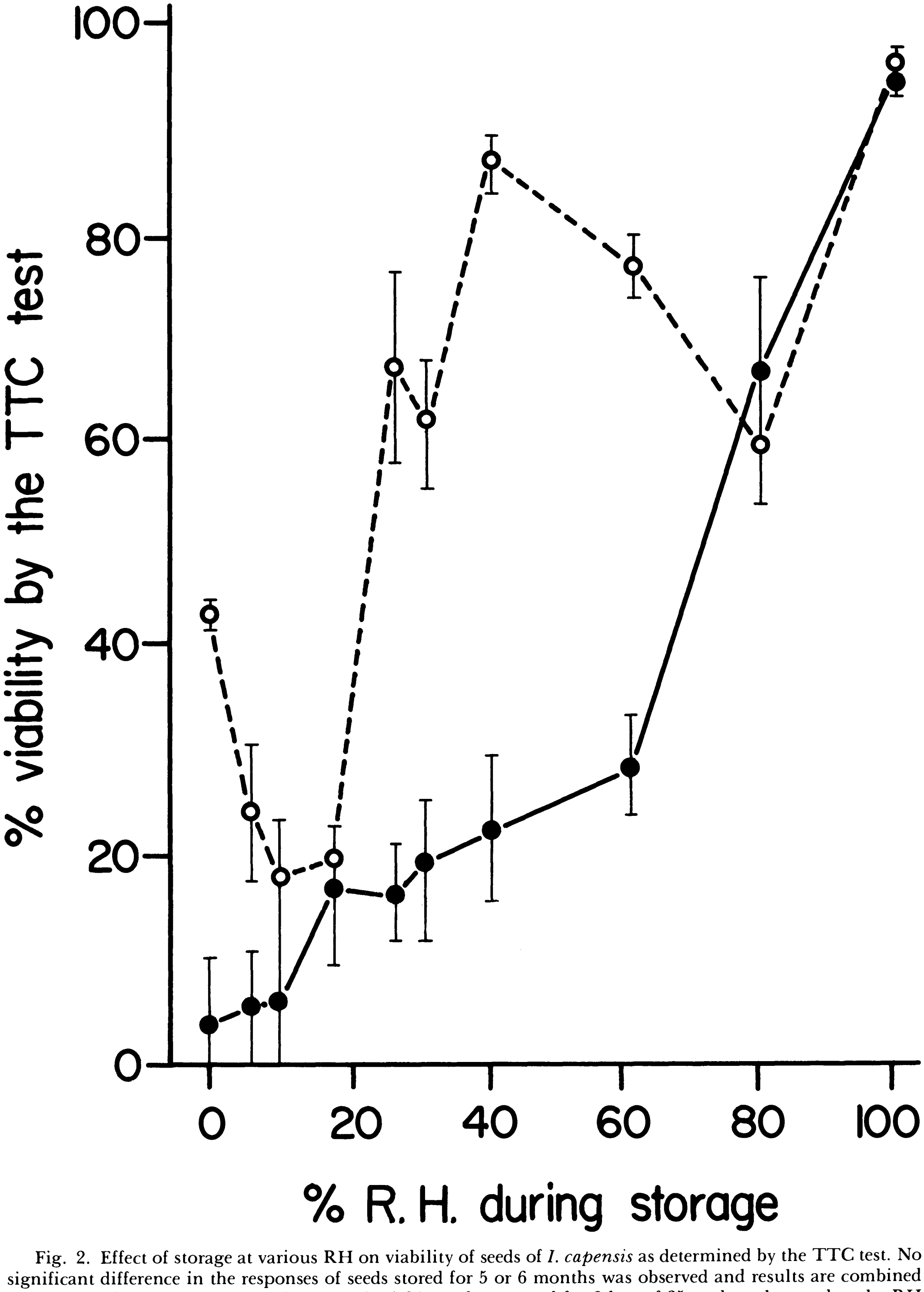


Fig. 2. Effect of storage at various RH on viability of seeds of *I. capensis* as determined by the TTC test. No significant difference in the responses of seeds stored for 5 or 6 months was observed and results are combined for the two time periods. Open circles are % viable seeds averaged for 2 lots of 25 seeds each stored at the RH indicated for 5 or 6 months. Closed circle, solid lines, are average values obtained with 8 lots of 25 seeds each after 1 month under germination conditions following storage at the % RH indicated for 5 or 6 months. Vertical bars are ±S.E.

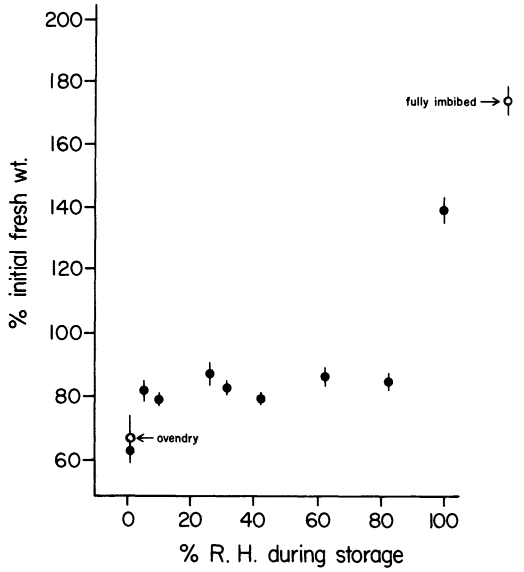


Fig. 3. Effect of cold storage at various RH on water content of *I. capensis* seeds. Results shown are average ±S.E. of 2 lots of 25 seeds stored for 5 or 6 mos at 5C.

hypocotyls grew out of one seed, each developing a root system. The cotyledons of one were lying underneath those of the other, so that it appeared at first glance that only one pair of cotyledons was present. Only one of hundreds of seeds observed developed in this fashion.

Discussion. STRATIFICATION PERIOD. Seeds of *I. capensis* f. *capensis* stored moist at 5C were ready to germinate after 132 to 153 days. This after-ripening period, averaging 4.5 months, was somewhat longer than the 4 month average reported by Leck (1979) in New Jersey and possibly reflects an ecological adaptation to the more severe Ottawa winters. Seeds of *I. capensis* f. *immaculata* tended to require either a few days more of after-ripening or were slower to germinate at the 5C temperature of the stratification condition. A few more days of cold exposure would have no ecological impact since seedlings do not usually appear in the Ottawa area until early May (unpublished observations) although, as the soil experiment showed, the seeds are ready to germinate long before that time. A slower germination response, however, would put the forma *immaculata* seedlings at a competitive disadvantage and may be a factor in the predominance of forma *capensis* in the study area.

EFFECT OF RH DURING STORAGE. Seeds stratified with a water content lower than that of complete imbibition were incapable of germination but still viable. This result supports the conclusion (Jouret, 1976) that a limited period of drying is possible before death occurs. The normal water content of about 35% was reduced to about 16% whether the RH of the storage environment was 10% or 80%, suggesting that the remaining 19% is very strongly bound. The positive TTC test indicated that at least some seeds were still alive at the end of the stratification period, even when all the bound water had been removed by storage over silica gel. The inability of those seeds stored in 100% RH to germinate suggested that availability of free water is essential to remove inhibiting substances, although no leaching tests were performed to determine if inhibiting substances could be removed. Loss of the positive TTC response under germination conditions of seed that had been stored at RH less than 65% suggested that a normal response to the stresses introduced by the rapid uptake of water during imbibition was no longer possible, and supported the conclusion of Leck (1979) that seeds allowed to dry lose viability. Becwar *et al.* (1982) reported that silver maple seeds showed a sharp drop in germinability as their moisture content declined from 42% to 37%, and attributed this to damage to the membranes by dehydration. For *Impatiens* seeds, with a water content of about 35% at maturity, a reduction to a moisture content of about 16% seemed to be the critical value. These data may explain the differences in the reports of Jouret (1976) and Leck (1979) in regard to drying and viability.

The use of sulfuric acid for humidity control possibly introduced toxic vapors into the atmosphere around the seeds. The lower initial % viability, 8 to 33%, of seeds stored over 80, 85, or 90% sulfuric acid (RH less than 20%), as compared to the 40% viability of seeds stored over silica gel, was perhaps a result of sulfuric acid damage rather than reduced humidity. However, seeds stored over 20 to 65% sulfuric acid (25 to 83% RH) retained a higher viability than

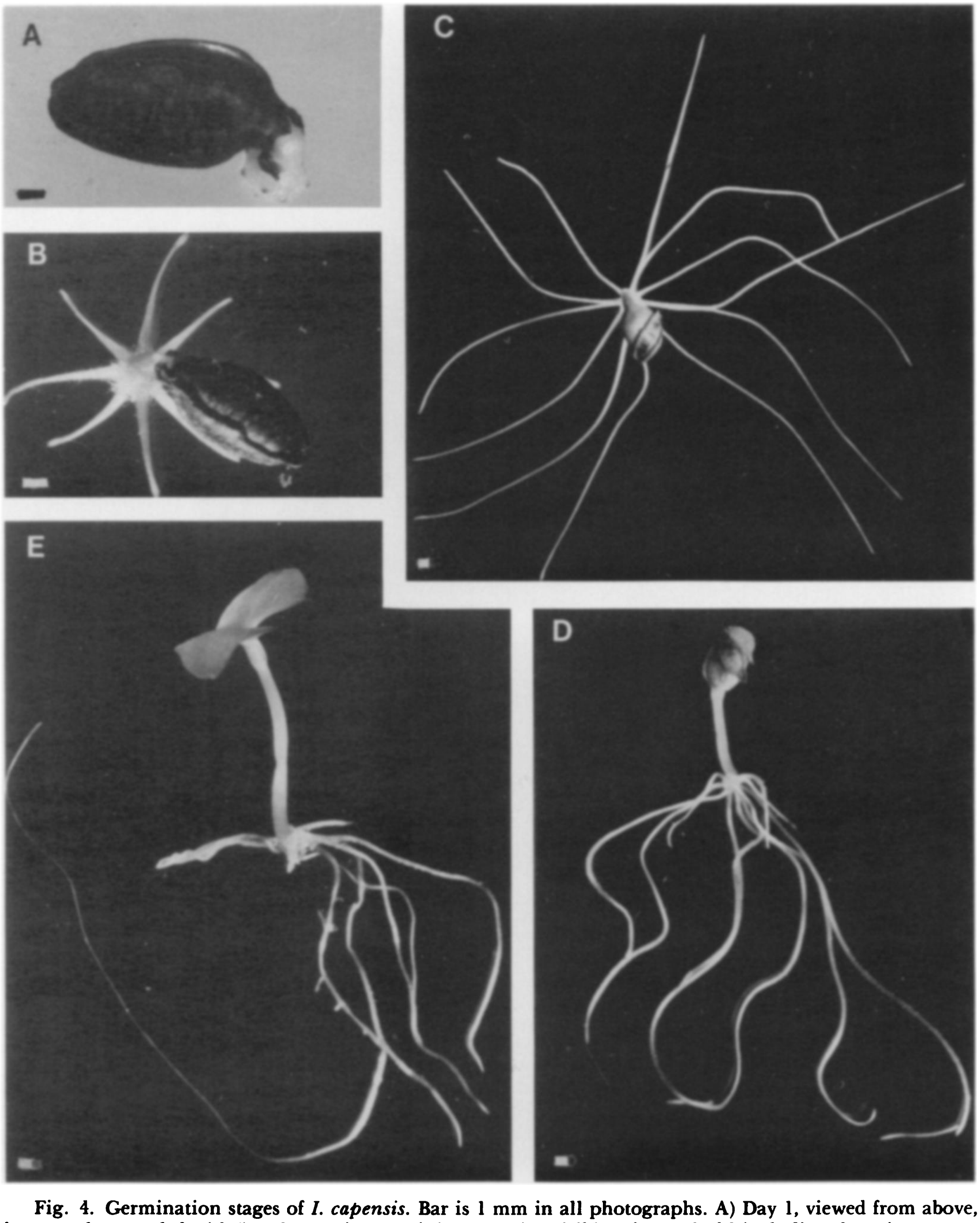
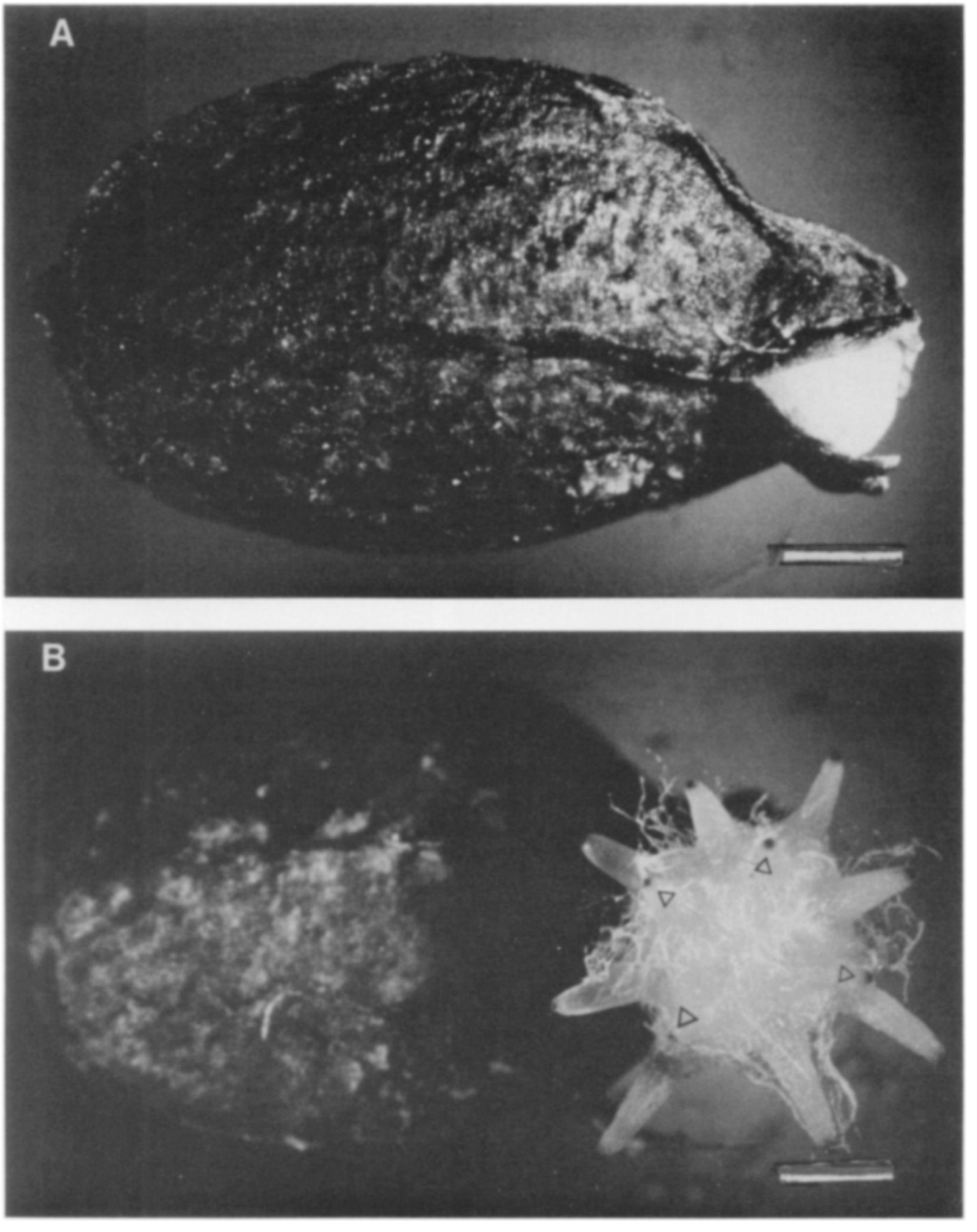


Fig. 4. Germination stages of *I. capensis*. Bar is 1 mm in all photographs. A) Day 1, viewed from above, hypocotyl protruded with 5 anthocyanin-containing root tips visible (of a total of 9 including the primary root tip which cannot be seen in this photograph). B) Day 3, viewed from above, all 8 adventitious roots growing at an equal rate in two tiers of 4 each, hairs on the base of the hypocotyl barely visible. No further growth of the primary root has occurred. C) Day 5, viewed from above, 12 adventitious roots, all of more or less equal length, hypocotyl hook still present, cotyledons green and emerging from the seed coats. D) Day 6, plant laid out on its side to show the straightened hook and unequal development of the roots. E) Day 8, plant laid out on its side to show opened cotyledons, lateral root development on the longer roots.


A
B

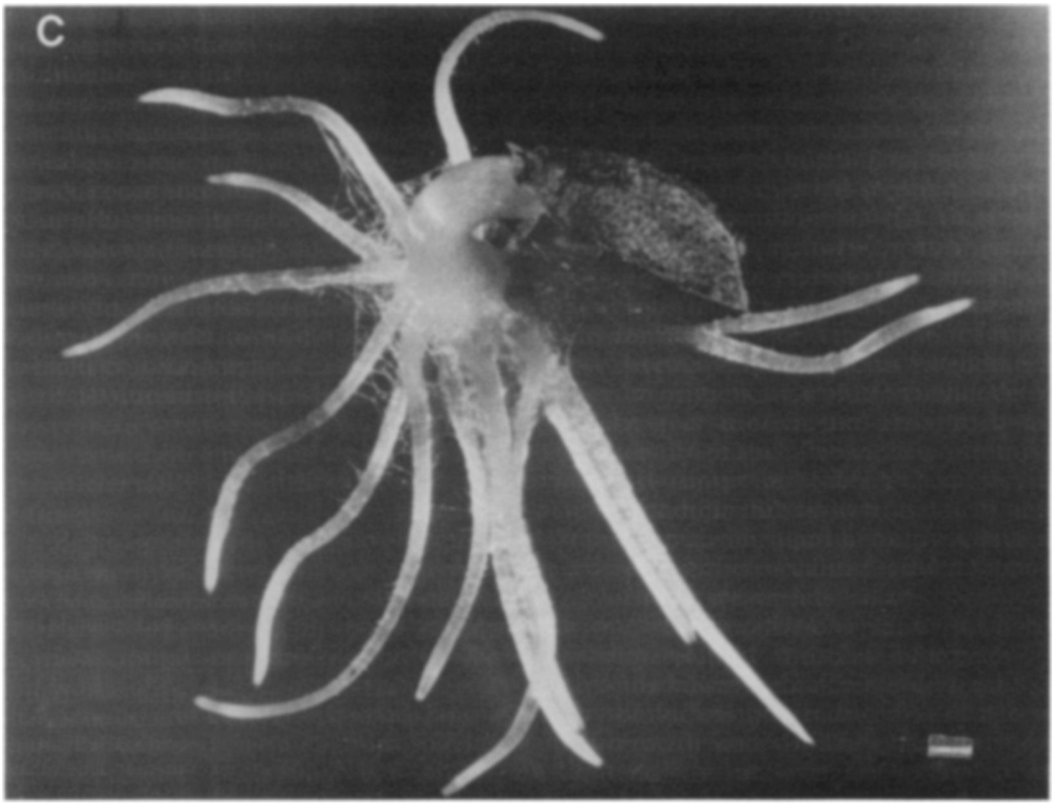


Fig. 5. Germination stages of *I. pallida*. Bar is 1 mm in all photographs. A) Day 1, hypocotyl barely protruding. B) Day 2, viewed from below to show 12 anthocyanin-tipped adventitious roots (inner row of 4 indicated by arrows), no evidence of primary root, and abundance of hairs on the swollen end of the hypocotyl. C) Day 4, viewed from above, cotyledons beginning to emerge from seed coats, hypocotyl hook still present, 16 adventitious roots present.

seeds stored over silica gel. After one month under germination conditions seeds stored over silica gel were no more viable than seeds that had been stored over 80 to 90% acid. This result, together with the 90% germinability of seeds of radish, lentil, and alfalfa stored for 6 months over 90% acid suggested that reduced humidity alone was responsible for loss of viability of the *Impatiens* seeds. The latter observation also negated the suggestion that the seeds suffered from anoxia.

SEEDLING DEVELOPMENT. Seedling development of these two wild species was similar to that of *I. parviflora* (Coombe 1958) and, except for the unusually large numbers of adventitious roots, typical of the genus (Thakur and Nozzolillo, 1978). Seedlings of *Impatiens balsamina* for example, had only 4 adventitious roots at the base of the hypocotyl but, as the experiments of Barker (1981) show, the production of additional tiers of roots awaited only the appropriate stimulus. Replacement of the primary root by a mass of adventitious roots is presumably an ecological adaptation to the moist locations these species prefer. They would provide quickly both support and a large absorptive surface on a wet clay soil, such as that at the site of seed collections, in which a normal tap root would die from anoxia.

Twinning is a relatively rare but not uncommon phenomenon and has never been reported for *Impatiens capensis*. In flax plants, twins are invariably a mix of haploid and diploid individuals (Murray, 1980). The cytogenetic status of the *Impatiens* twins was not determined as they did not survive beyond the seedling stage.

Anthocyanin pigmentation appeared in most seedlings of *I. capensis* f. *capensis*, and all seedlings of *I. pallida,* in early stages of seedling development, and increased progressively as roots, hypocotyls and cotyledons matured. Most seedlings grown from *I. capensis* f. *immaculata* lacked anthocyanins (Nozzolillo, 1983).

Literature Cited

BARKER, W. G. 1981. Patterns of regeneration in mutilated seedlings of garden balsam, *Impatiens balsamina* L. Ann. Bot. 47:661-668.

BARTON, L. V. 1939. Experiments at the Boyce Thompson Institute on germination and dormancy in seeds. Scient. Hort. 7:186-193.

BECWAR, M. R., P. C. STANWOOD, AND E. E. ROOS. 1982. Dehydration effects on imbibitional leakage from desiccation-sensitive seeds. Plant Physiol. 69:1132-1135.

COOMBE, D. C. 1958. *Impatiens parviflora* DC. J. Ecol. 44:701-713.

GLEASON, H. A. 1963. New Britton and Brown illustrated flora of the northeastern United States and adjacent Canada, Vol. 2, Hafner Publ. Co. Inc., New York and London.

JOURET, M.-F. 1976. Ecologie de la dormance séminale et de la germination chez diverses espèces du genre *Impatiens* L. Bull. Soc. R. Bot. Belg. 169:213-225.

LECK, M. A. 1979. Germination behaviour of *Impatiens capensis* Meerb. (Balsaminaceae). Bartonia 46:1-14.

MURRAY, B. E. 1980. Diploid, aneuploid, and triploid plants from haploid × diploid crosses in flax, *Linum usitatissimum.* Can. J. Genet. Cytol. 22:591-665.

NOZZOLILLO, C. 1983. Observations on anthocyanin pigmentation in seedlings and mature plants of *Impatiens capensis* Meerb. and its possible role as a determinant of floral form. Can. J. Bot. 61:633-638.

RUSSELL, A. 1976. Biosystematic studies on the genus *Impatiens* in northeastern North America. M. Sc. thesis. McGill University, Montreal.

THAKUR, M. AND C. NOZZOLILLO. 1978. Anthocyanin pigmentation in roots of *Impatiens* species. Can. J. Bot. 56:2898-2903.