GERMINATION REQUIREMENTS OF LABORATORY STORED SEEDS OF *SOLANUM NIGRUM* AND *SOLANUM PHYSALIFOLIUM*

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ABSTRACT

As part of a study of the biology of black nightshade (*Solanum nigrum*) and hairy nightshade (*Solanum physalifolium*) in New Zealand, the germination requirements of freshly harvested seed stored at 5°C were investigated. Previous studies of black nightshade germination have given conflicting evidence for optimum temperature regimes. In this study alternating temperature, pre-germination chilling and light were all required to germinate black nightshade seed. Seeds of hairy nightshade germinated only when treated with potassium nitrate (0.2%), suggesting the presence of a primary dormancy. The relevance of these results to field germination and management of freshly shed seed is discussed.

Keywords: *Solanum nigrum*, *Solanum physalifolium* var. *nitidibaccatum*, germination, primary dormancy, light requirement.

INTRODUCTION

Vegetable processors in New Zealand report processing losses of premium small grade 'baby' peas (*Pisum sativum* L.) due to contamination by fruit of *Solanum nigrum* L. (black nightshade) and *Solanum physalifolium* Rusby var. *nitidibaccatum* (Bitter) Edmonds (hairy nightshade). Organic growers of process peas in particular have had contamination problems. In the past two years over 7% of organic process pea fields in Canterbury had nightshade contamination (A. White, pers. comm.). There is a need for relevant biological information on problem weeds for developing ecologically based management strategies (Mortensen et al. 2000). Understanding the germination requirements of these weeds is useful to develop strategies to either minimise or maximise germination at different management phases.

Most previous studies on germination of black nightshade seeds after a period of storage report high levels of germination at constant temperatures (Givelberg et al. 1984; del Monte & Tarquis 1997; Kremer & Lotz 1998). However some studies report an alternating temperature requirement (Roberts & Lockett 1978; Wagenvoort & Opstal 1979). Similarly reports differ regarding black nightshade's germability when freshly harvested, with most studies reporting no primary dormancy (Givelberg et al. 1984; Bulcke et al. 1985; Agong 1993) but one study reporting primary dormancy (Roberts & Lockett 1978). Studies of black nightshade also report light as a germination requirement (Roberts & Lockett 1978; Givelberg et al. 1984). From these studies it was hypothesised that constant temperatures and light would provide high levels of germination in New Zealand populations of black nightshade and that freshly harvested seed is not dormant.

Reported germination requirements of hairy nightshade differ to those of black nightshade (del Monte & Tarquis 1997). The hypothesis for hairy nightshade germination

in this study was that alternating temperatures would provide high levels of germination (del Monte & Tarquis 1997) but that freshly harvested seed would be dormant (Roberts & Boddrell 1982).

METHODS

Seed collection and processing

Plants were collected from crops or field margins where herbicides had not been used for a minimum of 2 years. Three collections of fruit from black nightshade plants (one from a Gisborne farm and two from a Lincoln farm) and one from hairy nightshade plants (from a Lincoln farm) were made from February to April 2001. Both the Lincoln green and mature black fruit where collected from the same plants on the same date. Seed was extracted from fruit > 6 mm in diameter, and seed from green and black nightshade fruit was processed separately. The seeds were initially stored at 5°C, then in April they were dried at 30°C for 36 h, before being returned to 5°C. In May, 100 seeds from each collection were tested for viability with 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) using the procedure for the Solanaceae (Peters 2000) with a 24 h staining time at 25°C.

Four experiments using three replicates of 25 seeds/treatment were conducted with the seed collections. Experiments were initiated when seed had been stored for 6-12 weeks. The duration of all alternating temperature treatments was 16 h at the low temperature and 8 h at the high temperature.

Experiment 1

Three black nightshade collections and one hairy nightshade collection were germinated in 24 h light at constant temperatures of 10, 15, 20 or 25°C. After 14 days all material was transferred to 20/30°C with 16:8 h light:dark for 7 days.

Experiment 2

Two black nightshade collections in a factorial design were tested at two pre-germination chilling levels, 5 days at 5°C or no chilling, and 8 temperature regimes, constant temperatures of 10, 15, 20 or 25°C for 14 days, or alternating temperature of 10/15, 5/20, 10/25 and 20/30°C for 21 days. After 14 days material from the constant temperature regimes were transferred to 20/30°C for 7 days.

Experiments 3a and 3b

Two identical experiments used a hairy nightshade collection and two black nightshade collections which were treated with potassium nitrate (0.2% KNO_3), gibberellic acid (0.05% GA_3) or water. A repeat experiment was set up 3 days after the first experiment. Both experiments used a 24 h light regime and 20/30°C temperature.

Experiment 4

Seeds from a hairy nightshade collection and two black nightshade collections were pre-germination chilled at 10°C for 18 days under two light treatments, 24 h light or 24 h dark where germination containers were covered with black polythene. They were then subjected to three temperature regimes of 10/15, 5/20 and 20/30°C and the two light treatments for 14 days. Dark treatments were inspected after 14 days in the alternating temperatures. This final experiment used seeds that had been stored for 21-25 weeks.

All experiments used the following procedures or materials. Cuisine Queen 500 ml (internal size 142 x 97 mm) containers with moistened germination blotters were used to hold the seeds during germination. Moisture was maintained by the addition of water as required. Replicates from light treatments were inspected every 2-3 days. At the end of each assay (at 14 or 21 days) seed that resisted gentle pressure by tweezers was recorded as viable.

Statistical analysis used SYSTAT 1999 version. Only significant main effects and interactions are reported. When two species were included in the same assay, species were analysed separately.

RESULTS

Experiment 1

All black nightshade collections had 0% germination at 10 and 15°C, and minimal germination at 20 and 25°C (Table 1). Germination was high after 14 days pre-germination chilling at constant temperatures followed by 7 days at 20/30°C, with maximums of 56, 85 and 96% for seed from green Lincoln, black Lincoln and black Gisborne fruit respectively. Black fruit from Lincoln germinated to higher levels than that of green fruit from Lincoln. There was no germination of hairy nightshade at any temperature. In black nightshade there was a significant interaction between seed collection and pre-germination chilling temperature treatment (Table 1).

TABLE 1: Mean germination (%) of three black nightshade seed collections in experiment 1. Seeds were kept at constant temperatures for 14 days and then transferred to 20/30°C for 7 days.

		Lincoln		Gisborne
		Green fruit	Black fruit	Black fruit
Days prior to transfer to 20/30°C	Temperature			
14	20°C	0[1]	0	2.7
14	25°C	4.0	0	1.3
Days after transfer to 20/30°C	Temperature prior to 20/30°C			
7	10°C	56.0 a[2]	82.7 a	96.0 a
7	15°C	44.0 b	85.3 a	90.7 a
7	20°C	33.3 b	40.0 b	93.3 a
7	25°C	9.3 c	6.7 c	48 b

Interactions and main effects

14 day pre-germination chilling (PGC) $P<0.001$
Seed collection $P<0.001$
PGC x Seed collection $P<0.001$

[1]Values are the mean of 3 replicates.
[2]Means followed by the same letter within columns are not significantly ($P<0.05$) different using Fischer's Least Significant Difference test.

Experiment 2

In experiment 2 a 5 day 5°C pre-germination chilling and imbibition treatment had no effect on total germination (Table 2). Pre-germination chilling treatments for 14 days at 10, 15 or 20°C increased ($P<0.05$) germination in comparison to the control for both seed collections. There was a significant ($P<0.01$) interaction between seed collections and 14 day pre-germination chilling temperature. Germination at alternating temperatures other than at 20/30°C was negligible (data not presented). The maximum germination response to the 14 day pre-germination chilling treatment was at 10 or 15°C for seed from green Lincoln fruit. For seed from black Gisborne fruit it was at 10, 15 or 20°C.

Experiment 3

In the two chemical experiments there was no difference ($P>0.05$) between experiments for both species, therefore the means of both experiments are presented (Table 3). For black nightshade GA_3 significantly ($P<0.05$) increased germination of seed from green Lincoln fruit but not ($P>0.05$) that of seed from black Gisborne fruit. In both black nightshade seed collections KNO_3 increased ($P<0.05$) germination compared to the

TABLE 2: Mean germination (%) after 7 days at 20/30°C of black nightshade seeds in experiment 2. Seeds were given 0 or 5 days pre-germination chilling at 5°C, followed by 14 days pre-germination at constant temperatures of 10, 15, 20 or 25°C. Control seeds were germinated at 20/30°C for 21 days after pre-germination chilling at 5°C for 0 or 5 days.

		Green Lincoln fruit		Black Gisborne fruit	
Days of pre-germination chilling at 5°C		0	5	0	5
Days pre-germination chilling	Pre-germination chilling temperature				
0	-	12.0 c[1,2]	16.0 c	22.7 c	49.3 b
14	10°C	64.0 a	65.3 a	97.3 a	97.3 a
14	15°C	61.3 a	72.0 a	94.7 a	94.7 a
14	20°C	30.7 b	34.7 b	84.0 a	85.3 a
14	25°C	8.0 c	13.3 c	62.7 b	45.3 b

Interactions and main effects
Seed collection P<0.001
14 days pre-germination chilling (14d PGC) P<0.001
Seed collection x 14d PGC P<0.01

[1]Values are the mean of 3 replicates.
[2]Means followed by the same letter within columns are not significantly (P<0.05) different using Fischer's Least Significant Difference test.

control. There was a significant interaction (P<0.001) between seed collection and chemical treatment. For hairy nightshade germination was increased by KNO_3 (P<0.05), mean germination being 74.7% at 11 weeks.

TABLE 3: Mean germination of black nightshade and hairy nightshade seeds in experiment 3. Seeds had been treated with water, KNO_3 or GA_3 after 21 or 77 days of alternating temperatures of 20/30°C.

	Black nightshade after 21 days		Hairy nightshade Lincoln fruit	
	Green Lincoln fruit	Black Lincoln fruit	21 days	77 days
Control	9.3 b[1,2]	50.0 b	0.7 b	0.7 b
KNO_3	36.0 a	95.3 a	22.7 a	74.7 a
GA_3.	39.3 a	50.7 b	3.3 b	10.7 b

Interactions & main effects

Black nightshade		Hairy nightshade	
Seed collection	P<0.001	Chemicals	P<0.001
Chemicals	P<0.001		
Chemicals x seed collection	P<0.001		

[1]Values are the mean of two experiments, each having three replicates.
[2]Means followed by the same letter within columns are not significantly (P<0.05) different using Fischer's Least Significant Difference test.

Experiment 4

There was no germination of black nightshade at 10/15°C and hairy nightshade did not germinate at all. For black nightshade there was a significant (P<0.01) interaction

between temperature, seed collection and light treatments (Fig. 1). Light treatments gave higher ($P<0.05$) germinations than the dark treatments at 5/20 and 20/30°C, except for seed from green Lincoln fruit at 5/20°C.

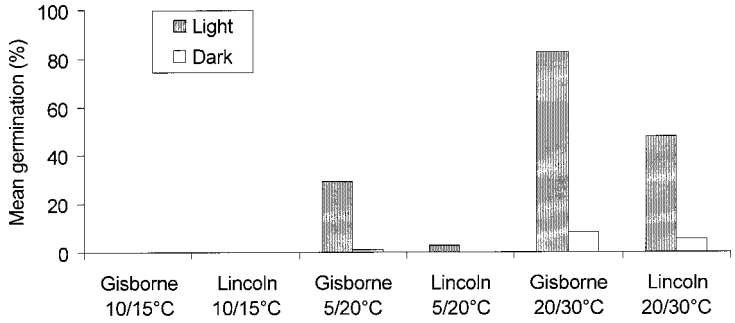

FIGURE 1: Mean germination after 14 days for black nightshade from two seed sources, green Lincoln fruit and black Gisborne fruit at 10/15, 5/20 and 20/30°C under 24 h light or dark conditions in Experiment 4.

DISCUSSION

Germination requirements

Previous studies of black nightshade reported high levels of germination at constant temperatures of 25 or 30°C for seed stored at room temperature for 2 months after collection (Givelberg et al. 1984) or stored at 5°C for 6 months (Benvenuti & Macchia 1993). In the present study testing at 10-25°C constant temperatures gave low germination (Table 1). Viability tests of the collections used indicated that the collections had adequate (mean TTC 77.2%) levels of apparent viability. Further an estimate of viability post-assay (data not presented) supported the assumption that only a small proportion of the apparently viable seed was germinating. Poor germination of freshly collected seed at constant temperatures has been reported for black nightshade. Freshly collected seed in 5 different years of collections did not germinate at constant temperatures ranging from 4-30°C (Roberts & Lockett 1978). The same seed germinated when tested at alternating temperatures of 10/25, 10/30, 15/25 and 15/30°C (Roberts & Lockett 1978). In the present study alternating temperatures of 10/15, 5/20, 10/25 and 20/30°C with black nightshade seed did not result in appreciable germination except at 20/30°C (Table 2). Pre-germination chilling for 14 days at 20°C and below prior to alternating temperatures of 20/30°C increased ($P<0.05$) germination (Table 2). This agrees with the report of Wagenvoort & Opstal (1979) where laboratory stored black nightshade seed stratified at 5°C prior to alternating temperatures of 9/25°C (8 and 16 h respectively) gave the highest germination.

In the present study the germination of hairy nightshade at alternating temperatures including pre-germination chilling was unsuccessful. Roberts & Boddrell (1983) obtained germination of 80% at 20/30°C for seed stored for 3 months. In the present study there was no germination at this temperature for seed stored for 3 months. It is possible that the dormancy status of the seed influenced their germination response. Only KNO_3 treatment resulted in significant germination ($P<0.05$) (Table 3). This effect of KNO_3 on germination of black nightshade has not been previously reported.

Primary dormancy

In the present study the requirement for pre-germination chilling prior to exposure to alternating temperatures provides evidence of dormancy restraints on germination in black nightshade (Tables 1 & 2). Dormancy restraints on germination of fresh black nightshade seed were reported by Roberts & Lockett (1978). Givelberg et al. (1984) proposed that some genotypes of black nightshade may have primary dormancy. Alleviating dormancy by pre-germination chilling and subsequent testing for light requirements showed that the light

requirement was retained after dormancy alleviation by pre-germination chilling (Fig. 1). This may have implications for reduction of weed seed germination in species that have a light requirement for germination (Scopel et al. 1994).

Hairy nightshade apparently exhibited primary dormancy. Previously successful testing conditions (Roberts & Boddrell 1982) did not induce germination in our study. However, it appears that KNO_3 may alleviate this dormancy restraint (Table 3). Dormancy had not been reduced after 6 months storage at 5°C in our New Zealand seed. One month was previously reported as the duration of primary dormancy of hairy nightshade seeds when stored at room temperature (Roberts & Boddrell 1982). The 5°C storage in the present study may have prolonged dormancy in this species.

Primary dormancy in freshly collected black nightshade and hairy nightshade seed implies that the seed will not germinate immediately after shedding. Weed seed numbers are reported to decline markedly from autumn to spring due to natural processes in uncultivated fields (Rahman et al. 2001). Delayed or inhibited germination of fresh seed of these two weed species may support the use of alternative management strategies, such as direct drilling or fallow over the autumn/winter period, following crops where the management of these species has not been successful. This would allow for predation and decay of fruit and seeds at the soil surface.

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