

**An evaluation of *Solanum nigrum* and *S. physalifolium* biology
and management strategies to reduce nightshade fruit
contamination of process pea crops.**

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An evaluation of *Solanum nigrum* and *S. physalifolium* biology and management strategies to reduce nightshade fruit contamination of process pea crops.

By S.L. Bithell

The contamination of process pea (*Pisum sativum* L.) crops by the immature fruit of black nightshade (*Solanum nigrum* L.) and hairy nightshade (*S. physalifolium* Rusby var. *nitidibaccatum* (Bitter.) Edmonds) causes income losses to pea farmers in Canterbury, New Zealand. This thesis investigates the questions of whether seed dormancy, germination requirements, plant growth, reproductive phenology, or fruit growth of either nightshade species reveal specific management practices that could reduce the contamination of process peas by the fruit of these two weeds.

The seed dormancy status of these weeds indicated that both species are capable of germinating to high levels (> 90%) throughout the pea sowing season when tested at an optimum germination temperature of 20/30 °C (16/8 h). However, light was required at this temperature regime to obtain maximum germination of *S. nigrum*. The levels of germination in the dark at 20/30 °C and at 5/20 °C, and in light at 5/20 °C, and day to 50 % germination analyses indicated that this species cycled from non-dormancy to conditional dormancy throughout the period of investigation (July to December 2002). For *S. physalifolium*, light was not a germination requirement, and dormancy inhibited germination at 5/20 °C early in the pea sowing season (July and August). However, by October, 100% of the population was non-dormant at this test temperature. Two field trials showed that dark cultivation did not reduce the germination of either species.

Growth trials with *S. nigrum* and *S. physalifolium* indicated that *S. physalifolium*, in a non-competitive environment, accumulated dry matter at a faster rate than *S. nigrum*. However, when the two species were grown with peas there was no difference in dry matter accumulation. Investigation of the flowering phenology and fruit growth of both species showed that *S. physalifolium* flowered (509 °Cd, base temperature (T_b) 6 °C) approximately 120 °Cd prior to *S. nigrum* (633 °Cd). The fruit growth rate of *S. nigrum* (0.62 mm/d) was significantly faster than the growth rate of

S. physalifolium (0.36 mm/d). Because of the earlier flowering of *S. physalifolium* it was estimated that for seedlings of both species emerging on the same date that *S. physalifolium* could produce a fruit with a maximum diameter of 3 mm ~ 60 °Cd before *S. nigrum*.

Overlaps in flowering between peas and nightshade were examined in four pea cultivars, of varying time to maturity, sown on six dates. *Solanum physalifolium* had the potential to contaminate more pea crops than *S. nigrum*. In particular, late sown peas were more prone to nightshade contamination, especially late sowings using mid to long duration pea cultivars (777-839 °Cd, T_b 4.5 °C). This comparison was supported by factory data, which indicated that contamination of crops sown in October and November was more common than in crops sown in August and September. Also, cultivars sown in the later two months had an ~ 100 °Cd greater maturity value than cultivars sown in August and September. Nightshade flowering and pea maturity comparisons indicated that the use of the thermal time values for the flowering of *S. nigrum* and *S. physalifolium* can be used to calculate the necessary weed free period required from pea sowing in order to prevent the flowering of these species. The earlier flowering of *S. physalifolium* indicates that this species is more likely to contaminate pea crops than is *S. nigrum*. Therefore, extra attention may be required where this species is present in process pea crops. The prevention of the flowering of both species, by the maintenance of the appropriate weed free period following pea sowing or crop emergence, was identified as potentially, the most useful means of reducing nightshade contamination in peas.

Keywords: *Pisum sativum*, *Solanum nigrum*, *Solanum physalifolium* var. *nitidibaccatum*, weed, dormancy, germination, thermal time, flowering, fruit growth.

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List of abbreviations

BA	bud appearance
CI	contamination index
d ₅₀	days to 50 % germination
DAS	days after sowing
DM	dry matter
FR	far-red light
FE	field emergence
GA	green area
GAR	green area ratio
HI	harvest index
HSD	Honestly Significant Difference
LER	low energy response
LA	leaf area
LAR	leaf area ratio
PAR	photosynthetically active radiation
PFD	photon flux density
PLA	plant leaf area
PAR	photosynthetically active radiation
R	red light
SDLE	short duration light exposure
T _b	base temperature
T _m	maximum temperature
T _{opt}	optimum temperature
TTC	tetrazolium chloride
VLFR	very low fluence response

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Figure A7-3. The arcsine transformed percent germination of viable seed of two *S. nigrum* seed lots in light (open symbols) and dark (filled symbols) on eight test dates. Seeds were germinated at 20/30 °C (in light ○ and dark ●), 5/20 °C (in light □ and dark ■) for 14 d. (Experiment 2).

Figure A7-4. The percent germination of arcsine transformed viable seed of two *S. nigrum* seed lots from green fruit (SN7A, open symbols) and black fruit (SN7C, symbols with cross hair, +). Seeds were germinated at 20/30 °C (○), 5/20 °C (□) on eight dates for 14 d. (Experiment 2).

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Chapter 1 General introduction

1.1 The problem of nightshade contamination of process peas

This research addresses the problem of contamination of process pea (*Pisum sativum* L.) crops by the immature fruit of two species of nightshade; *Solanum nigrum* L. (black nightshade) and *S. physalifolium* Rusby var. *nitidibaccatum* (Bitter.) (hairy nightshade) in Canterbury, New Zealand. *Solanum nigrum* and *S. physalifolium* compete with and contaminate both conventional and organic (Anon, 2001) process pea crops. Contamination of the harvested peas by the immature nightshade fruit necessitates separation of nightshade fruit from peas after harvesting, a difficult process, because the nightshade fruit is a similar shape, colour and density to peas. Currently, no technology is available which can successfully separate peas and nightshade fruit that are of similar size. The removal of contaminants in process peas is required for producing of a high quality product (Cawood, 1987). Separation is also required as nightshade plant material, including fruit, contains toxic alkaloids (Brain and Turner, 1971). As an example, small (0–3 mm diameter) *S. nigrum* fruit are reported to have a solasodine concentration of ~ 0.08 % of dry weight (Eltayeb *et al.*, 1997). However, this concentration reduces dramatically as the fruit diameter increases.

Processors have a nil tolerance for nightshade fruit in peas for processing (A. White, Heinz Wattie's Ltd, pers. comm.). Growers' payments for contaminated loads of peas are reduced by at least 15 %. Deductions are greater if losses in crop weight following nightshade fruit removal exceed 15 %. In cases of severe contamination, up to 50 % of the pea crop can be lost (A. White, Heinz Wattie's Ltd, pers. comm.). This is because, typically, in a nightshade-contaminated load of peas, the nightshade fruit are smaller than the largest size-grades of peas. To remove the nightshade fruit from loads, the load is size graded through screens. This results in the loss of all peas smaller than 8.7–9 mm. This smallest size-grade is called 'baby peas'. This is a serious problem as these 'baby peas' are the most valuable fraction of the crop. In some cases, additional screening with a larger mesh size may be required due to the continued presence of nightshade fruits after the first screening.

Product losses can be considerable. For example, the cost to a New Zealand processing company from nightshade contamination, in terms of product losses, was \$79,000 (\$70,000 for organic peas, \$9,000 for conventional peas) in the 1999/2000 season for the Gisborne, New Zealand operation (A. White, Heinz Wattie's Ltd, pers.

comm.). In the South Island of New Zealand production losses for the processing company Heinz Wattie's Ltd from nightshade contamination were about \$10,000 in the 1999/2000 season. Apart from losses associated with product loss, nightshade contamination also results in increased processing costs in comparison to non-contaminated loads, due to the extra separation procedures required for contaminated loads. Nightshade contamination is not confined to pea growers and processors in New Zealand alone (Healy, 1974; Cawood, 1987). There are problems with nightshade contamination of process peas in the United States of America (Heider, 1996), England (Gane, 1972; Knott, 1986) and France (Geoffrion, 2000).

Organic production of pea crops is currently increasing in importance in New Zealand. There were approximately 300 ha of certified organic peas, valued (to farmers) at approximately \$600,000 gross margin, grown for Heinz Wattie's Ltd in the 2000/01 season, in New Zealand. The management of nightshade weeds in organic process pea crops is more difficult than in conventional crops. Currently available weed management practices, such as mechanical weeding, have a number of limitations, such as operations being restricted to periods during early pea growth stages, as later weeding negatively affects pea yields due to crop damage (Rasmussen, 1993). Additional and/or alternative methods, other than repeated mechanical weeding, are required to assist with the management of weeds in organic peas while maintaining optimum pea yields.

1.2 Justification

The research presented in this thesis seeks to address the lack of information on seasonal nightshade contamination trends in process peas in Canterbury, and examines a number of factors that may contribute to seasonal contamination by nightshade. This information is required to obtain a better understanding of the problem and to assist with the identification of possible causal factors that may contribute to seasonal nightshade contamination trends. Information is also required on the biological attributes of *S. nigrum* and *S. physalifolium* contributing to process pea contamination in order to evaluate potential management strategies. The contamination of process peas at harvest by nightshade fruit occurs by the co-occurrence of three factors. First, the nightshade emerges in the field at a time relative to the crop, that given suitable conditions, the nightshade may flower and initiate fruit growth prior to pea maturity. Secondly, some nightshade plants escape or survive weed control measures and, thirdly, some plants successfully flower and produce fruit prior to the pea harvest. Therefore, information is required that can address these three requirements and help ameliorate the negative effects.

Despite *S. nigrum* being reported as one of the most common weed species in the world (Holm *et al.*, 1977) there is a lack of information on aspects of its biology relevant to the evaluation of non-herbicide management practices. *Solanum physalifolium* has relatively recently naturalised in New Zealand (Healy, 1974), and there is little information on its field germination requirements. The determination of the potential effects of seed dormancy on seasonal germination and emergence requirements for both species, during the process pea growing season in Canterbury, are required. Dark cultivation has been reported to reduce the field germination of *S. nigrum* and *S. sarrachoides* Sendt. in the United States of America (Scopel *et al.*, 1994). However, this practice has not been evaluated for nightshades in New Zealand, nor has the importance of light as a germination requirement during the pea sowing season been demonstrated.

The flowering of nightshade is an aspect of development that is important to understand. For *S. physalifolium* there is no published information on the phenology of its development and, for *S. nigrum*, there is also no published information on fruit growth after flowering. Identifying the role of environmental factors influencing *S. nigrum* and *S. physalifolium* bud appearance and flowering, and measuring when these developmental stages occur, is important. As well, describing fruit growth after flowering may facilitate estimates of when nightshade fruit will reach a size that will contaminate a process pea crop. This information will allow better management of the risk of nightshade contamination.

For *S. nigrum* and *S. physalifolium* growth, the effect of competition with a pea crop on nightshade growth is not well described. Yet, it is reported that weed growth affects the volume of reproductive output (Thompson *et al.*, 1991). Thus, growth success may affect the volume of fruit produced by a plant; this information gap also needs to be addressed.

Sowing date alone can have a substantial effect on the competitive balance between a crop and its weeds, particularly where environmental conditions differentially affect crop and weed growth rates (Mohler, 2001a). Environmental factors can affect weed development and can determine how successfully the weeds compete with (Oliver, 1979), or contaminate, a crop. Surprisingly, there is little information on seasonal trends in nightshade contamination of process peas. Anecdotal accounts from processing field staff from Heinz Wattie's Ltd, operating in Canterbury, New Zealand indicate that the contamination of process peas by nightshade fruit is more common for crops that are processed in January than for earlier processed crops. However, there has been no formal analysis of seasonal trends in nightshade

contamination of process peas and there has been no analysis of the possible causes of variation in seasonal nightshade contamination.

Finally, although this work focuses on nightshade biology and factors that affect nightshade contamination in pea crops in Canterbury, New Zealand the information gained will also be of use to pea growers and processors outside Canterbury.

1.3 Research objectives

These are :

1. Analyse factory nightshade contamination records for seasonal trends in nightshade contamination and consider factors that may contribute to these trends.
2. Describe the dormancy cycle and the field germination requirements of *S. nigrum* and *S. physalifolium* during the process pea growing season in Canterbury, New Zealand.
3. Describe the growth and dry matter accumulation of *S. nigrum* and *S. physalifolium* when grown with and without process peas.
4. Quantify the phenology in relation to bud appearance, and flowering and fruit growth of *S. nigrum* and *S. physalifolium*.
5. Develop recommendations for growers and processors about the management of *S. nigrum* and *S. physalifolium* in process pea crops.

Chapter 2 : Literature review

2.1 Introduction

This literature review provides a critical review of the relevant literature concerning the management of weeds in process pea crops, with particular attention to the management of weeds in organic pea crops. The practice of, and factors affecting, the success of dark cultivation to reduce weed germination and emergence, as a possible additional weed management tool in organic agriculture, are discussed. The literature on the dormancy, germination, emergence, growth, flowering phenology and fruit growth of *Solanum nigrum* and *S. physalifolium* is critically reviewed.

2.2 Production of process peas

In 2000, \$NZ 40.6 million (60,000 tonnes) of process peas were exported from New Zealand (Kerr and Aitken, 2000). They were the highest earning processed vegetable crop exported from New Zealand.

The volume of pea production in a region is determined by a number of factors including throughput processing capacity, area grown, mean yield of shelled peas per cultivar and the length of season suitable for process pea production (Ottoson, 1973). If the processing capacity of a factory is constant, there are limited options for maximising or increasing the volume of peas produced in a region. Extension to the season by late sowings is not a viable option due to the relative yield reductions from late sowings (Hardwick *et al.*, 1979). However, the season can be extended at the start, by sowing cultivars with a lower thermal time requirement early in the growing season, relative to the thermal time requirements of cultivars sown mid and late season (Cawood, 1987). Other factors, such as soil type, can also affect planning (Ottoson, 1973). In Canterbury, light soils are sown first and heavy soils are, generally, sown later in the season. Sowing starts in August and usually ceases in late November.

2.2.1 Production of organic process peas

Process peas grown in New Zealand for the organic market use large sieve size varieties to assist with potential screening to remove nightshade fruit (A. White, Heinz Wattie's Ltd, pers. comm.). Organic pea processors in the United States of America do

not use large sieve size peas due to customer requirements, and so are restricted to late season sowing to avoid nightshade contamination (A. McErlich, Small Planet Foods, Washington, United States of America., pers. comm.). Processors in the United States of America are moving towards the use of *afila* (leafless) cultivars. However, these have reduced the period within which tine weeding can be carried out due to their interlocking tendrils (A. McErlich, Small Planet Foods, Washington, United States of America., pers. comm.).

Pea cultivars used in Canterbury for the organic market are leafy type varieties. Sowing rates are 330 to 350 kg/ha, with a drilling rate aimed at giving a field emergence (FE) of 120 plants/m². This is 10 plants/m² higher than the FE for conventional peas to compensate for plant losses during tine weeding and to create a dense canopy. CEDENCO, a processing company in Gisborne, New Zealand, has increased sowing rates of organic peas even higher and aims at an FE of 135 plants/m² in an effort to make the crop more competitive with weeds (A. Holmes, CEDENCO, pers. comm.). Process pea density trials indicate that pea yield plateaued at 140 plants/m², in one year of trials and 180 plants/m² in the second year of trials (Lawson, 1982; Lawson and Topham, 1985). For weed competition effects, even the presence of low weed biomass at high pea densities (194 plants/m²) negatively impacted on pea yield relative to weed free plots (Lawson, 1982; Lawson and Topham, 1985). The authors reported that weed management practices were still required to avoid yield losses even at high pea densities.

Rolling of pea paddocks to assist harvesting with a pea viner is advocated either at pre-emergence or at a crop height of 50-100 mm (A. White, Heinz Wattie's Ltd, pers. comm.). Growers in Canterbury generally use 170 mm row spacings (A. White, Heinz Wattie's Ltd, pers. comm.).

2.2.2 Contamination of process peas

Weed management, in both conventional and organic crops, is usually directed at minimising the competitive effect/interaction that weeds have on the crop being grown. The competitive production principle is defined as the situation when one species has an effect on the environment, which causes a negative response in the other species (Radosevich *et al.*, 1997). In some cases, however, the yield of the crop can be compromised not through competitive interactions with weeds but by weeds indirectly reducing crop yield through contamination. With nightshade fruit the problem is the mixing of a contaminant that is similar to the harvested crop (Knott, 1986). Isolation of the contaminant increases processing requirements and leads to product loss.

Other contaminants of process peas crops can also mimic small green peas, such as the flower buds of *Cirsium arvense* L. (Scop.) and *Matricaria chamomilla* L., and fruit from volunteer *Solanum tuberosum* L. plants (Gane, 1972; Knott, 1993). However, green nightshade fruit are the most technically difficult to isolate (A. White, Heinz Wattie's Ltd, pers. comm.). Surprisingly, this problem has received little research attention, with one report studying nightshade *Solanum ptycanthum* Dun. (eastern black nightshade) growth in peas. Unfortunately, this study focused on yield reduction caused by competition rather than contamination (Croster and Masiunas, 1998).

Only one study directly addresses the problem of nightshade contamination in processing crops. The study was of *S. ptycanthum* and *S. sarrachoides*' thermal time requirements for bud initiation, flowering and fruit growth, to 0.5 mm, from the dicotyledonary stage (Heider, 1996). This study quantified the thermal time requirements for each species with and without competition from a mid season canning pea (cv. Rally, 738 °Cd maturity, T_b 4.44 °C) and a late season freezing pea (cv. Dual, 821 °Cd maturity, T_b 4.44 °C), each at two sites. One was irrigated on light sandy soils and one was unirrigated on a heavy soil. A repeat year of trials in 1995 followed those in 1994. For a single planting of each crop, nightshade seedlings were transplanted at weekly intervals into the crops or no competition plots. Relevant findings were: that only nightshades transplanted in the first three weeks from pea sowing developed 0.5 mm fruit; the nightshade species differed significantly and consistently in rates of development with *S. sarrachoides* having lower thermal time requirements for bud appearance, flowering and 0.5 mm fruit growth than *S. ptycanthum*. The presence or absence of pea competition had no significant effect on the thermal time requirements for bud appearance or flowering. From this data regression models were developed to predict nightshade development in pea crops. However, these models were noted by Heider (1996) to be limited in usefulness for growers or processors as they were dependant on highly specific information. This work provides useful information but does not include the nightshade species that are of concern in Canterbury, although *S. ptycanthum* is reported to have similar thermal time requirements for flowering as that of *S. nigrum* (McGiffen and Masiunas, 1992). The effect of seasonal planting dates of peas on nightshade development is also not addressed by this study, nor is the relevance of 0.5 mm nightshade fruit as a threshold contamination identified.

Some work on another contaminant species has been carried out. El Titi (1986) reported *Matricaria chamomilla* buds as a problem process pea contaminant in a study of conventional growers fields in Germany, and identified that *M. chamomilla* seedlings, established at a pea growth stage of 100 mm, had a mean of 28-50 buds/plant at pea maturity. *Matricaria chamomilla* seedlings that established one week later had a mean of only 5 buds/plant at pea maturity. This indicates that, in the case of *M. chamomilla*

contamination of process peas, the timing of weed establishment relative to the crop is an important factor influencing the potential contaminant level at harvest.

El Titi (1986) also reported that *Solanum nigrum* was not a problem contaminant in his study as flowering and fruiting of *S. nigrum* did not coincide with pea maturity. The reason for this was that the sowing date of these crops (mid March to end of May) largely preceded the seasonal emergence (late spring/summer) of *S. nigrum*. Elliot (1972) and Knott (1986) also identified the late seasonal emergence of *S. nigrum* in England as a factor, which may reduce the weed's potential to contaminate late sown versus early sown peas. An organic pea processor in the United States has ceased late season pea sowing because of the nightshade contamination problems of late season pea harvests (A. McErlich, Small Planet Foods, Washington, U.S.A., pers. comm.). A study of *S. ptycanthum* competition (5 nightshade plants/m²) with peas noted that the degree of pea yield reduction appeared to be linked to variations in mean temperatures between trial years, and to within season effects where high temperatures (average 23.9 °C over a two week period) favoured *S. ptycanthum* growth (Croster and Masiunas, 1998). Nightshades with a base temperature (T_b) of 6 °C have higher T_b s than those reported for most process pea cultivars (Alm *et al.*, 1988; Oliver and Annandale, 1998). If the flower and fruit development of *S. nigrum* and *S. physalifolium* respond to temperature in a similar manner to *S. ptycanthum* growth, then nightshade contamination problems in peas may be expected for peas grown in years with above average temperatures, or for sowing dates within a season that experience the highest average temperatures.

These reports raise several questions regarding nightshade contamination in New Zealand. Is there a seasonal emergence pattern in New Zealand that contributes to the potential for nightshade contamination? Are other factors involved, such as the differing pea cultivar maturity thermal time requirements of early and late sown peas, and/or the possible effects of differences in mean temperatures for early (e.g. August) and late (e.g. November/December) sown peas, affecting the potential for nightshade contamination? Finally, as with the case of *S. ptycanthum*, *S. sarrachoides* and *Matricaria chamomilla* seedlings, can a critical nightshade establishment period relative to the crop be identified, which also integrates sowing date and pea cultivar effects?

2.3 Weed management in process peas

2.3.1 Organic weed management

Organic production systems have a varied range of weed management methods and strategies (Parish, 1990), but in comparison to conventional production systems the number of curative rather than preventative management methods available is limited, due to the prohibition on the use of herbicides (Lampkin, 1990).

Weed management methods fall into three broad categories (Radosevich *et al.*, 1997). First, chemical control using herbicides and growth regulators. Secondly, biological control procedures that use microorganisms, invertebrates and vertebrates to damage weeds. Thirdly, cultural control procedures that manipulate cropping conditions to reduce the density and competitiveness of the weeds. This includes crop rotation, tillage practices, mechanical weeding, residue management for weed suppression, choice of competitive cultivars, and the density and arrangement of crop plants (Mohler, 1996). Organic weed control in broad acre crops currently relies, almost exclusively, on the last category, cultural methods. Multiple, or combinations of, methods are often required to achieve the best outcome. For example, suitable crop rotations must be planned, the timing of weeding operations should coincide with conditions that should desiccate weed seedlings. Particularly competitive cultivars may also be selected to assist with weed management (Radosevich *et al.*, 1997).

2.3.2 Recommended weed management in organic pea crops

The following are the weed management recommendations for organic pea growers in Canterbury, (A. White, Heinz Wattie's Ltd, pers. comm.). Growers are advised to: select fields for peas immediately after the pasture phase of their rotation; avoid fields with a high weed pressure; use stale seedbeds prior to sowing; and use pre-emergence tine weeding post sowing. Post-emergence tine weeding should then be carried out when the peas are established (~ 2.5-leaf stage) to reduce damage to the peas, and on hot dry days. Tine weeding should not be used after the crop has passed the 5-leaf stage.

2.3.3 Weed research relevant to organic pea production

Information on the competitive effects of weeds on process peas yields is somewhat mixed. Studies using natural field weed populations have reported that weeds cause yield reductions compared to weed free plots (El Titi, 1986; Croster and Masiunas, 1998). But for comparisons of natural weed populations at a range of densities, weed populations of < 80-100 plants/m² had no effect on yield relative to weed free or herbicide treated plots (El Titi, 1986). A comparison using sown *Brassica hirta* Moench at a range of densities (32-97 plants/m²) and emergence dates in un-weeded peas, reports that early emergence had a more significant effect on yield than did late emergence or weed density (Nelson and Nylund, 1962). The early emerging weeds had greater negative effects on pod number, leaf area and pea yields than an increase in weeds from 32 to 97 plants/m². This indicates that reports of weed and pea competition that do not describe the timing of weed emergence relative to the crop such as El Titi (1986) and Reddix *et al.*(2001) may incorrectly interpret the effects of density or weed biomass on pea yield.

In addition, to reducing the competitive effects of weeds, there are additional reasons to manage weeds in process peas. First, there is the risk of contaminants at harvest (Gane, 1972) and secondly, there are advantages to minimising weed seed inputs to the soil weed seed bank (Jordan *et al.*, 1995; Jordan, 1996). This is especially important as there is evidence that weed seed populations can increase under organic management. For example, after three years of organic management an increase in weed seed bank populations from 4,050 to 17,320 seeds/m² was reported in comparison to a conventionally managed trial (on the same site), where weed seed populations increased from 3,270 to 6,480 seeds/m² (Albrecht and Sommer, 1998). Therefore, weed management in process peas, as part of a total farm weed seed bank management strategy (Dekker, 1999), is required to limit weed seed increases and potential problems for later crops.

Agricultural soils hold large populations of weed seeds: for example, estimates of weed seed numbers in 15 *Zea mays* L. fields in the North Island of New Zealand ranged from 3,672 to 248,268 seeds/m² (Rahman *et al.*, 1997). Weed seed burdens can be considerably depleted: for example, monthly cultivations (to 100 mm) over four years reduced seed numbers to 1 to 2 % of the original number (Rahman *et al.*, 1998), and cultivation induced death of weed seeds may have important effects on long term weed seed populations (Jordan *et al.*, 1995). However, continual cultivation is not a practical or desirable method of crop production.

Research on the effects of crop rotation on weed seed banks under organic management indicates that the rotation sequence and choice of crops has a substantial

effect on weed seed accumulation in the soil and that weeds have species-specific responses to different crop rotations (Jordan *et al.*, 1995). Information is limited as to the effect of crop rotations of the type practised by Canterbury pea growers on nightshade weed populations. However, intensive *Lycopersicon esculentum* Mill. cropping with nil or minimal crop rotation is associated with increased nightshade weed populations in the United States of America (Lange *et al.*, 1986).

Herbicide resistance in nightshade weeds is a problem internationally (Lange *et al.*, 1986; Kremer and Kropff, 1999). A herbicide resistant (cyanazine, terbutylazine, atrazine and prometryn) *S. nigrum* population has also been reported in the Manawatu, New Zealand (Harrington *et al.*, 2001). Apart from the independent development of herbicide resistance, the dispersal of seed by birds has been identified as a possible factor in the spread of herbicide resistant *S. nigrum* populations between some regions in Europe (Stankiewicz *et al.*, 2001). These reports indicate the potential for this problem to establish independently, or to be spread to other regions in New Zealand.

The management of herbicide resistant weed populations often relies on cultural management methods in conjunction with specific herbicide strategies (Gorrdard *et al.*, 1996; Chauvel *et al.*, 2001). The requirement for successful cultural management strategies has led to research on the biology of resistant or susceptible populations of *S. nigrum* (Kremer and Lotz, 1998a; Kremer and Kropff, 1998c). This research contributes information relevant to organic and conventional management systems.

2.3.4 Germination reduction strategies

Cultivation during field preparation and after stale seedbed treatments can destroy newly germinated weed seedlings and weed seeds (Jordan *et al.*, 1995). However, the efficiency of such methods, particularly for final seedbed preparation prior to sowing of a crop, is questionable. Cultivation can further stimulate or expose weed seeds from the seed bank in the emergence zone, and contribute to additional weed germination and emergence in the establishing crop. For example, in over three years of trials for comparisons of beds, either rough harrowed (1), harrowing followed by raking (2), and harrowing followed by shallow rotary cultivation (3), the number of weeds for each treatment increased progressively, with, on average, 25 more weed seedlings/m² for treatment 2 in comparison to 1, and, on average, 80 more weed seedlings/m² for treatment 3 in comparison to 2 (Roberts and Hewson, 1971). An additional rolling of each of the above treatments provided an additional 29 % increase in weed seedling emergence compared with not rolling (Roberts and Hewson, 1971). This work

indicated that the finer and firmer a seedbed was prepared the greater the weed emergence. In relation to weed management objectives, the stimulation of the germination and emergence of weeds by cultivation is possibly only an advantage where seed bank depletion strategies (Dekker, 1999) are desired, such as with stale seedbeds (Johnson and Mullinix, 1995).

This disadvantage has led to research to reduce the effects of cultivation on the germination of weed seeds that lie within the emergence zone of the soil surface at particular times, such as just prior to, and at, the sowing of a crop. One possible method is the use of alleopathic cover crops to reduce weed seed germination. For example, incorporating residues of *Brassica napus* L, *Secale cereale* L. or *Triticum aestivum* L. before sowing peas and tine weeding after pea emergence reduced weeds to densities at 30 days after sowing (DAS) that did not differ significantly from a herbicide (Metribuzin) treatment (Al Khatib *et al.*, 1997). However, pea populations were significantly reduced by the *Secale cereale* or *Triticum aestivum* incorporation, and the pea yield for the three green manure incorporation treatments were all significantly less than for the respective herbicide treatment.

2.4 Dark cultivation

One cultivation based weed germination reduction strategy is that of dark cultivation. This practice has been referred to as night cultivation, but work has demonstrated that the use of lightproof covers over cultivation equipment during daylight cultivation can also reduce weed seed germination, in some species (Scopel *et al.*, 1994). This is because exposure to light can enhance germination in some plant species. Such species are defined as being positively photoblastic (Wessen and Wareing, 1969; Frankland and Taylorson, 1983; Milberg *et al.*, 2000). Cultivation in the dark can reduce the total weed seedling emergence and slow the germination rate of some light sensitive species relative to light cultivated soil (Scopel *et al.*, 1994; Jensen, 1995; Botto *et al.*, 1998). However, reductions in the emergence of light sensitive weed species through dark cultivation have proved variable among seasons and trial sites (Scopel *et al.*, 1994; Buhler, 1997; Botto *et al.*, 1998).

2.4.1 Theory of dark cultivation

The use of dark cultivation is based on the assumption that buried, positively photoblastic seeds, receive a brief exposure to light during soil cultivation before

reburial; and that this exposure provides an adequate stimulus for germination. For example, calculated laboratory threshold exposure times to induce germination of *Cerastium fontanum* Baumg and *Silene noctiflora* L. are 1 ms and for *Rumex obtusifolius* L. 0.5 s, for 1,000 $\mu\text{mole}/\text{m}^2/\text{s}$ (with a red to far-red ratio (R:FR) ratio of 0.85) of light reaching the soil surface under cultivation equipment (Milberg, 1997). This light exposure during cultivation results in a significantly greater proportion of seedlings emerging from depth. For example, in a trial area which was predominately *Stellaria media* Vill. significantly greater numbers of seedlings emerged from > 6 mm depth after light cultivation compared to dark cultivated plots (Jensen, 1995). For non-photoblastic species, or for positively photoblastic seeds left on the soil surface, or positively photoblastic seeds receiving light that penetrates the soil surface (~ 4 mm depending on soil type (Benvenuti, 1995), no differential response to light treatments during cultivation may be expected.

The germination responses of seeds to light have been classified through laboratory based testing. A full description of seed pre-treatment conditions is important as a seed's light requirement is often dependant on particular seed pre-treatments (Van der Woude, 1989; Pons, 1992). Species that germinate in response to a short duration light exposure (SDLE) (Milberg *et al.*, 1996; Milberg *et al.*, 2000) are classified as having a low energy response (LER) (Frankland and Taylorson, 1983; Pons, 1992). For seeds stimulated by red (R) light, a degree of inhibition can be demonstrated by subsequent exposure to far-red (FR) light (Schafer, 1976). This occurs where the requirement for germination is a short duration light exposures (<1 hour), at an appropriate wavelength (light quality), but is largely independent of photon flux density (PFD) above a minimum threshold value (Pons, 1992). An example of this type of response is: in *R. obtusifolius* after a pre-treatment of 25 °C for 24 h and germination for 3 d at 25 °C in the dark, total germination was 17.5 %. However, after a R light exposure (660 nm, 3.0 $\mu\text{mole}/\text{m}^2/\text{s}$ for 10 minutes) germination was 96.8 % (Kendrick and Heeringa, 1986). Thus a total PFD of 1800 $\mu\text{mole}/\text{m}^2$ provided a strong germination response. Further testing at lower PFD's established that a 50 % germination response only required 11 $\mu\text{mole}/\text{m}^2$ (Kendrick and Heeringa, 1986). Testing of weed seeds stored in the field then retrieved and exposed to a light source (210 $\mu\text{mole}/\text{m}^2/\text{s}$ for five seconds, R:FR 0.85), indicated that 24 out of 44 species had significantly enhanced germination following light exposure (Milberg *et al.*, 1996). This response was shown equally by summer annual, winter annual, and perennial species (Milberg *et al.*, 1996).

A secondary type of LER is also reported, which is distinguished as a very low fluence response (VLFR). Germination responses occur at PFD's of < 1 $\mu\text{mole}/\text{m}^2$, and germination responses can be elicited from R, FR and green light for species normally

only promoted by R light (Kendrick and Heeringa, 1986; Gallagher and Cardina, 1998b; Gallagher and Cardina, 1998c; Hartman and Mollwo, 2002). This response has been demonstrated in laboratory stored *Amaranthus retroflexus* L. seed (Gallagher and Cardina, 1998c). This VLFR mechanism is proposed to act as a germination mechanism for seed in the field (Gallagher and Cardina, 1998b; Gallagher and Cardina, 1998c; Hartman and Mollwo, 2002).

2.4.2 Evaluations of dark cultivation

A number of field trials have been undertaken that evaluated the effectiveness of dark cultivation (Scopel *et al.*, 1994; Jensen, 1995; Buhler *et al.*, 1997; Botto *et al.*, 1998; Gallagher and Cardina, 1998d; Fogelberg, 1999; Botto *et al.*, 2000). Some general findings can be drawn from these studies.

The emergence of particular weed species can be reduced by dark cultivation (Jensen, 1995; Botto *et al.*, 1998) including *S. nigrum* (Scopel *et al.*, 1994). However, success rates were mixed, with light cultivations carried out at the same site in late winter, late spring and late summer, providing weed seedling counts relative to the night time control of 0, 0.8, and 2.0 respectively (Botto *et al.*, 1998). While the weed species composition at the site changed with season it was apparent that the individual species response differed with the season. For eight weed species with seeds field stored and retrieved monthly, laboratory testing of light (40 $\mu\text{mole}/\text{m}^2/\text{s}$ for 12 h a day during germination), SDLE (210 $\mu\text{mole}/\text{m}^2/\text{s}$ for five seconds, R:FR 0.85) and dark germination responses indicated seasonal variations in light and SDLE responses, and some seasonal variability in dark responses (Milberg and Andersson, 1997). Such seasonal variability may be linked to annual changes in weed seed dormancy status (Vleeshouwers *et al.*, 1995; Baskin and Baskin, 1998). This would appear to provide an explanation of seasonal variation in dark cultivation responses.

However, the literature on dark cultivation indicates further temporal variability in response. For example, a dark cultivation in mid May resulted in no significant reduction in emergence of *S. ptycanthum* compared to cultivation in the light. With cultivation in late May, at the same site, there was a significant reduction in emergence of ~ 50 % for dark cultivation (Buhler, 1997). Gallagher and Cardina (1998d) also reported variability of success between trials in short succession. Such results are not consistent with the, generally, gradual annual cyclic light responses reported by Milberg and Andersson (1997). This may indicate that additional factors other than season may also affect a species germination response to light. There are a number of possible factors such as seed origin, mother plant, soil nitrate level, temperature/burial

depth and soil moisture effects in the literature, which may alter the light responses in positively photoblastic species.

The origin and individual mother plant source of the seed may be important pre-seed bank incorporation factors that influence the light sensitivity of seed. An evaluation of seed collected from three separate populations after pre-treatment at 3 °C for 18 weeks for 25 species, reported that 21 of the species had significant differences in responses to light (10 $\mu\text{mole}/\text{m}^2/\text{s}$ for 14 h/day during germination), SDLE (same methods as Milberg and Anderson (1997)), or dark treatments (Milberg *et al.*, 1996). For example, in one population of *Rumex longifolius* DC. 53 % of seed germinated in response to SDLE, while the other two populations had 5.5-6.1 % germination response to SDLE. The cause of this variability may be differing environmental factors between environments affecting seed development and maturation. This is known to influence germination requirements (Cresswell and Grime, 1981; Wulff, 1995). For example, seeds of *Chenopodium album* L. low in endogenous nitrate are more dormant but more responsive to exogenous nitrate than seeds with high endogenous nitrate levels (Saini *et al.*, 1986). It is possible that different cohorts of weeds in a season produce different quality seed with differing responses to germination stimuli. Even comparisons of seed from individual plants ($n = 8$) in a population ($n = 8$), for three species (*Sinapis arvensis* L., *Spergula arvensis* L., and *Thlaspi arvense* L.) showed intraspecific variability in dormancy (Milberg and Andersson, 1998a). In addition, there was a significant plant by stratification interaction and a population by stratification interaction. This indicated differential responses to seed pre-treatments among both plants and populations. Seed pre-treatments such as stratification have a strong influence on a seed's response to light (Van der Woude, 1989; Pons, 1992). Germination responses to pre-treatments can differ for seed from different plants (Milberg and Andersson, 1998a) and populations (Milberg and Andersson, 1998b), indicating that these factors may contribute to variable responses to dark cultivation for both within site and among site comparisons.

Dark cultivation may, solely, control the light that a seed may receive during cultivation, but other factors (often acting in conjunction) within the soil environment also affect seed responses to germination stimuli, including light. One such factor is soil nitrate, which, for example, stimulates *Chenopodium album* germination most when the seed is least dormant (Bouwmeester and Karssen, 1993a). However, near maximum germination responses are obtained when KNO_3 (50 mM) and R light (30 minute R irradiations) act in conjunction (Bouwmeester and Karssen, 1989; Bouwmeester and Karssen, 1993a). The action of light and nitrate providing near maximum germination responses are common in a number of weed species (Roberts and Benjamin, 1979; Karssen and Hilhorst, 1992). Analysis of the effects of three

nitrate levels (0, 0.01, 0.1 mol/l) prior to pre-treatment at 3 °C for 10 d, and subsequent, exposure to a range of PFD's (0, 0.4, 400, 4000, and 40,000 $\mu\text{mole}/\text{m}^2$) for four weed species resulted in significant interactions between light and nitrate for three species (Milberg, 1997). This indicated that germination responses for the three species in the presence of nitrate could not be predicted solely from PFD exposure. From these results Milberg (1997) proposed that soil nitrate level can modify seed responses to light and may require consideration in dark cultivation trials. However, how soil nitrate levels relate to field germination is complex. The rate of nitrate mineralization depends on soil temperature and soil moisture levels. In addition, there are a number of influential site effects: soil type; soil pH; soil depth; soil disturbance; plant uptake; and microbial communities (Karssen and Hilhorst, 1992; Ritz *et al.*, 1994).

Soil temperature and soil moisture affect nitrate mineralization, these factors are also reported to affect seed light sensitivity. The soil temperature or the storage temperature of imbibed seeds, stored in the laboratory, affects seed dormancy status (Vleeshouwers *et al.*, 1995; Martinez Ghera *et al.*, 1997). Generally, increases in soil temperature during early spring to mid summer are positively linked to increased germination capacity for summer annuals, until the induction of secondary dormancy (Bouwmeester and Karssen, 1989). Gallagher and Cardina (1998c) reported significant temperature (20 or 30 °C) by light (0 or 300 $\mu\text{mole}/\text{m}^2$ R light) interactions for buried seed of *Amaranthus hybridus* L., retrieved on 10 dates over two years. Temperature had a strong effect on the proportion of seed germinating at 0 $\mu\text{mole}/\text{m}^2$ R light. For example, at 20 °C only 30 % of seed germinated in the dark, while at 30 °C 70 % of seed germinated. These results indicate a temperature dependant light requirement. Gallagher and Cardina (1998c) proposed that such responses may function in the field as a depth perception mechanism; that seed in warmer soils near the soil surface had a low proportion of seed requiring light, and that the seed deeper in soil experiencing lower temperatures does have a light requirement. This mechanism would also assist seed persistence. This links with observations that cultivation equipment that inverts the soil such as mouldboard ploughing in daylight, generally, promoted greater relative weed emergence than night time ploughing (Scopel *et al.*, 1994; Botto *et al.*, 1998).

Soil moisture is another environmental factor that may affect seed light responses, as reduced soil moisture has implications for nitrate mobility. Daylight tillage consistently gave higher *Chenopodium album* germination if the soil moisture was maintained at > -0.5 MPa (Botto *et al.*, 2000). The proposed cause of these soil moisture effects on seed light requirements is that dark germination was less sensitive to decreased soil moisture than that of light-induced seed germination.

2.5 The target weeds

2.5.1 Taxonomy of the target weeds

There is uncertainty over the taxonomic identity of the target weed species in this study, especially in different parts of the world (Edmonds and Chweya, 1997) (Table 2-1). The species named *Solanum sarrachoides* was, in 1986, identified as two distinct species, with the name *S. sarrachoides* remaining and a new species *S. physalifolium* named (Edmonds, 1986). The species, previously known as *S. sarrachoides* in New Zealand, was identified as *S. physalifolium*, and only this species is reported as present in New Zealand (Edmonds, 1986; Webb *et al.*, 1988).

Table 2-1. Latin and common names of three *Solanum* nightshade species.

Latin name	Common name
<i>Solanum nigrum</i>	black nightshade
<i>Solanum sarrachoides</i>	hairy nightshade
<i>Solanum physalifolium</i>	hairy nightshade

Differentiation between *S. sarrachoides* and *S. physalifolium* is important in terms of their biology. For example, *S. sarrachoides* can develop into an erect bushy plant, is frost resistant and potentially an annual but will perennate (Edmonds, 1986). In contrast, *S. physalifolium* is strictly annual, with a prostrate spreading growth habit (Edmonds, 1986). There is the possibility that other biological attributes may also differ significantly.

Most descriptions of *S. sarrachoides* in the literature, particularly those discussing this species in Australia, New Zealand and North America, conform to those for *S. physalifolium* (Edmonds and Chweya, 1997). Edmonds and Chweya (1997) specifically refer to the descriptions of Healy (1974), Henderson (1974), Ogg *et al.* (1981), Schilling (1981) and Symon (1981) as conforming to the morphology of *S. physalifolium*. Accordingly, references to *S. sarrachoides* in later literature that refer to the above authorities for identification, will be treated as *S. physalifolium*.

Solanum sarrachoides is reported to have a sporadic distribution in North America, and *S. physalifolium* is more common especially in the Plains and Pacific States and adjacent Canadian provinces (Edmonds, 1986; Edmonds and Chweya, 1997). However, since the identification of *S. physalifolium* in 1986, no North American based study refers to *S. physalifolium*, but *S. sarrachoides* is regularly cited (Hermanutz and Weaver, 1991; Scopel *et al.*, 1994; Boydston and Hang, 1995; Heider, 1996; Forcella *et al.*, 1997). In other regions of the world such as Australia, where prior

to 1986 *S. sarrachoides* was recognised, now only report the presence of *S. physalifolium* (Lazarides *et al.*, 1997). Or, in other regions such as Spain, the existence of both *S. physalifolium* and *S. sarrachoides* is reported (Sobrinho and del Monte, 1994) and the particular species used in studies from this region is indicated (del Monte and Tarquis, 1997). There is a distinct possibility that some post 1986 North American based studies were actually made on *S. physalifolium* rather than *S. sarrachoides*, due to identification problems (Edmonds, 1986; Edmonds and Chweya, 1997). Because the identification of references to *S. sarrachoides*, especially from North America, is not certain, some references to the literature on *S. sarrachoides* will be made for two reasons. First, due to taxonomic confusion the species described could be *S. physalifolium* and, secondly *S. sarrachoides* and *S. physalifolium* may behave in the same way under some circumstances.

2.5.2 *Solanum nigrum* biology and ecology

Solanum nigrum is a spring and summer annual weed with a worldwide distribution (Edmonds and Chweya, 1997). This section of the review will concentrate on the literature about *S. nigrum*'s seed biology, plant growth, and flower and fruit development.

Germination requirements - temperature

Laboratory temperature requirements: There are contrasting results reported for the importance of constant versus alternating temperatures for the germination of laboratory-stored seed. Germination at constant temperatures was reported as successful in studies carried out in Israel, Spain, Kenya and Italy (Givelberg *et al.*, 1984; Agong, 1993; Benvenuti and Macchia, 1993; del Monte and Tarquis, 1997). However, work in the United Kingdom, the Netherlands, Sweden and New Zealand reported poor (< 10 %) or no germination at constant temperatures (Roberts and Lockett, 1978; Wagenvoort and Opstal, 1979; Teketay, 1998; Kremer and Lotz, 1998a; Bithell *et al.*, 2002) (Appendix 1). For example, Roberts and Lockett (1978) reported that at constant temperatures in the range 4-30 °C there was no germination of freshly harvested *S. nigrum* seed from five years of collections, or after 18 months storage of dry seed, or for seed stored in moist sand for 15 weeks (Roberts and Lockett, 1978). Givelberg *et al.* (1984) suggested that the reported differences in germinability with regard to alternating or constant temperature treatment differences may be due to some accessions of freshly harvested *S. nigrum* having primary dormancy.

Alternating temperatures are reported to increase germination for *S. nigrum* accessions that germinate poorly under constant temperatures (Bithell *et al.*, 2002) (Appendix 1). For example, comparisons of constant 20 °C and alternating temperature treatments of 20/12, 30/12 (12/12 h) gave 0 % germination at 20 °C but 100 % germination at 20/12 °C and 30/12 °C (Teketay, 1998). Stratification (5 °C for 0-6 weeks) of some *S. nigrum* accessions prior to testing at alternating temperatures of 9/25 °C (16/8 h) gave optimal germination responses. The germination rate also responded positively to increased stratification period (Wagenvoort and Opstal, 1979).

The minimum germination temperature has been examined in two studies of laboratory-stored seed, with a base temperature (T_b) of 8.4 °C reported for *S. nigrum* (Benvenuti and Macchia, 1993). A comparison of *S. nigrum* accessions from three regions (cool/tropical, semi-hot/subtropical, hot temperate) in Spain reported respective T_b values of 7.5, 7.6, and 10 °C, and respective optimal temperatures (T_{opt}) of 30, 20-25, and 25 °C (del Monte and Tarquis, 1997). The maximum temperature (T_m) at which the germination rate reached zero ranged from ~37-43 °C. The results of Givelberg *et al.* (1984) broadly support this T_m range, with germination occurring at temperatures over 35 °C, and only some seed germinating at 40 °C.

Laboratory testing of field stored seed: Most of the literature regarding germination temperature requirements is based on the use of laboratory-stored seed. The temperature requirements of seed stored in the field prior to laboratory testing may be more relevant to understanding the germination temperature requirements of *S. nigrum* seed in the soil seed bank. This seed is exposed to natural conditions of soil temperature fluctuations, soil chemicals and moisture. Two studies reported the temperature requirements of field-stored seed. The first for seed buried one and two years in England reported a strict alternating temperature requirement. Seed was tested from April to March, and a seasonal depression in germinability occurred from August to December each year for seed tested at 15/25 and 10/25 °C (16/8 h) (Roberts and Lockett, 1978). Testing this seed at 15/30 and 10/30 °C produced minor germinability depression in August. In all other months at all four test temperatures germinability approached 100 %.

A second study in the Netherlands reported positive responses to constant temperatures (5-30 °C) following seed retrieval, but with differing minimum temperature requirements at the different seed retrieval dates (Kremer and Lotz, 1998a). For example, for a triazine-susceptible accession (Achterberg) the lowest temperature germination was observed at 20, 15 and 10 °C for the February, March and May retrievals, respectively. This indicated seasonal dormancy temperature thresholds, which were consistent with a summer annual type two response pattern, where, with an

additional loss of dormancy, the minimum temperature at which the seed will germinate declines (Baskin and Baskin, 1998). Germination in May at 10 °C corresponded with soil temperatures (50 mm depth) at the burial site reaching 15 °C (Kremer and Lotz, 1998a). In this study germination percentage at 30 °C was lower than at 25 °C for all four accessions, and retrieval dates. But germination rates were consistently higher at 30 °C. This indicated rapid germination of a possible non-dormant portion of the population and the possible induction of dormancy in the remainder of the population. These results differ from studies with laboratory-stored seed at, comparable, constant temperatures (Givelberg *et al.*, 1984).

Germination requirements - light

Laboratory studies: *Solanum nigrum* seed exhibits positively photoblastic light responses (Roberts and Lockett, 1978; Givelberg *et al.*, 1984; Kazinczi and Hunyadi, 1990; Teketay, 1998; Bithell *et al.*, 2002) (Appendix 1). For example, freshly harvested *S. nigrum* seed exposed to natural light for short periods during transfer between incubators, or germinated in light proofed dishes, was tested at alternating temperatures (10/25 °C, 10/30 °C, 15/25 °C and 15/30 °C, 16/8h light/dark durations). The mean germination for all temperatures from two seasons (1971, 1972) for light and dark treatments was 73 % and 11 %, respectively (Roberts and Lockett, 1978). Studies of seed exposed to R and FR light indicated that *S. nigrum*, like many other species, has a positive germination response to R light and was inhibited by FR light (Roberts and Lockett, 1978; Kazinczi and Hunyadi, 1990). Both studies indicated that for seed imbibed at high temperatures (15 or 20 °C) for 9 or 12 d, there was some sensitivity to FR light. Such results may indicate that *S. nigrum* seed can exhibit VLFR responses after particular seed pre-treatments (Hartman and Mollwo, 2002). This mechanism has been proposed to affect *Amaranthus retroflexus* seed that is close to the soil surface and is subjected to high temperatures in late spring and summer (Gallagher and Cardina, 1998c).

Solanum nigrum seed germination is inhibited by light with a low R:FR ratio. In a glasshouse study of filtered light treatments through *Bergeria crassifolia* (L.) Fritsch leaves (R:FR 0.08), after 21 d *S. nigrum* seed had not germinated under the leaves but 71 % of seeds germinated under non-filtered light (Teketay, 1998). The germination requirement for light of *S. nigrum* is not absolute, and the degree of additional proportional germination for light versus dark comparisons is moderated by a number of factors. Storage of seed in moist sand in incubators at 4, 15 and 30 °C, or outdoors for 15 weeks and then exposed to intermittent light or darkness and tested in alternating temperatures of (4/25 °C, 17/23 °C, 10/25 °C, 10/30 °C, 15/25 °C, and

15/30 °C, 16/8 h) gave germinations of nearly 100 % regardless of light, storage temperature or site (Roberts and Lockett, 1978). With one exception, for seed at all four storage conditions germinated at 17/23 °C, germination was 81.8 % and 15 % for the light and dark treatments respectively. This suggests that in the absence of light, *S. nigrum* may have a more restricted germination temperature range. In this case small temperature amplitudes may be a limitation to germination.

Some chemicals such as gibberellin (GA) and potassium nitrate (KNO₃) also positively, affect the germination of laboratory-stored seed of *S. nigrum* in the dark. For example, *S. nigrum* seed germinated fully in the dark at 25 and 30 °C in the presence of either GA₃ or GA₄₊₇ (Givelberg *et al.*, 1984). The addition of KNO₃ (0.2 %) to dark treatments of laboratory-stored seed also gave high germinations (36-94 %) compared to dark only treatments (2-12 %) (Roberts and Lockett, 1978). In these tests, seed age comparisons indicated that older seed had a greater germination response to KNO₃. Tests with seed stored in the field and retrieved also indicated that both GA₃ (100-400 ppm) and KNO₃ (0.2 %) increased the germination of dark treatments of *S. nigrum* seed (Roberts and Lockett, 1978).

Field studies: A study of dark cultivation carried out in the United States of America in early summer reported a combined species emergence of *S. nigrum* and *S. sarrachoides* density of 4 plants/m². Daylight cultivation gave significantly more nightshade seedlings (19 plants/m²) (Scopel *et al.*, 1994). However, this study did not report the proportion of *S. nigrum* to *S. sarrachoides* seedlings, making it difficult to identify the relative species contributions to the result. The study also did not report on soil temperature, soil nitrate levels at the site, or an assessment of the dormancy status of the species. This makes it difficult to identify the effect of the possible contributing factors to the result.

Germination requirements - moisture

A soil moisture content of 35-40 % has been reported as necessary for optimal germination. Only 8 % germination was observed at 20 % soil moisture (Wakhloo, 1964). There are some studies on the effect of water potential (ψ) on *S. nigrum* seed. Using polyethylene glycol (PEG) to produce ψ 's of -0.1, -0.2, -0.4, -0.6, and -0.8 MPa, *S. nigrum* germination at 25 °C after two weeks in solution was 29, 35, 15, 2 and 0 % respectively (Givelberg *et al.*, 1984). Similarly, for ψ 's of -0.1, -0.3, -0.5, and -1.0 MPa, *S. nigrum* germination ranged from 35-75 % at -0.1 MPa for four accessions, but for all accessions at -0.3 MPa the germination was < 10 %, and at -0.5 MPa there was no germination (Kazinczi and Hunyadi, 1990). Germination of *S. nigrum* may be hampered at -0.3 and may fail at -0.5 to -0.6 MPa. These results indicate that

S. nigrum germination may be more susceptible to water deficits compared with other species such as *Chenopodium album* and *Marrubium vulgare* L. For these species base ψ 's are -0.64 and -1.5 MPa, respectively (Lippai *et al.*, 1996; Roman *et al.*, 2000).

Seasonal emergence

In the United Kingdom a maximum daily soil temperature, at 20 mm depth, approaching 20 °C was associated with appreciable emergence of *Solanum nigrum* (Roberts and Lockett, 1978). The start of emergence in the United States of America was observed when the maximum air temperature reached 20 °C (Ogg and Dawson, 1984). A mean soil temperature, at 50 mm depth, of 15 and 17 °C preceding the initiation of emergence was observed over two years in the Netherlands (Kremer and Lotz, 1998a). An American study, in California, also reported that a 17 °C soil temperature, at 50 mm depth, was linked with the beginning of the seasonal emergence of *S. nigrum* (Keeley and Thullen, 1983).

An 11 year study of seasonal *S. nigrum* emergence after monthly cultivation of plots in field studies at Levin, New Zealand reported a longer annual emergence period (ten months) in New Zealand than in the United Kingdom (five months) (Roberts and Lockett, 1978; Popay *et al.*, 1995). Emergence in New Zealand started in August when the mean maximum air temperature was ~ 14 °C. The proportion of annual seedling emergence on a monthly basis was, September ~ 4 %, October ~ 7.5 %, November ~ 11 % and December ~10 %. The mean maximum temperature at the annual peak period of emergence (November) was ~ 18 °C (Popay *et al.*, 1995). May was the last month in autumn in which emergence was observed.

Proportion of the seed bank to emerge, and seed viability

In a two year study, based in Hawke's Bay, New Zealand the overall mean proportion of *S. nigrum* seed sown in trial plots that emerged from different cultivation treatments for combined spring and autumn seedling counts was 6.3 % (Hartley, 1991a). However, higher emergence totals were reported for a study using spring cultivation in the Netherlands where emergence over the three months of spring and early summer was 45 % (Kremer and Lotz, 1998a). Roberts and Lockett (1978) in three separate (sown 1966-68) studies of *S. nigrum* seed mixed with soil to a depth of 50 mm (and cultivated three times a year) in the field, demonstrated that in the first year 30-57 % of sown seed emerged, in the second year 9-13 %, and in the fifth year emergence was 1-3 %. Of the remaining seed 5-19 % was demonstrated to be still viable in subsequent glasshouse evaluations.

Emergence depth

Grundy and Mead (1998) studied *S. nigrum* emergence depths, using lots of 300 seeds, each buried at a range of depths in narrow bands. The mean number of seedlings that emerged in the first year from five depths were: 0.1 for surface sown seed, 6.6 from 1.25 mm, 71.4 from 12.5 mm, 99.8 from 25 mm, 43.8 from 50 mm and no seed emerged from 100 mm (Grundy and Mead, 1998). This indicated a non-monotonic response for this species, with the maximum number of seeds not emerging from the surface. A study of *S. nigrum* emergence for two triazine susceptible accessions report little difference in the emergence fraction for germinated seed buried at 10 or 20 mm, for seed at 40 mm emergence was generally halved and no seed emerged from 60 mm (Kremer and Lotz, 1998b).

Cultivation effects

A trial in which soil containing *S. nigrum* seed in pots where all pots were cultivated in March was carried out in the United Kingdom. Additional cultivation in the late spring (May) increased *S. nigrum* emergence compared with later season additional cultivations in June, July or August (Roberts and Boddrell, 1983). Field plot trials in New Zealand, using *S. nigrum* (at rates of 0, 2,500 to 5,000 seed/m²) surface sown in autumn, three cultivation treatments (autumn and spring, autumn only, spring only) with a rotary hoe to 75 mm depth, and a non-cultivation treatment, were undertaken (Hartley, 1991b). For the 0 seed/m² rate, the total number of unsown resident *S. nigrum* seedlings over two years was significantly greater in the autumn and spring treatment compared with the other treatments. This indicated that frequency of cultivation may have a bigger effect than seasonal timing. In the same trial, but with *S. nigrum* seed sown on the surface, cultivation after burial (autumn cultivation treatments) tended to produce the greatest emergence the following spring (Hartley, 1991b). This suggests that burial protects seeds from predation and decay over the winter, and/or that burial enhances the potential for later emergence.

Dormancy

Solanum nigrum has a non-deep physiological type of dormancy, and has a dormancy cycle that moves seeds from conditional dormancy to non-dormancy (Baskin and Baskin, 1998). During progression from conditional dormancy to non-dormancy, dormancy alleviation is characterised by a widening of the germination temperature range and a reduction in the requirements for other germination promoters (Hilhorst *et al.*, 1996). Temperature is identified as the key factor influencing dormancy of spring and summer annual seeds (Vleeshouwers *et al.*, 1995; Baskin and Baskin, 1998). The

indication for *S. nigrum* seed is that minimum temperature requirements for germination are higher early in the season and lower late in the season (Kremer and Lotz, 1998a). There is evidence for the induction of secondary dormancy occurring at the end of the emergence season in England (Roberts and Lockett, 1978). Popay *et al.* (1995) suggest that the secondary dormancy of species such as *S. nigrum* is induced by low autumn temperatures and broken by low winter temperatures. While Roberts and Lockett (1978) proposed that secondary dormancy is induced by high autumn temperatures and broken by low winter temperatures.

Plant growth

Solanum nigrum varies from a spreading habit to erect forms. It has a fibrous root system with slender and herbaceous stems, leaf and stem shape are highly variable, being strongly affected by environment and genotype (Ogg *et al.*, 1981). Nightshade species have sympodial growth (dicototymous branching), where the lateral meristems overtake the apical meristem (Bassett and Munro, 1985). Some studies have been made of the growth of widely spaced plants. For plants widely spaced (0.5×0.7 m) and grown in West Java, plant leaf area peaked at 0.8 m^2 78 d after transplanting. The maximum relative growth rate of the whole plant occurred from 13-33 days after transplanting, with a mean relative growth rate of 0.13 g/g/d (Fortuin and Omta, 1980). The net assimilation rate during this period was $11 \text{ g m}^2/\text{d}$. A thermal time study of *S. nigrum* and *S. ptycanthum* growth (spaced at 0.3×0.9 m) reported that both species exhibited their most rapid growth after $900 \text{ }^\circ\text{Cd}$ (McGiffen and Masiunas, 1992). Plant growth responses to full sun or shade differ: *S. nigrum* plants produce larger leaves when in the shade (Fortuin and Omta, 1980). Plants under a canopy receive low R:FR light. *Solanum nigrum* plants growing in low R:FR (0.14 at $210 \text{ } \mu\text{mole}/\text{m}^2/\text{s}$) exhibited significant increases in stem weight, internode length, plant height and stem to total weight ratios in comparison to plants grown in high R:FR (4.1 at $220 \text{ } \mu\text{mole}/\text{m}^2/\text{s}$) light (Croster *et al.*, 2003).

Development of flowers, fruit and viable seeds

Flowering: In a United Kingdom based study the number of days from seedling emergence to first flower, differed with the time of year. Early season (April) glasshouse plants took 50-55 d, glasshouse plants that emerged later in the season took 40 d (June and July), while marked field plants took > 60 d for plants that emerged in May and 50 d in June (Roberts and Lockett, 1978). *Solanum nigrum* flowers in California appeared 50-65 d after emergence for spring emerged seedlings; while summer emerged seedlings flowered in 35-45 d (Keeley and Thullen, 1983). The

results of Keeley and Thullen (1983) are cited as demonstrating that *S. nigrum* is a short day plant (Bassett and Munro, 1985; Croster *et al.*, 2003). However, the shorter time to flowering in the summer may be the results of seasonal temperature effects confounding a possible photoperiod effect. Other workers cite nightshade species as being day neutral (Hinckley, 1981). Flowering of *S. nigrum* plants in a field trial occurred at approximately 600 °Cd (T_b 6 °C) for plants established over a six week period (McGiffen and Masiunas, 1992). However, this period is not sufficient to establish the effect of photoperiod. Because of the sympodial growth of nightshade, plants can continue to produce flowers and fruit until the end of the growing season (Bassett and Munro, 1985).

Canopy shading reduces the PFD received by plants within the canopy, and it also affects light quality. A growth chamber study (18/12 °C 14/10h) of constant light quality but variable light intensity (100 (377 $\mu\text{mol}/\text{m}^2/\text{s}$), 56 (213 $\mu\text{mol}/\text{m}^2/\text{s}$) and 35 % (133 $\mu\text{mol}/\text{m}^2/\text{s}$) full light) on *S. nigrum* reported flowering was delayed by 5 d at the two lower light levels (Kremer and Kropff, 1999). Another growth chamber study of *S. nigrum* and *S. ptycanthum* plants growing in low R:FR (0.14 at 210 $\mu\text{mol}/\text{m}^2/\text{s}$) and high R:FR (4.1 at 220 $\mu\text{mol}/\text{m}^2/\text{s}$) reported no significant effect of light quality on the time of flowering (Croster *et al.*, 2003). Heider (1996) reports that *S. sarrachoides* and *S. ptycanthum* °Cd requirements for development stages such as flowering was not significantly affected by competition with process peas. However, *S. ptycanthum* flowering was delayed by up to two weeks in some study years for plants grown in competition with un-defoliated *Glycine max* L. (Merr.), compared with defoliated *G. max* (Quakenbush and Andersen, 1984). The results of Croster *et al.*, (2003) indicate that light quality does not affect the time of flowering, but it is difficult to reconcile whether the results of Quakenbush and Andersen (1984) and Kremer and Kropff (1999) indicate that differences in light intensity or temperature delays the time of flowering in nightshades. Work with density effects on pea development indicated that delayed development in high density plots of peas may be due to lower temperatures within the canopy of the high density plots (Moot, 1993). Thus delays to nightshade flowering could also be caused by canopy effects on temperature.

Fruit and seed: *Solanum nigrum* is predominantly self pollinated (Edmonds and Chweya, 1997). Tagging individual flowers and testing seed from the resulting, marked, fruit showed some germination of *S. nigrum* seed from 27 d after flowering and 100 % germination from 32 d after flowering (Roberts and Lockett, 1978). In California, viable seed was produced 63 d after seedling emergence for all except an early spring emergence, which took longer (Keeley and Thullen, 1983). Seed maturity of *S. nigrum* was recorded at approximately 1000 °Cd (McGiffen and Masiunas, 1992). Mean seed

number/fruit in New Zealand was reported to be 70 in *S. nigrum* plants grown in *Phaseolus vulgaris* L. (Hartley, 1991a). Overseas studies reported only about 60 seeds/fruit for individually grown plants (Keeley and Thullen, 1983). Crop competition is also reported to reduce seed numbers/fruit (Kremer and Kropff, 1998c).

Solanum nigrum is reported to produce fewer fruit in response to increased shade. At 50 % shade fruit production was half of that under full sun. At 95 % shade fruit production was only 10-20 % of that in full sun (Fortuin and Omta, 1980). Studies of the effect of shade on *S. ptycanthum* report similar results with the reduction in fruit number being identified as the result of fewer peduncles produced rather than a reduction in the number of fruit/peduncle (Croster *et al.*, 2003). Crop competition also affects fruit production, with the number of fruit/plant dropping from 12.6 to 3.4, and 37.9 to 4.3 for comparisons of *S. nigrum* grown in pure stands, to plants grown with *Zea mays*, respectively, for two atrazine susceptible biotypes (Kremer and Kropff, 1998c).

2.5.3 *Solanum physalifolium* biology and ecology

Solanum physalifolium

There is less published information on the germination requirements and growth of *S. physalifolium* than for *S. nigrum*. A study of laboratory stored *S. physalifolium* seed germination in Spain reported the optimal germination temperature was 30/15 °C over 8/16 h (with a 12/12 h light/dark regime) (del Monte and Tarquis, 1997). This study and a study in New Zealand report the presence of primary dormancy (Bithell *et al.*, 2002) (Appendix 1). High temperatures were required to overcome germination inhibition. It appears that dormancy also affected the estimation of T_b as a T_b of 21 °C was reported (del Monte and Tarquis, 1997). Such a high T_b value does not conform with the field behaviour of the plant, or with reports for other closely related species. Sobrino and del Monte (1994) reported that seed germination was enhanced by treatment with 1,000 ppm of GA. Edmonds (1986) noted that *S. physalifolium* seed was difficult to germinate, even after a pre-treatment with 2,000 ppm of GA (type not defined). *Solanum physalifolium* seed in New Zealand responded to KNO_3 (0.2 %) but not GA_3 (0.05 %) (Bithell *et al.*, 2002) (Appendix 1). The number of seeds/fruit ranged from 15-26 in fruits ranging in diameter from 4.3-6.7 mm (Sobrino and del Monte, 1994)

Solanum physalifolium is a herbaceous annual, with a prostrate growth habit and abundant branching (Sobrino and del Monte, 1994; Edmonds and Chweya, 1997). There are two leaf types: one with toothed leaves and an entire leaved variant. Both are present in New Zealand (Edmonds and Chweya, 1997). *Solanum physalifolium*

plants in the field flower and fruit throughout the autumn and summer until killed by the first frosts (Edmonds, 1986). Besides seed dispersal by birds, fruit and seed dispersal is assisted by ripe fruit dropping from the plant if it is knocked or shaken (Sobrinho and del Monte, 1994). Mature fruit are dark green to brownish green and 6-9 mm in diameter (Edmonds and Chweya, 1997).

Solanum sarrachoides

Information on the biology of *S. sarrachoides* is presented due to problems of its taxonomic identification, as it is assumed that some of this work may refer to *S. physalifolium* (Edmonds and Chweya, 1997), alternatively that some aspects of the biology of the two species may be similar.

Moist storage at low temperatures enhanced the germination of laboratory-stored *S. sarrachoides* seed (Roberts and Boddrell, 1983). Fresh laboratory stored seed exhibited primary dormancy (Roberts and Boddrell, 1983; Hermanutz and Weaver, 1991; Heider, 1996). This dormancy was reported to abate after six months (Hermanutz and Weaver, 1991). Baskin and Baskin (1998) report that *S. sarrachoides* had a dormant/non-dormant dormancy cycle, without a period of conditional dormancy. Germination studies of *S. sarrachoides* report that germination was achieved at alternating temperatures of 4/25 °C, 10/25 °C, 10/30 °C and 20/30 °C cycles of 8/16 h, or at a constant 25 °C and 30 °C once the primary dormancy of fresh seed had abated (Roberts and Boddrell, 1983).

Comparison of dark and intermittent light treatments during germination tests gave similar results for the two treatments (Roberts and Boddrell, 1983). Similarly comparisons between 14/6 h light and dark treatments tested at 15/5 °C, 20/10 °C, 25/15 °C and 30/20 °C for two *S. sarrachoides* accessions indicated a significant light by temperature by accession interaction, where dark germination at the 15/5 °C temperature was low (~5 %), but ~70 % germination in the alternating light treatment for seed from one accession (Hermanutz and Weaver, 1991). At the other temperatures for this accession, and the other accession at all temperatures, there was no significant effect of light treatments. However, a mixed population of *S. sarrachoides* and *S. nigrum* were reported to have significantly reduced germination following dark cultivation (Scopel *et al.*, 1994).

The seasonal emergence patterns for *S. nigrum* and *S. sarrachoides* are reported to be similar, with seedling emergence greatest in May and June (Roberts and Boddrell, 1983). A study of the yearly variation in seasonal emergence in United States cornfields for *S. sarrachoides* found an average emergence of 5.2 % of the known *S. sarrachoides* seed bank, with a minimal seasonal variation of 1.3 % and a maximum of

9.3 %. Analysis of seasonal variation linked differences in germination to seasonal temperature differences (Forcella *et al.*, 1997). Trials of *S. sarrachoides*, using sown plots, had 15 % seedling emergence from seed over two years. However, 97 % of these seedlings emerged in the first year (Ogg and Dawson, 1984). Depth of tillage of *S. sarrachoides* plots had no effect on relative seedling emergence (Ogg and Dawson, 1984). *Solanum sarrachoides* can flower and fruit quickly under some conditions. Seedlings in growth rooms at 28/20 °C (14/10 h) flowered and produced some immature fruit by 35 d after emergence (Hermanutz and Weaver, 1991). Heider (1996) noted that *S. sarrachoides* can exhibit rapid growth rates producing 2-3 times the biomass of *S. ptycanthum* for seedlings grown with or without peas. *S. sarrachoides* also had significantly lower thermal time requirements than *S. ptycanthum* for bud appearance, flowering, and initial fruit growth (Heider, 1996).

2.6 Summary

The literature reviewed indicated a number of information gaps and particular issues that need to be addressed in order to meet the objectives stated in the General Introduction. There is limited information available for *S. physalifolium*. However, some useful publications from North America on *S. sarrachoides* (Scopel *et al.*, 1994; Heider, 1996) may actually be referring to *S. physalifolium* due to identification problems (Edmonds and Chweya, 1997). Points regarding the five research objectives are:

1. That seasonal nightshade emergence, pea cultivar, pea sowing date and seasonal temperatures are potential factors that may contribute to nightshade contamination.
2. Knowledge of the dormancy cycle and germination requirements of *S. nigrum* and *S. physalifolium* in New Zealand is poor. A number of factors (seed origin, soil temperature, soil moisture and soil nitrate levels) may affect the light responses of *S. nigrum* seed. There is little information available on the dormancy of *S. physalifolium* seed.
3. The growth of *S. nigrum* and *S. physalifolium* in peas has not been studied, but some relevant work on related nightshade species in peas has been undertaken.
4. No information on the fruit growth of either the study species was apparent.
5. There is, currently, an information gap with regard to specific *S. nigrum* and *S. physalifolium* management practices available for pea processors and growers.

Chapter 3 Processing factory contamination records – analysis for causal factors

3.1 Introduction

Investigations of weed problems often focus on the factors governing crop/weed competition at a single field or plant community level within a field. Access to the nightshade contamination records from the Heinz Wattie's Ltd, Hornby, Christchurch processing factory provided the opportunity to investigate a broader range of potential factors that may influence the occurrence of nightshade contamination.

Process peas are contract crops managed and grown by farmers. However, the choice of pea cultivar, sowing date and harvest timing are largely determined and managed by the requirements of the processing factory, and is influenced by site factors such as soil type (Cawood, 1987). Fields with light or heavy soil are generally sown early and late in the growing season, respectively. Sowing date, crop cultivar and harvest timing are factors reported to influence both crop/weed competitive interactions and the reproductive success of annual weed populations (Oliver, 1979; Quakenbush and Andersen, 1984; Ghera and Holt, 1995; Khan *et al.*, 1996; Anderson, 2000). Nightshade contamination can occur only if nightshade plants in a pea crop can flower and produce fruit prior to the pea harvest. This chapter addresses the first research objective of this study, which is to analyse factory nightshade contamination records for seasonal trends in nightshade contamination and to consider causal factors for the contamination. The hypothesis investigated is that factors independent of those that can be managed by farmers, such as sowing date, cultivar type and harvest timing do not influence the occurrence of nightshade contamination in process pea crops.

3.2 Materials and methods

Three seasons of process pea yield and contamination records (2000/01 (00/01), 2001/02 (01/02), and 2002/03 (02/03)) were obtained from the Heinz Wattie's Ltd Hornby factory. The distribution of nightshade contamination cases during the pea processing season was investigated. Monthly sowing date, using 1 to 5 to represent the months August to December, and year, were examined as factors in relation to the mean monthly proportion of contaminated crops using logit regression of binomial proportions:

$$\log(p/(100-p))$$

where p = mean monthly proportion of contaminated crops (Genstat Sixth Edition[®] 2002). Predicted values for p were calculated by back transformation of y values (percentage contamination):

$$100/(1 + \exp(y))$$

For each season the pre-contamination period was defined as the period prior to any case of contamination occurring, and the contamination period as the period from the first case of contamination to the end of the sowing season. Group and paired T -tests were made using the Bonferroni test for 95 % confidence intervals (Systat[®] Version 9.01, 1998).

3.3 Results

3.3.1 Monthly sowing date effects

Contamination records presented in terms of the sowing dates for process pea crops indicated some evidence of sowing date effects on the presence of nightshade contamination (Appendix 2). Over the seasons 00/01, 01/02 and 02/03 no pea crops sown in August (mean number of crops sown 68.3) had nightshade contamination (Table 3-1). Nightshade contamination was also observed only in one season for crops sown in September. Mean values for the three seasons of contamination data indicated that the proportion of crops contaminated increased progressively from August to November (Table 3-1). A small number of crops were sown in December in the 00/01 and 02/03 seasons, and of the 02/03 December sowings, 7.1 % were contaminated (Appendix 2).

Table 3-1. Mean number (no.) of crops sown and contaminated, and percentage of crops contaminated over three seasons (00/01, 01/02 and 02/03), data from the Heinz Wattie's Ltd, Hornby factory.

	August	September	October	November	December
No. sown	68.3	92.3	172.7	145.0	16.0
No. contaminated	0	0.7	8.0	7.0	1.0
% contaminated	0	0.6	4.8	5.6	3.6

Two cases of nightshade contamination from organic farms were observed, one each in the 00/01 and 01/02 seasons (Appendix 2). In 00/01, 01/02, 02/03 the number of organic fields was 26, 17 and 10 respectively. For each season the proportion of

nightshade contaminated organic pea crops was 3.8, 5.9 and 0 %. For conventional pea crops over the same period the respective values were 2.9 (n = 475), 5.4 (n = 484), and 1.8 % (n = 508). A *T*-test indicated that there was no significant difference ($P > 0.05$, d.f. = 2) in mean contamination levels between the two management types.

For the period in each season, starting from when cases of nightshade contamination were first observed (11 October 00, 1 September 01 and 16 October 02) to the end of each season, organic pea crops had 9.1 (n = 11), 7.1 (n = 13) and 0 % (n = 10) crops contaminated, respectively. For the conventional crops over the same period, the figures were 4.3 (n = 303), 6.2 (n = 403), and 3.1 % (n = 287). A *T*-test indicated that there was no significant difference ($P > 0.05$, d.f. = 2) in contamination levels between the two management types for the period in which nightshade contamination occurred.

Logit regression of binomial proportions of the mean proportion of crops contaminated each month indicated that sowing month as a variate was significant ($P < 0.001$, d.f. = 13). A separate test for year effects indicated that year was not a significant variate ($P > 0.05$). Predicted values and s.e. for the mean proportion of crops contaminated each month, from August to December, were 0.8 (0.36), 1.6 (0.44), 3.0 (0.48), 5.6 (0.91) and 10.2 (2.71) respectively. Figure 3-1 presents predicted and actual contamination values of the mean proportion of crops contaminated each month.

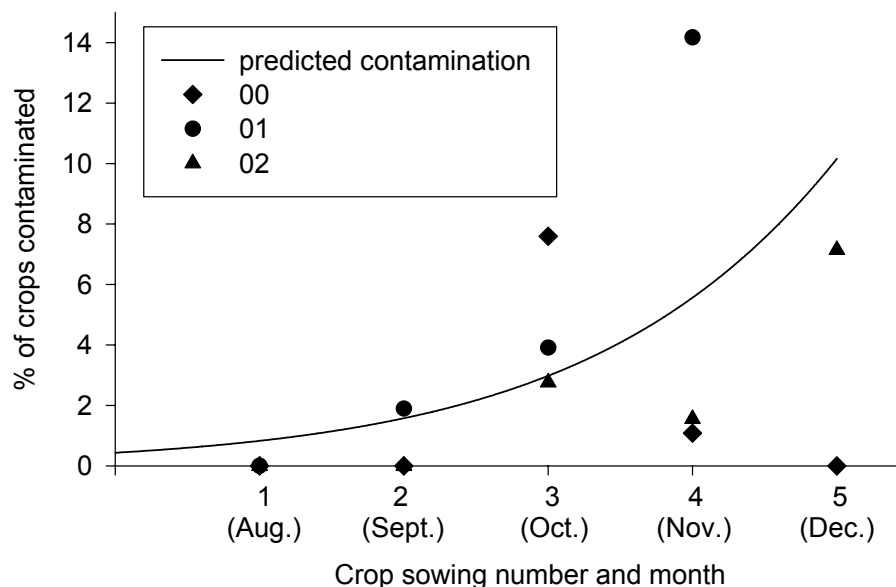


Figure 3-1. The relationship between % of crops contaminated and time of sowing, based on a logarithmic regression: $y = -5.44 + 0.652x$. The line is for the back transformed values for $100/(1 + \exp(y))$.

3.3.2 Cultivar effects within the period of nightshade contamination

Comparisons of the pea cultivar composition, for the period in each season when nightshade contamination was first observed in crops, were made for each year. For the six cultivars sown (Bolero, Bounty, Durango, Orbit, Prolific and Tyne) during the nightshade contamination period in each of the three seasons, each was contaminated in, at least, one season. Some cv.s such as Bounty and Prolific were associated with nightshade contamination in every season. For this period in the three seasons 20.5, 10.9, and 4.9 % of crops respectively were sown with cv. Bounty, and 48.2, 34.0 and 34.5 % of crops, respectively, were sown with cv. Prolific. For the same seasons 3.2, 9.1 and 14.3 % of cv. Bounty crops and 1.4, 10.2, and 4 % of cv. Prolific crops were nightshade contaminated.

The cv. Bolero was associated with nightshade contamination in both the 00/01 and 01/02 seasons, when the proportion of crops over this period which were sown in cv. Bolero was 15.8 and 25.3 %, respectively. There was no contamination associated with this cv. in the 02/03 season when the proportion of crops sown in cv. Bolero was 3.5 % (n = 10 crops). Of the other cultivars (Durango, Orbit and Tyne), nightshade contamination occurred only in a single season. For these cultivars, in each of the three seasons (00/01, 01/02, 02/03), the percentage of crops sown in each cultivar was low. Less than 5 % of crops in the contamination period were sown with Durango and Orbit in the 01/02 season, and with cultivar Tyne in the 00/01 and 02/03 seasons (Table 3-2).

In terms of cultivar contributions to the number of nightshade contamination cases observed each year, Bolero in 00/01 and 01/02 contributed 71.4 and 15.5 %, respectively. For cv.s Bounty and Prolific the figures for 00/01, 01/02, and 02/03 were 14.3, 15.4 and 22.2 % for cv. Bounty and 14.3, 53.8, and 44.4 % for cv. Prolific. Over the three seasons mean values for the cultivar contributions to the number of nightshade contamination cases in a season was 17.3 and 37.5 % for Bounty and Prolific, respectively. *T*-tests of the yearly percentage contribution values to the total number of contamination cases versus their respective percentage values for the number of crops sown in each cultivar, over the contamination period indicated, for the cv.s Bounty and Prolific, gave no significant ($P > 0.05$, d.f. = 2) difference between percentage contribution and percentage sowing for either cultivar.

Table 3-2. Cultivars (Cv.s) and % crops sown (for cultivars that contribute $\geq 5\%$ of crops sown) in two periods, when no nightshade contamination was observed (Pre-con.) or the period in which contamination was observed (Con.), and the number of crops sown (No. sown) in both periods for the 2000/01-2002/03 seasons, data from the Heinz Wattie's Ltd, Hornby factory.

Cv.s	2000/01			2001/02			2002/03		
	No. sown	Pre-con.	Con.	No. sown	Pre-con.	Con.	No. sown	Pre-con.	Con.
Bolero	99	29.1	15.8*	132	36.3	25.3*	126	52	3.5
Bounty	63	0.6	20.5*	44	0	10.9*	<		*
Brule	-			-			26	0	9.1
Durango	<			<		*	28	0.4	9.4
Epic	42	24.0	0	28	35.0	2.2*	<		
Orbit	<			<			40	0	13.9*
PF 400	<			34	0	8.4*	28	12.1	4.2
Princess	25	14.3	0	<			-		
Prolific	147	1.7	48.2*	137	0	34.0*	103	1.8	34.5*
Resal	<			-			<	0	8.7
Talbot	10	5.7	0	11	13.8	0	25	11.2	0
Tere	36	20.6	0.7	22	15.0	2.5	24	10.8	0
Tyne	<			24	0	6.0*	<		

* = crop contaminated, - = not sown that year, < = $< 5\%$ crops sown with the cultivar in either the pre-contamination or contamination periods.

3.3.3 Cultivar effects between pre-contamination and contamination periods

The distribution of cultivars through the season was grouped in relation to the first case of nightshade contamination each season. Cultivars were selected that contributed $\geq 5\%$ of the total number of crops sown for either the period in each season prior to the first contamination case (pre-contamination period), or occurring after the first case of contamination (contamination period) (Table 3-2). Three groups of cultivars were apparent. First, cultivars that were sown in both the pre-contamination and contamination periods (type 1). These were the cv.s Bolero and PF 400. Secondly, cultivars that were predominately sown in the pre-contamination period (type 2). These were the cv.s Epic, Princess, Talbot and Tere. Thirdly, cultivars that were predominately sown in the contamination period (type 3). These were the cv.s Bounty, Prolific, Tyne, Brule, Durango, Orbit and Resal. A two group *T*-test comparing the thermal time maturity values for the type 2 and type 3 groups indicated the two groups differed significantly ($P < 0.01$, d.f. = 3). Type 3 cultivars had ~ 100 °Cd ($T_b = 5$ °C) greater mean values than the type 2 cultivars. Five out of seven type 3 cultivars were associated with nightshade contamination; the two type 3 cultivars sown in 02/03 (cv.s Brule and Resal) were not associated with contamination. No type 2 cultivars were nightshade contaminated.

3.4 Discussion

The factory nightshade contamination records, when presented in terms of monthly sowing dates, appeared to support evidence of seasonal trends. In particular, in each of the study years there was no contamination of August sown crops, and in only one of the three years there was no contamination of a September sown crop (Table 3-1). To correct for differences in the number of crops sown each month, analysis was made of the proportion of crops contaminated each month. The proportion of crops contaminated peaked in October, for the 00/01 season, in November, for the 01/02 season and in December in 02/03 (Figure 3-1). However contamination of December sowings was irregular among years. Binomial logit regression indicated that sowing month was a significant ($P < 0.001$) variable, with a progressive increase in the predicted mean monthly proportion of crops contaminated (Figure 3-1).

There was no significant evidence that there was greater nightshade contamination in organic fields over the whole season, or during the period of contamination. However, the small number of organic fields in these results (i.e. $n = 10$ in the 02/03 season), and the small number of contamination cases means there is considerable uncertainty in this conclusion.

Two possible causes for the effects of sowing month on contamination of crops are proposed. The first is of seasonal effects on nightshade phenology, the second pea cultivar effects. *Solanum nigrum*, in particular, is identified as germinating late in the spring/early summer in European studies (Hakanson, 2003). This late season emergence is considered to influence the ability of *S. nigrum* to contaminate early versus late sown process pea crops in Europe (El Titi, 1986; Knott, 1986). However, in New Zealand at a study site in Levin, *S. nigrum* was reported to emerge over a ten month period compared with a five month period for a comparable study in England (Roberts and Lockett, 1978; Popay *et al.*, 1995). Canterbury processing factory field records indicate that nightshade weeds are present at the early crop growth stages for August and September sown crops (pers. comm. A. White, Heinz Wattie's Ltd). Established *S. nigrum* plants, with reproductive buds and some open flowers, were observed in a pea crop close to harvest maturity, which had been sown in August 2001 (S.L. Bithell, pers. observation). These observations indicate that the seasonal emergence of *S. nigrum* may not be a contributing factor to monthly pea crop contamination trends in Canterbury. The seasonal emergence of *S. physalifolium* has not been studied in New Zealand. However, studies in the United States of America and the United Kingdom report that the timing of emergence for *S. sarrachoides* closely matched that of *S. nigrum* (Roberts and Boddrell, 1983; Ogg and Dawson, 1984).

Flowering and fruit growth are necessary for nightshade weeds to be able to contaminate pea crops. Temperature may affect the flowering phenology of the nightshade relative to the pea crop. *Solanum nigrum* is reported to be day neutral and to flower at approximately 600 °Cd ($T_b = 6^{\circ}\text{C}$) (McGiffen and Masiunas, 1992). Differences in accumulated thermal time values prior to crop harvest for *S. nigrum* plants in crops sown, for example, in August versus those sown in November may contribute to the differential contamination observed for crops sown in these months (Table 3-1). However, the pea cultivars sown in these months differ in terms of their thermal time maturity requirements, indicating that contamination may be linked to cultivar thermal time maturity effects and/or sowing date effects.

Some field pea crop cultivars are reported to have differential competitive abilities with weeds (Wall and Townley-Smith, 1996; Pester *et al.*, 1999). However, evaluations of process pea cultivars for differential weed competitiveness are not apparent in the literature. The nightshade contamination records indicated that two cv.s, Bounty and Prolific, were consistently associated with contamination when sown in the contamination period (Table 3-2). Analysis of the percentage contribution to contamination cases each season for these two cultivars, during the period of nightshade contamination, indicated that for both cultivars, in proportional terms, the number of cases of nightshade contamination did not differ significantly ($P < 0.05$) from the proportion of crops sown with these cultivars. That is, there is no cultivar effect. Contamination was also observed with other cultivars (Table 3-2), but a similar analysis was not attempted due to the largely single year cultivar contamination associations observed. However, at least for the cv.s Bounty and Prolific, there is no evidence to suggest that nightshade contamination is more likely to occur for either cultivar. On this basis, the results generally suggest that the risk of nightshade contamination for a cultivar is related to the number of crops sown with that cultivar i.e., a degree of exposure effect for crops sown during the nightshade contamination period. However, in two cases, cultivars sown at less than the 5 % levels were contaminated (Table 3-2).

Comparison of cultivar sowing distribution data for the pre-contamination and contamination periods each season indicated three types of cultivars (Table 3-2). Type 1 was sown in both the pre-contamination and contamination periods, while type 2 occurred only in the pre-contamination period, and type 3 in the contamination period only. Comparison of the thermal time maturity values for types 2 and 3 indicated that type 3 cultivars had significantly greater thermal time values. This result is in agreement with reported process pea crop scheduling practices, where cultivars with comparatively low thermal time requirements are reported to be used early in the sowing season in comparison with the thermal time requirements of cultivars used later in the season (Cawood, 1987). These differences in cultivar thermal time maturity

values could be a factor contributing to the observed seasonal distribution of nightshade contamination. Nightshade weeds may have an increased ability to flower and produce fruit in pea cultivars with longer thermal time maturity values. This is reinforced by the fact that these cultivars are sown mid to late season when higher mean temperatures may allow more rapid nightshade development relative to the pea crop.

3.5 Conclusions

1. There was evidence to support the alternative hypothesis that some factors not able to be managed by pea growers, such as sowing date and harvest timing may influence the occurrence of nightshade contamination.
2. There is evidence that the proportion of crops contaminated with nightshade fruit increases progressively with pea sowing dates from August to December.
3. There was no strong evidence to indicate that particular cultivars sown during the nightshade contamination period were more susceptible to contamination than others sown during this period.

Chapter 4 Germination requirements

4.1 Introduction

The dormancy status of seed with physiological dormancy can be assessed from the range of temperatures that seed will germinate at, germination rates, and from responses to factors such as light and nitrate (Karssen and Vries, 1983; Karssen and Hilhorst, 1992; Murdoch, 1998). The germination requirements of field stored and laboratory tested weed species can be used to understand seasonal dormancy cycles and germination requirements (Bouwmeester and Karssen, 1993b). From such an understanding specific management practices and/or predictive models of the germination behaviour of these species can be developed (Kremer and Lotz, 1998a; Vleeshouwers and Bouwmeester, 2001). Establishing the germination requirements of a species by the use of freshly collected seed sources can, however, be made difficult by the presence of primary dormancy. Primary dormancy is the failure of freshly collected seed to germinate in conditions under which non-dormant seed will readily germinate.

New Zealand collected *Solanum nigrum* and *S. physalifolium* laboratory stored (5 °C) seed is reported to have primary dormancy (Bithell *et al.*, 2002) (Appendix 1). Studies of *S. physalifolium* also report that primary dormancy impairs germination (Edmonds, 1986; del Monte and Tarquis, 1997). However, primary dormancy of *S. nigrum* is reported in some studies (Roberts and Lockett, 1978; Wagenvoort and Opstal, 1979) but not in others (Givelberg *et al.*, 1984; Bulcke *et al.*, 1985; Agong, 1993). Givelberg *et al.* (1984) proposed that the differences between studies in the primary dormancy status of *S. nigrum* seed may be due to genotype effects. Another factor that may contribute to these conflicting reports is the maturity of fruit from which the *S. nigrum* seed was processed. *Solanum nigrum* fruit changes from green to black with increasing maturity. Nightshade plants are indeterminate, and flower and produce fruit until they are limited by the environmental conditions (Edmonds and Chweya, 1997). Thus, nightshade plants can bear fruit with a range of relative maturities, including fruit colour.

Seed maturity in relation to fruit maturity may be a contributing factor to dormancy polymorphism, as there is a report that *S. nigrum* seed from black fruit can be more dormant than seed from green fruit (Kazinczi and Hunyadi, 1990). However, this study did not report if the fruits came from the one collection site. Studies of other weed species indicate that seed dormancy status can differ significantly between collection sites (Milberg and Andersson, 1998a). A comparison of seed from black and green *S. nigrum* fruit, from a single plant collection, indicated that seed from both green

and black fruit was dormant, but that seed from black fruit had a greater response to pre-germination chilling (Bithell *et al.*, 2002) (Appendix 1). This indicates, that in this case, seed from green fruit may be more dormant. To confirm the relevance of this finding it is necessary to investigate the dormancy status of seed from black and green fruit from a number of collection sites. The quantification of differences in seed viability between populations is also required to prevent differences in viability confounding apparent differences in dormancy status.

A genetic study of *S. nigrum* populations identified *S. sarrachoides* as being not as closely related to *S. nigrum* as another common Solanum weed, *S. luteum* (Stankiewicz *et al.*, 2001). It appears that *S. physalifolium* may be more closely related to *S. sarrachoides*, given their similar morphologies, than *S. nigrum* (Edmonds, 1986; Edmonds and Chweya, 1997). These differences may explain differences in germination requirements reported between the two species. Studies of *S. nigrum* seed report that light is a germination requirement (Roberts and Lockett 1978; Givelberg *et al.* 1984). However, light is not reported to be an important factor for the germination of *S. physalifolium* (del Monte and Tarquis, 1997). Cultivation at night is reported to reduce the field emergence of *S. nigrum* compared with cultivation in daylight (Scopel *et al.*, 1994). Light was shown to be an important requirement for the germination of freshly harvested *S. nigrum* seed. However, for seed that had been stored in moist sand for 15 weeks, light was only important for germination at alternating temperatures with narrow amplitudes (Roberts and Lockett, 1978). These results indicate that the importance of light for germination may be affected by seed age and test conditions. Furthermore, in other species, germination responses to light can interact with nitrate and soil moisture (Milberg, 1997; Botto *et al.*, 2000). Studies of some annual weed species report differences in light requirements following field burial or laboratory stratification among populations of the same species (Milberg and Andersson, 1998b). The ability to identify the importance of light for germination in relation to some of these factors, including seasonal effects, would give information relevant to dark cultivation as a potential management tool. Further, information on the dormancy of *S. nigrum* and *S. physalifolium* during the pea processing season in New Zealand may have implications for practices that seek to maximise weed seed germination as in the use of the stale seed bed technique (Johnson and Mullinix, 1995).

This chapter addresses the second research objective of this study, to describe the dormancy cycle and field germination requirements of *S. nigrum* and *S. physalifolium* seed during the process pea growing season in Canterbury, New Zealand. From the literature a number of hypotheses were investigated:

1. The germination requirements of freshly harvested seed from black and green *S. nigrum* fruit does not differ, and the requirements of seed of *S. nigrum* collected from different sites does not differ.
2. Field stored *S. nigrum* seed has a light requirement for germination that is constant and a static dormancy status.
3. *Solanum physalifolium* seed does not have a light requirement.
4. The germination of *S. nigrum* seed following SDLE at different dates would indicate no change in light requirements, and no variation in SDLE responses between *S. nigrum* seed lots from green and black fruit. It was also hypothesised that *S. physalifolium* germination would not be affected by SDLE.
5. The dormancy of field stored *S. physalifolium* seed and *S. nigrum* seed does not differ, as indicated by percentage germination at the sub-optimal temperature of 5/20 °C, and time to 50 % germination.
6. There are no differences in the germination requirements of field stored seed from black and green *S. nigrum* fruit.
7. Dark cultivation will not reduce the field germination of *S. nigrum*, or *S. physalifolium*.

4.2 Experiment 1 – A comparison of primary dormancy status

This experiment was conducted to test the hypothesis that the germination requirements of freshly harvested seed from black and green *S. nigrum* fruit does not differ, and the requirements of seed of *S. nigrum* collected from different sites does not differ (hypothesis 1).

4.2.1 Materials and methods

Seed lots

Three collections of *S. nigrum* seed from crops or field margins were made on 20 March 2002. Fruit were removed from plants by hand and fruit that were < 6 mm in diameter were rejected. The fruit from each collection was sorted by colour (green or black) then processed separately. The seed from the black (coded A) and green (coded B) fruit from the three collections provided six seed lots SN11A, SN11B, SN12A, SN12B, SN13A, and SN13B in total (Appendix 3). For processing, the fruit were crushed and soaked in water for 24 h at room temperature. Fruit material and seed

were separated by floatation and washing in cold water, then dried in indirect light at room temperature. The seed was then dried at 30 °C for 36h, then cleaned with an Ottawa type seed blower. It was then sieved (0.85 mm) and seed that passed through the sieve was rejected, the seed was then stored at room temperature for six days.

Germination

Six days after the completion of seed processing a factorial experiment with three replicates of 40 seeds per treatment was set up. The four factors used were: seed lots, as listed above; nitrate, seed was placed on germination blotters dipped in KNO_3 (N^+) (0.2 %) or dipped in water (N^-); light, where un-imbibed seeds were placed on germination blotters in the presence of light; where dark (L^-) treatments were placed in a clear zip lock bag (Mini grip 255 × 305 mm), inside a black polythene bag (510 × 380 mm, 60 µm thickness) after counting 40 seeds into a Petri dish and sealing of the dish. The bags surrounding the dark treatments seeds were sealed immediately after the last seed had been placed on to the germination pad on completion of the last replicate of three. The light (L^+) treatment dishes were sealed in clear zip lock bags only. The fourth factor was prechilling, where the seed, as prepared above, was prechilled (PC^+) for 14 d at 10 °C in 24 h light, or was not prechilled (PC^-).

The non-prechilled seed treatments were placed directly into the germination cabinets at 20/30 °C (16 h low temperature/8 h hour high) with 24 h light. The L^+ treatments were placed on the top two shelves (under the light source) and L^- treatments were placed on the bottom shelf. The experiment was conducted in a Sanyo MIR 152 incubator with 24 h light, based at the Field Service Centre, Lincoln University. The incubator was illuminated with a single 15 W (Toshiba FL 15 D) tube. The 8 h period at the higher temperature had a higher light intensity value than the lower 16 h temperature. Mean light values and the range over a 24 h period for the incubator was 12.7 µmole/m²/s (range 10.1–15.5). The light source provided a R:FR of 3.6 at 20 °C.

The prechilled treatments were placed into the cabinet at 20/30 °C after 14 d at 10 °C with 24 h light. In the cabinet, the L^+ and L^- treatments were placed as described above. Germination of L^+ treatments was assessed daily for 14 days. An additional 1 ml of water was supplied to all light treatment replicates on two inspection dates over the 14 days. The L^- treatments were inspected at day 14. Germination was defined as radicle emergence, and germinated seeds were removed on inspection. At the end of each experiment, at 14 d, the remaining seed was assessed for viability. Seed that resisted gentle pressure by tweezers were recorded as being viable (Forcella *et al.*, 1992).

Analysis

The percent of viable seed that had germinated was calculated. Percentage data were arcsine transformed for statistical analysis. Statistical analysis of the germination was by ANOVA using Systat® Version 9.01 (1998). Where arcsine transformation yielded significantly different results from the analysis of non-transformed values, the arcsine results are presented in an appendix and non-transformed values are presented in the main text. The results and discussion section only cite non-transformed values. Means separation tests were made using the Tukeys honestly significant difference test (HSD) (Zar, 1984).

4.2.2 Results

Seed lot had a significant effect ($P < 0.001$) on percentage of non-viable seed, with seed lot SN12B having significantly fewer viable seed than lots SN11A, SN12A and SN13A (transformed values Appendix 4, non-transformed values Table 4-1). Lot SN11B had significantly more non-viable seed than lots SN11A, SN12A, SN12B and SN13A, but lot SN13B had significantly more non-viable seed than all of the other seed lots.

Table 4-1. Percent of non-viable seed as determined by the pressure test (Forcella *et al.*, 1992) for six seed lots (A = seed of black fruit, B = seed of green fruit) of forty seeds (Experiment 1).

Seed lot	Non-viable %
SN11A	1.1
SN11B	23.3
SN12A	1.1
SN12B	7.0
SN13A	1.9
SN13B	35.9

Analysis of the arcsine transformed percentage germination (transformed values Appendix 6) of viable seed at 14 d for the four factors nitrate, prechill, light and seed lot indicated a significant ($P < 0.001$) four way interaction between the factors (non transformed values Figure 4-1). In the absence of nitrate, prechill and light, seed from black Gisborne and green Manawatu fruit had significantly higher germination levels than both Lincoln seed lots (Figure 4-1, a). In the presence of light only, seed from black Gisborne fruit and both the Manawatu seed lots germinated at higher levels than both Lincoln seed lots (Figure 4-1, b). In the presence of prechilling only, all seed lots responded with significantly higher germination than seed from green Lincoln fruit,

and seed from black Gisborne and green Manawatu fruit had significantly higher germinations than the other seed lots (Figure 4-1, c). In the presence of prechilling and light seed from green Lincoln fruit had a significantly lower germination than seed from black Gisborne and black Manawatu fruit (Figure 4-1, d). In the presence of nitrate only, seed from green Gisborne fruit had a significantly higher germination than both the Lincoln seed lots (Figure 4-1, e). Seed from green Manawatu fruit had significantly higher germination than both the green Gisborne and black Manawatu fruit. Seed from black Gisborne fruit had a significantly higher germination than all other seed lots.

In the presence of both nitrate and light, both Lincoln seed lots had significantly reduced germination compared with the other four seed lots (Figure 4-1, f). Seed from black Gisborne fruit also had significantly higher germination than seed from green Lincoln fruit. In the presence of nitrate and prechilling seed from black Lincoln fruit had significantly lower germination than seed from black Gisborne fruit and both Manawatu seed lots (Figure 4-1, g). Seed from green Lincoln fruit had a significantly lower germination than all the other seed lots. In the presence of nitrate, prechilling and light there were no significant differences among seed lots and the germination approached 100 % for all seed lots (Figure 4-1, h).

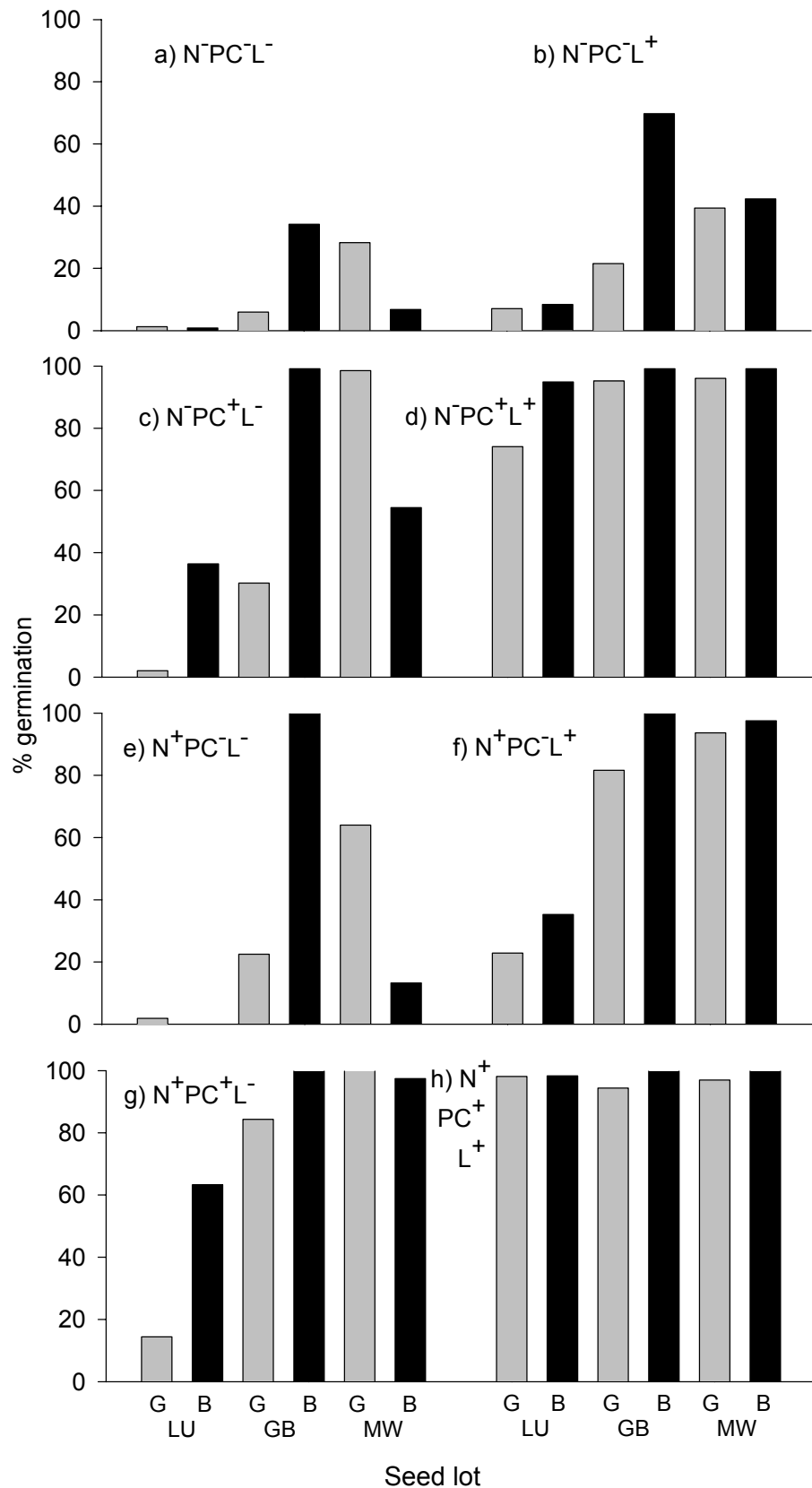


Figure 4-1. The percentage germination of viable seed from green (G) and black (B) *S. nigrum* fruit collected from Lincoln (LU), Gisborne (GB) and the Manawatu (MW). Seed was germinated at 20/30 °C in the presence (+) or absence (-) of light (L), and/or prechilling (PC) and/or nitrate (N), (Experiment 1).

4.3 Experiment 2 – The germination requirements of field stored *Solanum nigrum* and *S. physalifolium* seed

This experiment was conducted to test the hypotheses that field stored *S. nigrum* seed has a light requirement for germination that is constant and a static dormancy status (hypothesis 2). *Solanum physalifolium* seed does not have a light requirement (hypothesis 3). The dormancy of field stored *S. physalifolium* seed and *S. nigrum* seed does not differ, as indicated by the percentage germination at the sub-optimal temperature of 5/20 C, and time to 50 % germination (hypothesis 5). There are no differences in the germination requirements of field stored seed from black and green *S. nigrum* fruit (hypothesis 6).

These hypotheses relate to the objective of describing the dormancy cycle and the germination requirements of the study species during the process pea sowing season in Canterbury. To do this seed of both species was buried in 2001, and retrieved on ten dates, in 2002. At each retrieval date seed was tested in light and dark at two temperatures.

4.3.1 Materials and methods

Seed lots

Solanum nigrum and *S. physalifolium* plants were collected from crops or field margins between February and April 2001 (Appendix 3). Seed from collections were processed and dried as described in Experiment 1, then stored at 5 °C. Except that the fruit of *S. physalifolium* was processed as a single seed lot, as no separation for fruit colour could be made. In May 2001, 100 seeds (four sub-samples of 25 seeds) from each lot were tested with 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) using a procedure for the Solanaceae (Peters, 2000) with a 24 h staining time and with treated seed stored at 25 °C. Three seed lots (SN7A, SN7C, and SP3) with high levels of viability and sufficient seed quantities for the experiment were selected (Appendix 5).

Field storage

Two separate lots of seed for germination experiments were prepared for burial. The first was of 450 seeds from the seed lots SN7A, SN7C, and SP3. The second, of 225 seeds was from the same seed lots. The seed from each lot was placed in individual nylon mesh bags and tied with colour coded electrical wire. Eight bags each with 0.23 g of seed lot SP3 seed and eight bags each with 0.5 g of seed lot SN6A seed were also

prepared, to monitor seed moisture level. Various combinations of seed lots, as indicated in Table 4-2, were placed into large 'carrier' nylon mesh bags with ~ 350 ml of soil. At every second seed retrieval date a bag of SP3 and SN6A seed was included for moisture determination. The small bags of individual seed lots were placed together in the centre of the soil mass, the large bag was then tied tightly with electrical wire. Separate carrier bags were prepared for seed lot combinations of the 225 and 450 seed batches.

Table 4-2. Carrier bags buried containing 2–3 seed lot combinations of either 450 or 225 seeds/seed lot in individual bags. Seed from *S. nigrum* (SN) (A = seed of black fruit, B = seed of green fruit) and *S. physalifolium* (SP) plants.

Combination	Seed lots of 450 seeds	Seed lots of 225 seeds
1	SP3/SN7A	SP3/SN7A
1	SP3/SN7A /SN7C	SP3/SN7A /SN7C
2	SP3/SN7A /SN7C	SP3/SN7A

The carrier bags were buried in Wakanui Silt Loam on 8 August 2001 in area H19, of the Horticultural Research Area, Lincoln University, at a depth of 75 mm to the centre of the bags, in a randomised design. For each retrieval date a bag containing seed lots with 450 seeds, and a bag containing seed lots with 225 seeds were buried, together, side by side. Bags for different retrieval dates were buried ~ 0.15 m apart. A length of electrical wire from the neck of the carrier bag was attached to a coded metal disc at the soil surface. Hourly soil temperature at a depth of 75 mm was recorded by a Tinytag™ data logger, placed 0.4 m to the east of the burial area. During the trial the area was hand weeded.

Seed retrieval

Bags were retrieved at ~ 2 week intervals from 15 July to 2 December 2002. All bags retrieved in July were for those indicated as combination 1 in Table 4-2. Combination 2 bags were retrieved on 19 August, 3 September, 18 October and 2 November. Combination 3 bags were retrieved on 18 September, 3 October, 17 November and 2 December. The location of seed bags was made with the metal disc and retrieved a minimum of one hour after sunset, without the use of any lighting. On retrieval bags were placed in double layer black polythene bags and were placed inside a light proof plastic container.

The seed was taken to a dark room where seed lots were removed from the bags. A 23 W (Phillips Ecotone PL Electronic T 827 PRO) light source, filtered through a dark green filter (Ilford 907 safe light filter), was used in the dark room. This light

source, and a number of other light sources, were evaluated with a spectroradiometer (Licor 1800), with three replicate scanings, repeated four times. At 50 mm from the filter the PAR was $0.030 \mu\text{mole}/\text{m}^2/\text{s}$, with a R:FR ratio of 0.6. All seed lot bags except for the current lot being prepared were then returned to a light proof plastic container.

Light and dark treatments

The carrier bag containing lots of 450 seeds were used for this experiment, with the dark (L^-) treatments being prepared first. Twenty five seeds were counted into pre-prepared glass Petri (90 mm) dishes containing a moistened Whatman grade 181 90 mm paper under a water dipped seed germination blotter (Anchor Paper Co. Steel Blue Germination Blotters). Water was then spray misted over the seeds and the Petri dish was sealed with plastic cling film. A mean of 7 ml of water was used to pre-moisten the germination papers and sprayed over the seeds and the germination blotter. Three dishes each containing 25 seeds were placed together on top of each other in a clear plastic zip lock bag. The bag was sealed and then placed in a black polythene bag (380×510 mm, 60 μm thickness). Following preparation of the L^- treatments, seeds for the light treatments (L^+) were transferred into an adjoining lighted (PAR of $21.0 \mu\text{mole}/\text{m}^2/\text{s}$, R:FR 7.0) room where they were prepared as above. However, no black polythene bag was used to cover the clear zip lock bag. Light and dark treatments were prepared for the *S. nigrum* seed lot SN7A at each of the ten test dates this seed was retrieved from the field, and for lot SN7C for the eight dates this was retrieved. Seed of the *S. physalifolium* was retrieved on ten dates and light treatments were prepared at each date, but following the second retrieval date dark treatments for this species were prepared monthly instead of fortnightly.

Soil nitrate testing

For the retrievals from 31 July, except for 3 October, three 10 g soil samples were taken from the soil in each of the two carrier bags. The six 10 g soil samples and two blanks were prepared using the 2M KCl extractable nitrate method of Blakemore *et al.* (1987). This process was carried out on the day following a night retrieval of seed. The soil extract was stored at 4 °C before spectrophotometrical analysis with a Flow Injection Analyser (Alpkem FS3000). Soil moisture was measured for each sample, by drying a 100 ml soil sample from each carrier bag at ~ 103 °C for 48 h.

Temperature treatments and germination conditions

After treatment the prepared seed lots were transferred to Sanyo MIR 152 incubators with 24 h light, as described for Experiment 1. Light levels in the germination cabinets were monitored periodically with a quantum sensor (Licor or Lambda Inst. Corporation). For these light regimes light intensity, was affected by the operating temperature of the incubator. Mean light values and ranges over a 24 h period for the two alternating temperatures of 5/20 and 20/30 °C, were 7.3 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 3.6–15.1) and 12.7 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 10.1–15.5) of PAR respectively.

The seed from lots SN7A, SN7C, and SP3 for the L⁺ and L⁻ treatments was germinated at two temperatures (5/20 and 20/30 °C) for 14 d. Germination in the light treatments was inspected daily between 5 and 7 PM. The dark treatments were inspected at 14 d. All light treatments at 20/30 °C received 1 ml of water at 5 and 10 d after trial establishment. All light treatments incubated at 5/20 °C received 1 ml of water 8 d after the trial was set up. The definition of germination and counts of germinated and viable seed were performed as per Experiment 1.

Analysis

Analysis was performed and results presented as in Experiment 1. With the addition that ANOVA with missing values, was used for the analysis of days to 50 % germination in Genstat Sixth Edition[®] (2002). Means separation tests were made using the Tukey HSD test (Zar, 1984)

4.3.2 Results

Soil temperature

Hourly temperature records from the seed burial site indicated that maximum temperatures of ~ 28 °C were recorded in December 2001. The lowest temperatures of ~ 2.5 °C were recorded in July 2002 (Figure 4-2).

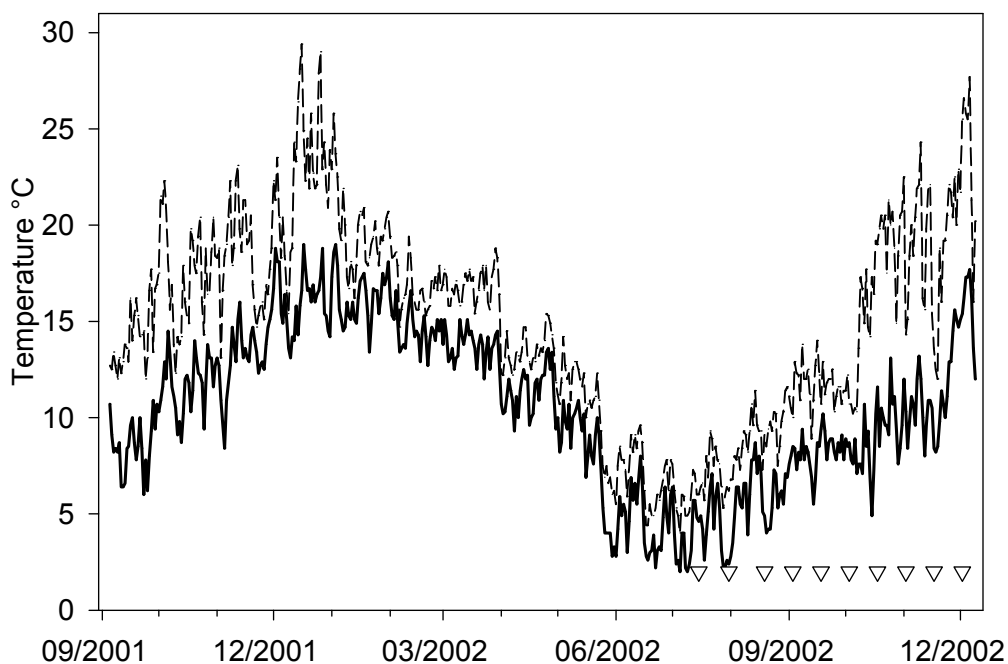


Figure 4-2. Maximum and minimum daily soil temperatures recorded at a depth of 75 mm at the seed burial site. Dates of seed retrievals are indicated by □.

Retrievals – *Solanum nigrum* seed

Seed moisture values for *S. nigrum* seed ranged from 35.8 to 42.6 %, respectively. The soil moisture level in the soil burial bags ranged from 12.5 to 21.4 %.

Germination percent of seed lot SN7A: Seed lot SN7A had a mean 28.6 % non-viable seeds for the ten test dates (15 July to 2 December). Analysis for the factors test date, temperature and light provided three significant ($P < 0.001$) two way interactions. The transformed values for these three interactions are presented in Appendix 6, selected interactions are presented in this section. For the interaction of test date and light (transformed values Appendix 6, non transformed values Figure 4-3), for germination in light, the percentage germination (59 %) significantly declined on the seventh test date, in comparison to the sixth (97 %) and eighth (98 %) test dates. But for germination in darkness, significant increases in germination occurred on the third (79 %) and sixth (67 %) test dates in comparison to the preceding test dates (3 and 12 %, respectively). Percentage germination in darkness also significantly declined following the sixth test date. At all test dates, germination in darkness was significantly lower than the germination in light.

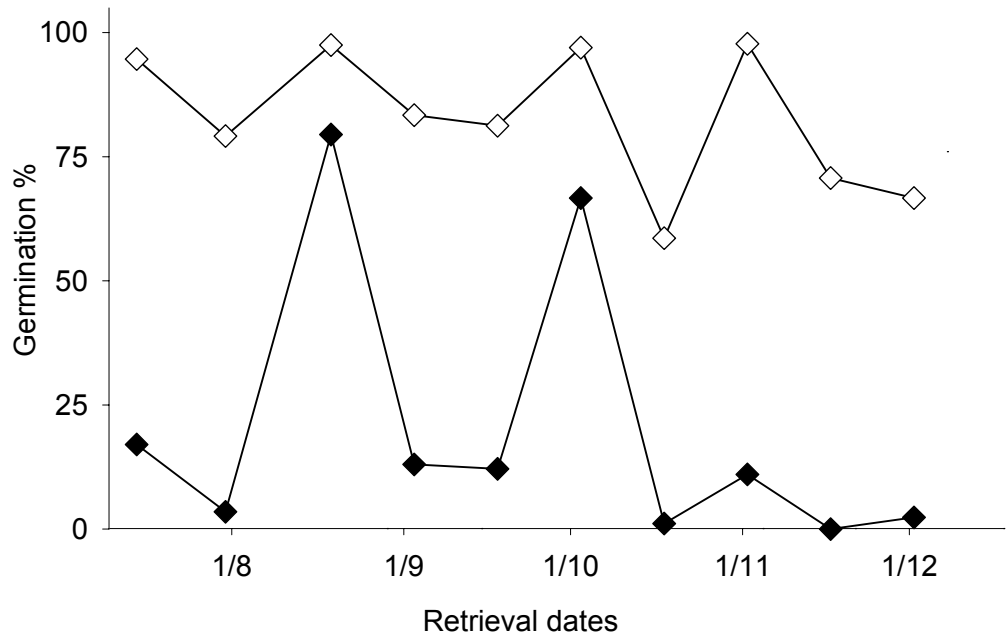


Figure 4-3. The percentage germination of viable seed of *S. nigrum* seed lot SN7A retrieved in 2002. Seeds were germinated in light \diamond and dark \blacklozenge for 14 d. (Experiment 2).

For the interaction of test date and temperature, at 20/30 °C, there was significant variation between test dates, with germination at the third test (85 %) significantly greater than the preceding test (47 %) (transformed values Appendix 6, non transformed values Figure 4-4). Germination at the sixth test date (79 %) also significantly increased in relation to the fourth test date (50 %), and percentage germination significantly declined for the four test final dates (range 41-52 %) following the sixth test date. For germination at 5/20 °C, significant increases in germination were also observed at the third and sixth test dates in relation to the preceding test date. However, at this temperature, a third significant increase was observed at the eighth test date (58 %) in comparison to the preceding test date (13 %), germination at this temperature also significantly declined for the last two test dates in comparison to the eighth test date. Only at the seventh test date did germination differ significantly between germination at 5/20 °C (13 %) and 20/30 °C (47 %).

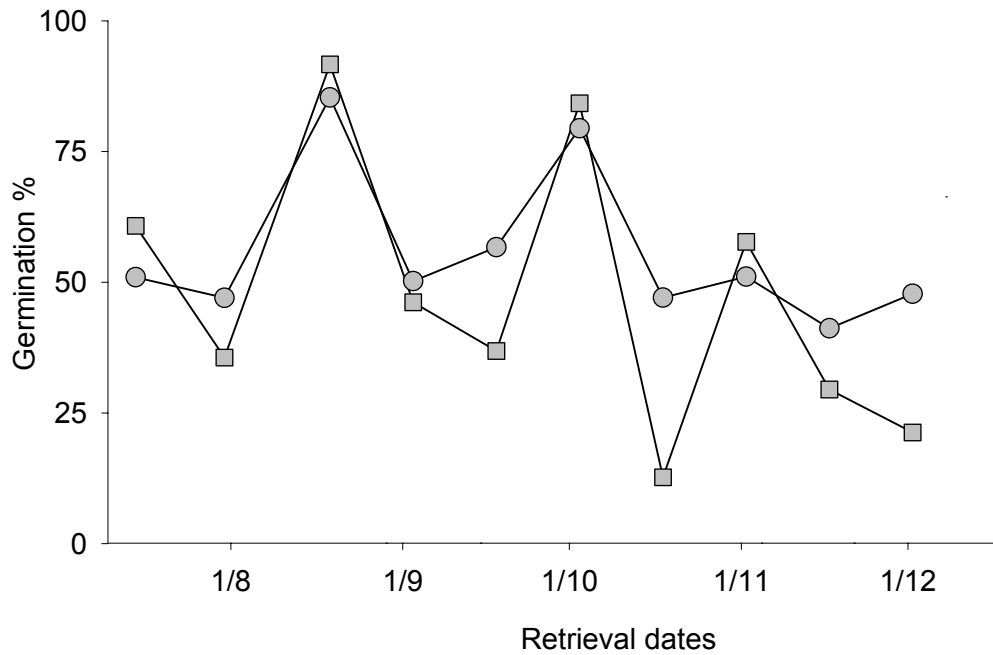


Figure 4-4. The percent germination of *S. nigrum* seed lot SN7A at 20/30 °C (○), 5/20 °C (□) for 14 d. (Experiment 2).

Germination percent comparisons between seed lots SN7A and SN7C: For the eight dates (19 August to 2 December) where seed from lots SN7A and SN7C was retrieved and tested, an analysis of the factors seed lot, temperature, light and test date indicated a significant ($P < 0.001$) four way interaction between these factors, this interaction is presented in Appendix 7. There were also three significant three way interactions, each is presented in Appendix 7. The interactions were: test date, seed lot and temperature ($P < 0.05$); test date, temperature and light ($P < 0.001$); and seed lot, temperature and light ($P < 0.001$). There was no significant interaction between test date, seed lot and light. Selected interactions are presented in this section.

For the interaction between seed lot, temperature and light, percentage germination at all temperatures for both seed lots was significantly lower than for germination in light (transformed values Appendix 7, non transformed values Figure 4-5). However, for germination in darkness at 5/20 °C lot SN7C from black fruit was significantly higher (55 %) than for lot SN7A from green fruit (27 %). The percentage germination of lot SN7C in darkness at 5/20 °C was also significantly greater than the germination of both lots germinated at 20/30 °C in darkness (SN7C 23 % and SN7A 20 %). Percentage germination in light was significantly higher at 20/30 °C than at 5/20 °C for both seed lots, but at neither temperatures did the percent germination in light differ between the two seed lots.

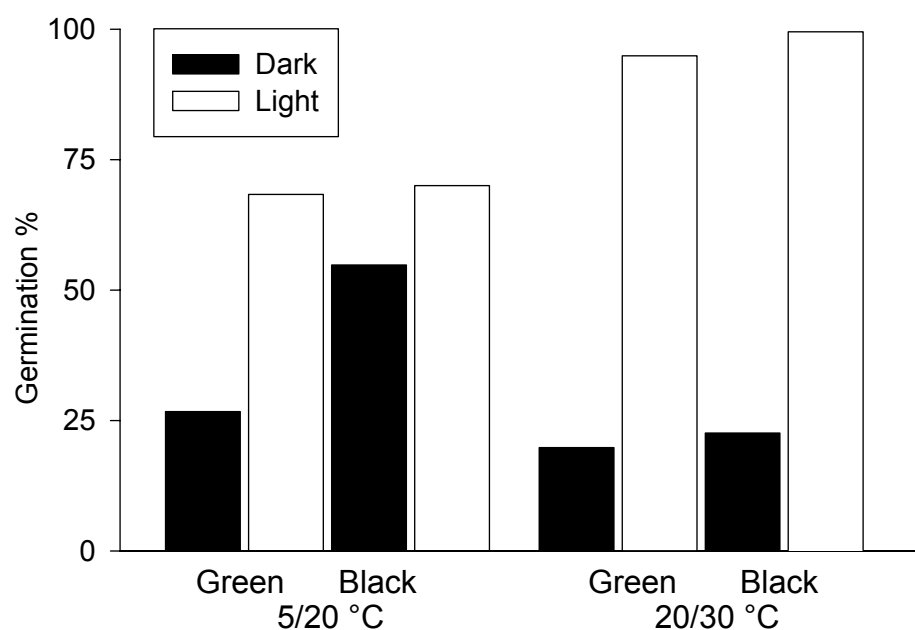


Figure 4-5. The percent germination of two *S. nigrum* seed lots from green fruit (SN7A) and black fruit (SN7C) in light (open bars) and dark (filled bars) at 5/20 °C and 20/30 °C (Experiment 2).

For the interaction between test date, temperature and light, the percentage germination at 20/30 °C in light did not differ between test dates, but germination in light at 5/20 °C differed significantly with test date (transformed values Appendix 7, non transformed values Figure 4-6). With percentage germination declining between the first (96 %) and second (72 %) test date, and fourth (92 %) and fifth (60 %) test dates, and sixth (95 %) and seventh (55 %) test dates. Also significant increases in germination were observed between the third (40 %) and fourth (92 %) test date, and the fifth (60 %) and sixth (95 %) test date. For germination in the dark at 5/20 and 20/30 °C significant declines between the first and second test date were observed, and also from the fourth to the fifth test date. For seed tested at 20/30 °C following the fifth test date there was a significant decline, and for seed tested at 5/20 °C following the sixth test date there was another significant decline. For seed at both temperatures, when tested in darkness, germination significantly increased between the third and fourth test dates.

Germination in light at 20/30 °C in light was significantly greater than germination at 5/20 °C in light except for the first, fourth and sixth test dates. Germination in light at 20/30 °C was significantly greater than germination in darkness at both test temperatures on all test dates. For seed at 5/20 °C, however, germination in light was significantly higher than germination in darkness at this temperature at five of the eight test dates. Seed germinated at 5/20 °C in light had significantly higher germination than that in darkness at 20/30 °C, at all test dates. For seed germinated at

5/20 and 20/30 °C in darkness, germination at 5/20 °C was significantly higher than at 20/30 °C on two test dates.

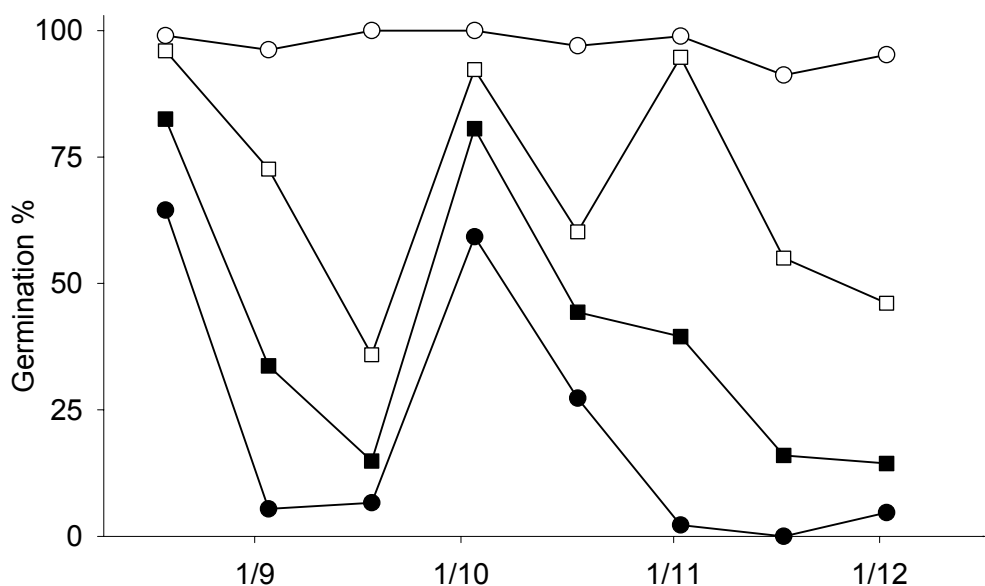


Figure 4-6. The percent germination of viable seed of two *S. nigrum* seed lots in light (open symbols) and dark (filled symbols) on eight dates. Seeds were germinated at 20/30 °C (in light ○ and dark ●), 5/20 °C (in light □ and dark ■) for 14 d (Experiment 2).

Days to 50 % germination of SN7A and SN7C: The day at which 50 % germination (d_{50}) was observed for lots SN7A and SN7C was significantly ($P < 0.01$) affected by the interaction of seed lot, retrieval date and temperature (Table 4-3). For seed lots germinated at 5/20 °C it was notable that for a number of retrieval dates individual replicates or sets of three replicates did not reach 50 % germination prior to the final count at 14 d. There was no significant variation between seed lots at 20/30 °C but there was at 5/20 °C, with individual seed lot d_{50} affected by retrieval date. Lot SN7C had a faster germination than lot SN7A at the 17 November retrieval.

Soil nitrate: There was a nearly linear decrease in soil NO_3 levels from the first to the third sample date (Table 4-4). After this it was unchanged.

Table 4-3. Days to 50% germination (d_{50}) at 5/20 and 20/30 °C for two seed lots (SN7A, seed of green fruit and SN7C, seed of black fruit) of *S. nigrum* (s.e. = 0.36, d.f. = 53, Tukey HSD = 1.99). Means followed by the same letter in columns are not significantly different. (Experiment 2).

Seed lot	d50 at 5/20 °C		d50 at 20/30 °C	
	SN7A	SN7C	SN7A	SN7C
Retrieval date				
19 Aug	9.3 a	11.0 b	3.0 a	3.0 a
3 Sept	12.3 c	12.3 ab	3.3 a	3.3 a
18 Sept	12.3 c	- ³	3.0 a	3.3 a
3 Oct	10.0 ab	10.7 b	3.0 a	3.0 a
18 Oct	- ³	7.3 ab	4.0 a	3.0 a
2 Nov	9.7 a	9.7 a	3.0 a	3.0 a
17 Nov	13.0 c ¹	11.0 ab ²	3.7 a	4.0 a
2 Dec	- ³	- ³	4.0 a	4.0 a

superscript value = number of replicates not reaching 50 % germination at 14 d.

Table 4-4. Soil NO₃ levels (mg/kg dry soil) on eight sample dates, as indicated by a KCL extractable nitrate method (s.e. = 24.8, d.f. = 40). Means followed by the same letter are not significantly different (Tukey HSD = 148.70). (Experiment 2).

Sample date	NO3
13 Sept.	296.1 a
27 Sept.	154.8 b
25 Oct.	44.7 bc
8 Nov.	9.2 c
19 Nov.	21.9 c
28 Nov.	9.6 c
10 Dec.	7.2 c
13 Jan.	15.3 c

***Solanum physalifolium* seed**

Comparisons of percent germination: The seed moisture content of *S. physalifolium* seed ranged from 25.0 to 38.9 %. Germination at 5/20 °C progressively and significantly increased between the first, second and third test dates, but at 20/30 °C it was constant at about 100% (transformed values Appendix 8, non transformed values Figure 4-7). There was no significant difference between light treatments at 20/30 °C, but at 5/20 °C on the third retrieval date (3 September) germination in the dark (94.7 %) was significantly higher than in the light (72.0 %). At this, and the fourth test date (3 October), germination in dark at 5/20 °C did not differ significantly from the germination in light or dark at 20/30 °C. However, for both of these test dates the germination in light at 5/20 °C was significantly lower than germination in light or dark at 20/30 °C.

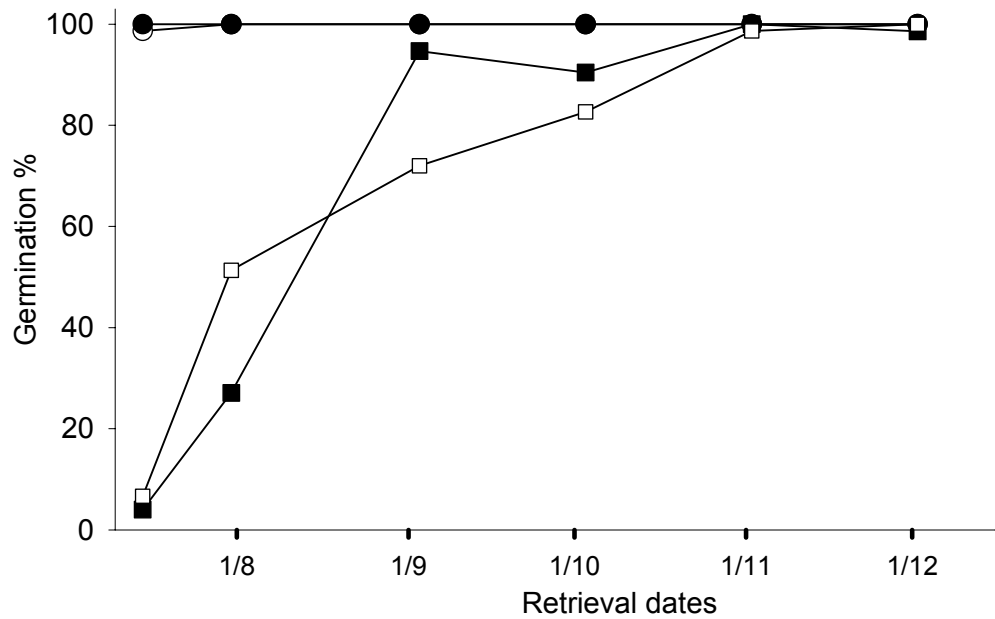


Figure 4-7. The percentage germination of viable seed of field stored *S. physalifolium* seed lot SP3. Seeds were germinated at 20/30 °C (in light ○ and dark ●) or 5/20 °C (in light □ and dark ■) for 14 d. (Experiment 2).

Species d_{50} comparisons

Fifty percent germination was not achieved for *S. physalifolium* seed retrieved on 15 July. This also occurred for the *S. nigrum* lot SN7A at the 18 October retrieval and at the 2 December retrieval (Table 4-5). For seed tested at 20/30 °C the d_{50} values for the *S. physalifolium* and *S. nigrum* did not differ significantly. For seed tested at 5/20 °C the d_{50} values of the *S. physalifolium* seed lot were not significantly affected by retrieval date. Germination of the *S. physalifolium* seed lot at 5/20 °C was significantly faster than both *S. nigrum* lots at all retrieval dates except on 2 November, and for lot SN7A on 19 August. There was significant variation in d_{50} values for *S. nigrum* between test dates with the values for the 2 November and 19 August retrievals significantly lower than at other test dates, except the 3 October retrieval. In addition, the 31 July and 17 November retrieval was significantly greater than the 3 October retrieval.

Table 4-5. Days to 50 % germination (d_{50}) at 5/20 and 20/30 °C for seed lot SN7A of *Solanum nigrum* and of *S. physalifolium* (SP3) (s.e. = 0.32, d.f. = 72, Tukey HSD = 1.84). Means followed by the same letter in columns are not significantly different. (Experiment 2).

Seed lot	d_{50} at 5/20 °C		d_{50} at 20/30 °C	
	SN7A	SP3	SN7A	SP3
Retrieval date				
15 July	11.7 bc	- ³	4.0 a	3.0 a
31 July	13.7 d	9.5 a ¹	3.3 a	3.0 a
19 Aug	9.3 a	9.7 a	3.0 a	3.0 a
3 Sept	12.3 cd	9.3 a	3.3 a	3.0 a
18 Sept	12.3 cd	8.3 a	3.0 a	2.0 a
3 Oct	10.0 ab	8.0 a	3.0 a	2.0 a
18 Oct	- ³	9.0 a	4.0 a	2.7 a
2 Nov	9.7 a	8.7 a	3.0 a	2.7 a
17 Nov	13.0 d ¹	8.3 a	3.7 a	2.0 a
2 Dec	- ³	8.3 a	4.0 a	3.0 a

superscript value = number of replicates not reaching 50 % germination at 14 d.

4.4 Experiment 3 – The effect of short duration light exposure effects on *Solanum nigrum* and *S. physalifolium* germination

This experiment was conducted to test the hypothesis that the germination of *S. nigrum* seed following SDLE at different dates would indicate no change in light requirements, and no variation in SDLE responses between *S. nigrum* seed lots from green and black fruit. It was also hypothesised that *S. physalifolium* germination would not be affected by SDLE (hypothesis 4). Testing used the same field storage procedure as in Experiment 2, with burial of seed of both species in 2001, and testing on ten dates in 2002.

4.4.1 Materials and methods

The seed lots used for this experiment were buried with those used in Experiment 2. For each retrieval date in Experiment 2 the bags containing batches of 225 seeds, as described in Experiment 2, were used for this experiment (Table 4-2). Twenty five seeds were counted into Petri dishes and, at the completion of each count of 25 seeds,

water was misted over the seeds and the dish was placed into a light box. The box contained an air cooled incandescent light (Eye Multi-metal Lamp, MF 400 X/U) with a remotely activated mechanical shutter with a timer control. The shutter when open, exposed the Petri dish, which was in a light proof draw at the base of the light box, to the light. The measured light intensity at the level of the base of the Petri dish, and with the shutter open, gave a PAR of 221.0 $\mu\text{mole}/\text{m}^2/\text{s}$ and a R:FR of 2.4. Light measured with the shutter closed gave a PAR of 0.005 $\mu\text{mole}/\text{m}^2/\text{s}$. The replicates were exposed to one of three light treatments:

1. 10 seconds with the shutter closed (PAR, 0.050 $\mu\text{mole}/\text{m}^2$)
2. exposed to 4.6 s light (PAR, 1,017 $\mu\text{mole}/\text{m}^2$)
3. exposed to 25.8 s light (PAR, 5,701 $\mu\text{mole}/\text{m}^2$)

After light exposure, dishes were sealed and covered with black polythene, as described in Experiment 2. For each seed lot being tested at a retrieval date three replicates were exposed to each of the above three treatments. The seeds were germinated at 20/30 °C for 14 d, as described for the L⁻ treatments in Experiment 2. Testing of *S. nigrum* seed lot SN7A was made on ten test dates and lot SN7C, on four test dates. Testing of *S. physalifolium* was not continued after the first two test dates. The analysis was performed, and presented as in Experiment 1.

4.4.2 Results

The results for each species are presented separately.

Solanum nigrum

Seed lot SN7A: There was a significant interaction ($P < 0.001$) between retrieval date and light duration (transformed values Appendix 9, non transformed values Figure 4-8). On no test date was there a significant difference between the 25.8 and 4.6 s light exposures. For both of these treatments germination increased significantly at the third, fourth and fifth test dates compared with the first and second test dates. Germination declined significantly at the final test (2 December) compared with the seventh, eighth and ninth test dates. Germination also declined significantly in the 4.36 s light treatment at the sixth test date (65 %) compared with the third test date (100 %). The germination of the 0 s light treatment was significantly lower than both the 25.8 and 4.6 s light treatments at the third (19 %), and the sixth to ninth test dates (range 0-13 %). Germination for this treatment was also significantly less (2 %) than the 25.8 s (52 %) treatment at the second test date. Throughout the experiment germination in

the nil light treatment's germination was generally lower than both the other light treatments.

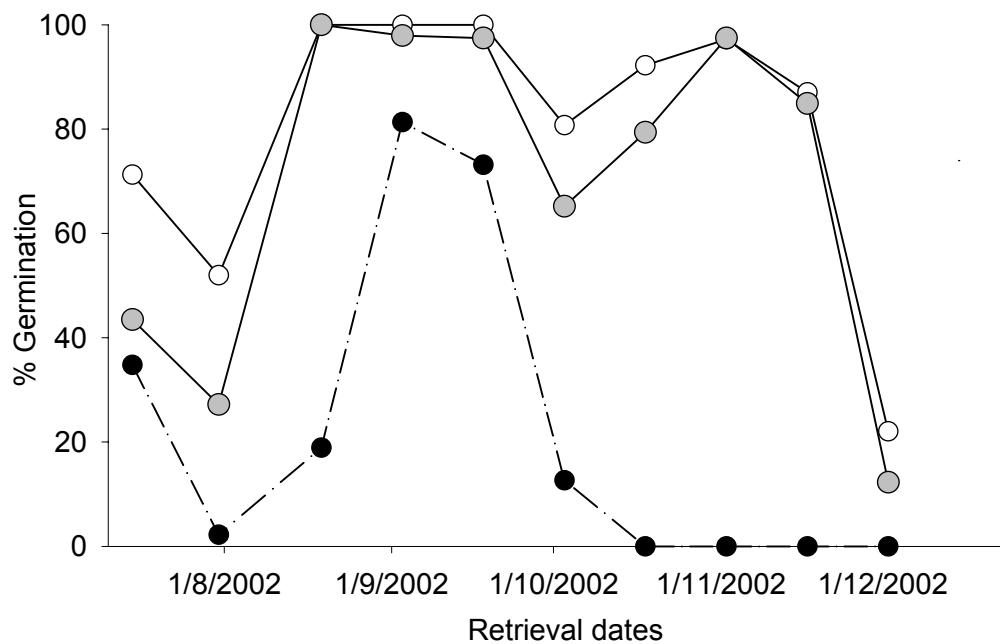


Figure 4-8. The percent germination of viable *S. nigrum* seed (SN7A) at 14 d. Seeds were germinated at 20/30 °C, following exposure to 25.8 seconds light (○), 4.6 seconds light (grey fill), and 0 s light exposure (●). (Experiment 3).

Seed lot comparisons: There was a significant ($P < 0.001$) interaction between light duration and test date, and a significant ($P < 0.001$) interaction between seed lot and light duration (transformed values Appendix 9, non transformed values Table 4-6). Test date, seed lot and light duration did not interact significantly. For the interaction between light duration and test date at no test date did light duration differ with test date for the light durations of 4.6 and 25.8 s, but for the nil light duration treatment the November test (3 %) had significantly lower germination than the preceding four test dates (Table 4-6, a). And the September test (68 %) had significantly higher germination than the August (32 %) and November (28 %) tests. In addition the September value for the nil light duration treatment did not differ significantly from that of the 25.8 s exposure (85 %). For the interaction between seed lot and light duration, seed lot SN7C had significantly higher germination for the nil light duration than lot SN7A (Table 4-6, b)

Table 4-6. The percent germination of viable seed after 14 d at 20/30 °C for two seed lots (SN7A, seed of green fruit and SN7C, seed of black fruit) of *S. nigrum* on four dates and exposed to 25.8, 4.6 or 0 s of light prior to germination testing in the dark. a) Test date and light duration interaction. b) Seed lot and light duration interaction. (Experiment 3).

a) Light duration (s)	0 s	4.6 s	25.8 s
Test date			
19 Aug	33	99	100
3 Sept	85	99	95
18 Oct	33	88	95
2 Nov	1	96	97

b) Light duration (s)	0 s	4.6 s	25.8 s
Seed lot			
SN7A	25	94	97
SN7C	51	98	97

Solanum physalifolium

Seed of SP3 was tested on 15 and 31 July 2002, the germination in each of the three light treatments was 100 % of viable seed. Statistical analysis was therefore not possible due to the lack of variation in the variable. Testing of SP3 seed in 2002 was discontinued after these tests.

4.5 Experiment 4 – Dark cultivation effects on *Solanum nigrum* and *S. physalifolium* field germination

This work was conducted to test the hypothesis that dark cultivation will not reduce the field germination of *S. nigrum*, or *S. physalifolium* (hypothesis 7). To test this hypothesis two dark cultivation experiments (4A and 4B) were conducted, in 2001 and 2002, respectively.

4.5.1 Materials and methods

Both experiments were carried out at Lincoln University, Lincoln (latitude 43°38' longitude 172°28').

Dark cultivation - Experiment 4A

Trial area and design: An area of field H19 in the Horticultural Research Area of Lincoln University was used for the trial. The trial design consisted of five blocks (18 m

× 10 m). Each block had two main plots (each plot 9 × 10 m) of *S. nigrum* or *S. physalifolium*. In each main plot there were three sub plots (3 × 10 m) which were: un-covered cultivation and un-covered pea drilling by day; covered cultivation and covered pea drilling by day; un-covered cultivation and un-covered pea drilling after sunset. Prior crop and trial use of the area was 1998-9 *Avena sativa* L., 1999-00 *Zea mays* and *Chamaecytisus palmensis* (Christ) Bisby et K. Nicholls, 2000-01 was *Brassica campestris* ssp. *Pekinensis* L. Soil samples (20 × 150 mm depth cores), taken from the test area on 12 February 2003, gave soil test values of 17, 10, 14, 6 (MAF units) and 5.7 for Olsen P, K, Mg, Ca, and pH respectively. The area was ploughed in the autumn, and was grubbed six weeks prior to sowing nightshade seed.

Nightshade establishment and treatment methods: The main plots were sown either with a mixture of *Solanum nigrum* (SN1, SN2 and SN9B seed lots) or with a mixture of *S. physalifolium* (SP1, SP2, SP3 and SP4 seed lots) on 27 July 2001 (Appendix 3). The sowing rate was 100 viable nightshade seeds/m². The seed was drilled at a depth of 3.5-4 cm with a tractor driven Öyjord cone seeder. The area was sprayed on the 26 September with Glyphosate (active ingredient 360 g/l), 1 l/h with a Cropland boom sprayer fitted with 11-004 Teejet nozzles. Water delivery rate was 232 l/hour at 300 KPa, and the unit was driven at 8 km/h.

The cultivation and sowing treatments were carried out on 1 October 2001. Prior to cultivation, a single soil moisture reading, at a depth of 120 mm, and soil temperature, at a depth of 105 mm, were recorded from every sub-plot. Cultivation was with a Duncan Vibrotiller fitted with a rotating crumbler.

The covers of the cultivator and the seed drill are shown in Appendix 10. Covered daylight cultivation and un-covered daylight cultivation was carried out between 1:20 and 2:00 PM. A Sunfleck Ceptometer (Decagon) was used to record PAR under the covered and uncovered cultivation and sowing equipment. To limit potential transfer of nightshade seed between main plots the coulters of the cultivator and drill were brushed and wiped clean of adhering dirt, as required. Peas (cv. Bounty) were drilled at 296 kg/ha at 170 mm row spacings with a Fiona F873 (Schery Maskinfabrik) seed drill in daylight, for both treatments, between 3:10 and 3:45 PM. Night cultivation was initiated 1 h 30 min after solar sunset, cultivation and drilling occurred between 8:00 and 8:20 PM. Night operations were completed with only the front lights of the tractor on.

On 2 October 2001, in daylight, the exterior area from the plot edge to fence (~ 6 m) surrounding and between the two areas of plots was sown with peas at twice the

plot density. A 0.28 m edge area of each sub-plot, not sown by the Fiona seeder, was drilled with a hand operated cone seeder (Hans-Urlich Hege-Maschinbau) with three rows drilled into the 0.56 m between neighbouring sub-plots. Twenty four mm of irrigation water was applied to plots on 19 December 2001.

Sampling: A sample area of 8.0 × 0.5 m in each sub-plot (10 × 3 m) was marked out. The sample area was divided into 8, 0.5 × 1 m sample areas, and a random number table was used to allocate the eight areas. Three weed emergence sample areas, and five weed and pea dry matter (DM) and leaf area (LA) sample areas were allocated. Within the three emergence sample areas a 0.1 m² rectangular quadrat was placed and the interior corners marked with plastic stakes (3 mm D). These stakes were used to reposition the quadrat at later samplings. In all emergence experiments, when analysed seedling density was calculated on a 1 m² basis.

Only nightshade species were counted in the three fixed quadrats on 13 October and 21 October, and nightshades and some other weed species were counted between 1 and 4 November. On 2 January 2002 the number of nightshade and *Chenopodium album* plants were counted in two fixed quadrats in three blocks only. The five DM and LAI areas were sampled on the 7 to 8 November, 16 November, 24 to 25 November, 30 November to 1 December and 17 December. On the 29 December one of the fixed emergence quadrats was also sampled. At each of these dates the number of *S. nigrum* and *S. physalifolium* seedlings was counted.

Analysis of the results of this experiment used the repeated measures analysis of variance, split plot procedure in Genstat 6th Edition © (2002) for counts of seedlings in fixed quadrats. The analysis of variance split plot procedure was used for counts of seedlings from DM and LAI destructive sample quadrats.

Dark cultivation - Experiment 4B

Trial area and design: Two separate experimental test areas were established for *S. nigrum* and *S. physalifolium*. The *S. nigrum* area was a randomised block split plot design. There were 10 blocks with six main plots per block. Each main plot had a split plot treatment of *S. nigrum* seed sown, or a control. This gave 120 experimental plots (sub-plots), of 2.65 × 0.6 m. The main plot treatments were cultivation × *S. nigrum* seed source. There were three cultivation treatments; un-covered cultivation during the day, covered cultivation during the day, and un-covered cultivation after sunset. Two *S. nigrum* seed sources were used. Three of the main plots were sown with seed from black fruit (SN11A) of *S. nigrum*, and three of the main plots were sown with seed from

green *S. nigrum* fruit (SN11B) (Appendices 3 and 5). The three cultivation treatments and the two seed sources gave six main plot treatments per block.

The *S. physalifolium* area was a randomised split plot design. There were 20 main plots and two main plot treatments; un-covered cultivation during the day, and un-covered cultivation after sunset. Each main plot had a split plot treatment of *S. physalifolium* seed (SP4) sown, or a control. This gave 40 sub-plots of 2.65 × 0.6 m.

Establishment and treatment methods: The area used was in H18 of the Horticultural Research Area, Lincoln University. This had previously consisted of grazed pasture (ex. pasture composition experiment, March 1995 to December 1999). The pasture was sprayed off with Glyphosate (2 l/ha) in February 2002, and was top worked and ploughed in the third week of August. In the first week of September the area received a final grub and roll. Seed lot SN11A (from black fruit) and SN11B (from green fruit) *S. nigrum* seed for seeded plots were broadcast onto a 1.65 × 0.6 m area on 10 September 2002 at 1000 viable seeds/m². On the same date, *S. physalifolium* SP6 seed (area 3b) was sown using the same method at 193 viable seeds/m² per plot. Seed was hand sown on to the soil surface. After sowing all plots received two passes (in opposite directions) with a hand operated rotary hoe (S.E.P 1700 Special). The rotary hoe shield and tines were cleaned when changing from seeded to unseeded plots. The mean cultivation depth was 100 mm.

On 11 September 2002 seven additional plots, adjacent to area 3b and of the same size as above, were established to monitor soil nitrate level. These plots were cultivated on 11 September and 21 November 2002, with two passes in opposite directions of the rotary hoe on each date. A soil corer (50 mm D) was used to take a single soil sample to 80 mm depth from the centre of each plot. Soil samples were taken on the 13 September, 27 September, 25 October, 8 November, 19 November, 28 November, 18 December, and 13 January. The seven samples and two blanks were prepared on the day of sampling using the 2M KCl extractable nitrate method as described for Experiment 2. Soil moisture was measured for each sample, by drying a single ~ 150 ml sample from each plot at 105 °C for 48 h.

For this experiment cultivation treatments were made on 14 November 2002. Weeds in the trial area were sprayed off on 13 November 2002 with an application of Interceptor (active ingredient pine oil 650g/litre) at the equivalent of 147 l/ha in 2,069 l of water/ha using a back pack sprayer with a foaming applicator nozzle. On 14 November 2002 soil moisture and soil temperature records, using the same equipment as for Experiment 4A, were taken from 10 plots diagonally across the experimental area. Cultivation was with a S.E.P rotary hoe; a Perspex shield was fitted over the

rotary hoes tines in place of the usual metal shield. The PAR transmission through this shield was measured at 55 % of incident PAR. When covered with polythene no PAR was transmitted through the shield (Appendix 10).

For the November 2001 cultivation, day light cultivation treatments of both areas was between 12:25–3:00 PM. The PAR, using a Sunfleck Ceptometer, during cultivation of each sub-plot was recorded. Night cultivation started 2 h 40 min after solar sunset, with cultivation occurring from 11:10 PM–2:15 AM. Between sub-plots, the hoe tines and the shield were cleaned to limit nightshade seed transfer between plots. No light sources were used during the night cultivation. A quantum sensor (Licor) positioned at the site recorded PAR levels during the course of the day and the night. Heavy rain, after sowing, caused soil capping; a light application of water (2 mm) was applied on the 26, 27, 28 and 30 December and on 2 January, at dusk, to soften the soil surface over night and facilitate seedling emergence.

Sampling: Emergence counts were made, using a randomly placed 0.05 m² circular quadrat. Three counts were taken per plot. Nightshade emergence prior to the cultivation treatments in the area sown with *S. nigrum* seed was sampled on the 10, 11 and 13 November and in the area sown with *S. physalifolium* seed on 13 November. After the November 2002 cultivation, on 10 December, the *S. nigrum* area was sampled and the position of the quadrats was marked. This sampling was made in the *S. physalifolium* area on 12 December 2002. On 17 December 2002 the marked quadrats in both experimental areas were sampled.

Statistical analysis: The three 0.05 m² quadrat emergence counts from each sub-plot were averaged and weed numbers calculated on a per m² basis for statistical analysis. Analysis of the results of these experiments used the repeated measures analysis of variance, split plot procedure in Genstat 6th Edition (2002). Analysis of seedling counts after the cultivation treatments for the *S. nigrum* test area, used the precultivation treatment *S. nigrum* seedling counts as a covariate. This covariate was significant ($P < 0.01$) at the level of block and the block/main plot stratum. Means separation tests were made using the Tukey HSD test (Zar, 1984)

4.5.2 Results

Results for experiments 4A and 4B are presented in chronological order.

Dark cultivation - Experiment 4A

The mean soil moisture prior to the cultivation was 14.9 % (range 9-23 %) and mean soil temperature was 11.8 °C (range 11.6-12.2 °C). The mean PAR recorded during uncovered daylight cultivation was 1,502 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 1,158-1,652 $\mu\text{mole}/\text{m}^2/\text{s}$), and during periods of covered daylight cultivation it was 1,496 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 1,427-1,536 $\mu\text{mole}/\text{m}^2/\text{s}$). During the uncovered sowing the mean PAR was 299 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 254-360 $\mu\text{mole}/\text{m}^2/\text{s}$) and for the covered sowing it was 518 (444-583 $\mu\text{mole}/\text{m}^2/\text{s}$). During cultivation and sowing at night the mean PAR was 0.3 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 0-0.7 $\mu\text{mole}/\text{m}^2/\text{s}$). The mean PAR under the stationary cultivator, when covered, was 0 $\mu\text{mole}/\text{m}^2/\text{s}$ and under the stationary uncovered cultivator it was 899.4 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 649-1,179 $\mu\text{mole}/\text{m}^2/\text{s}$). Similarly, under the stationary covered seeder the mean PAR was 0 $\mu\text{mole}/\text{m}^2/\text{s}$, and under the stationary uncovered seeder it was 38.8 $\mu\text{mole}/\text{m}^2/\text{s}$ (range 36.8-76.3 $\mu\text{mole}/\text{m}^2/\text{s}$). Values for PAR at night were 0 $\mu\text{mole}/\text{m}^2/\text{s}$ under both the cultivator and seeder.

Weed seedling counts from fixed quadrats: Only time had a significant effect ($P < 0.001$) on the number of nightshade seedlings present (Table 4-7). No other factor had a significant main factor effect and there was no significant interaction. There were also no significant treatment effects for *Chenopodium album* and *Spergula arvensis* L. numbers (Table 4-8).

Table 4-7. Counts of nightshade (NS) seedlings/ m^2 (sample date s.e. = 25.0, cultivation s.e. = 23.9, d.f. = 48) for three cultivation treatments on three dates. Means followed by the same letter in rows are not significantly different (Tukey HSD = 110.7). (Experiment 4A).

DAS	12	20	34
Cultivation			
Cover	10 a	177 b	202 b
Night	12 a	191 b	244 b
Day	10 a	171 b	265 b

Table 4-8. Counts of *Chenopodium album* (s.e. = 24.5, d.f. = 16, Tukey HSD = 89.3) and *Spergula arvensis* seedlings/ m^2 (s.e. = 30.9, d.f. = 16, Tukey HSD = 112.7) for three cultivation treatments. Means followed by the same letter in columns are not significantly different as indicated by the Tukey HSD. (Experiment 4A).

Cultivation	C. album	S. arvensis
Cover	177 a	226 a
Night	181 a	254 a
Day	178 a	226 a

Weed counts from single non-fixed quadrats: There was no effect of cultivation on weed counts from the non-fixed quadrats (Table 4-9). However, there was a significant interaction ($P < 0.01$) between sample date and the main plot (sown with *S. nigrum* or *S. physalifolium*) (Table 4-10). There was a significant decline in the number of *S. nigrum* seedlings for the final two sample dates compared to counts at 37, 54 and 60 DAS of peas. There were significantly more *S. nigrum* seedlings in the plots sown with *S. nigrum* at 46 DAS, than in plots sown with *S. physalifolium* on the same day.

Table 4-9. Counts of *S. nigrum* seedlings/m² (s.e. = 15.0, d.f. = 16) for three cultivation treatments. Means followed by the same letter in columns are not significantly different (Tukey HSD = 133.7). (Experiment 4A).

Cultivation	<i>S. nigrum</i>
Cover	279 a
Night	275 a
Day	222 a

Table 4-10. Counts of *S. nigrum* seedlings/m² in plots sown with *S. nigrum* or *S. physalifolium* seed on six dates indicated as days after sowing (DAS) of peas (s.e. = 56.7, d.f. = 120). Means followed by the same letter in rows are not significantly different (Tukey HSD = 197.4). (Experiment 4A).

DAS	37	46	54	60	79	89
Main plot						
<i>S. nigrum</i>	391 c	429 c	366 bc	425 c	172 ab	97 a
<i>S. physalifolium</i>	227 a	215 a	208 a	292 a	163 a	117 a

The analysis for the number of *S. physalifolium* seedlings indicated that only sample date had a significant effect ($P < 0.05$) (Table 4-11). The greatest number of *S. physalifolium* seedlings was observed at 46 DAS of peas, and at 79 DAS the number of *S. physalifolium* seedlings was significantly lower than at 46 DAS.

Table 4-11. Counts of *S. physalifolium* seedlings/m² on six different intervals (days after sowing (DAS)) (sample date s.e. = 7.69, d.f. = 120). Means followed by the same letter are not significantly different (Tukey HSD = 22.28). (Experiment 4A).

DAS	37	46	54	60	79	89
No. seedlings	17.3 ab	34.0 b	21.0 ab	27.0 ab	11.0 a	13.7 ab

Dark cultivation - Experiment 4B

Pre-light cultivation emergence counts: For the *S. physalifolium* test area, seed sown had a significant effect ($P < 0.001$) on counts of *S. physalifolium* (Table 4-12). Analysis indicated that 4.5 % of the sown *S. physalifolium* seed had emerged at the pre-cultivation count at 13 November. For the *S. nigrum* test area, only one

S. physalifolium seedling was recorded (where half of the sub-plots were sown with *S. nigrum*). Analysis of seedling counts for the factor seed sown and seed source (seed from green or black *S. nigrum* fruit) indicated that seed source had a significant effect ($P < 0.05$) but that seed sowing was not significant (Table 4-13). Further analysis of main plot effects from this area indicated that main plots had a significant ($P < 0.001$) effect (Table 4-14). This indicated that one side of the trial area had a significantly greater density of nightshade seedlings.

Table 4-12. The number of *S. physalifolium* seedlings/m² in sown and unsown plots prior to cultivation treatments (s.e. = 1.32, d.f. = 19, Tukey HSD = 3.90). (Experiment 4B).

Sown	Not sown
8.7	0.3

Table 4-13. a) The number of *S. nigrum* seedlings/m² in main plots designated as black or green *S. nigrum* seed sources (s.e. = 13.45, d.f. = 58, Tukey HSD = 38.05), b) and plots sown or not with *S. nigrum* seed (s.e. = 5.53, d.f. = 29, Tukey HSD = 15.98). (Experiment 4B).

a) Seed source	Black	Green
	66 a	105 b
b) Seed sown	Sown	Not sown
	88 a	82 a

Table 4-14. The number of *S. nigrum* seedlings/m² (s.e. = 21.11, d.f. = 45, Tukey HSD = 89.32). Means followed by the same letter are not significantly different. (Experiment 4B).

Main plots	Seedlings
1	14 a
2	294 a
3	32 a
4	93 ab
5	194 b
6	150 b

November cultivation: The mean percent soil moisture prior to cultivation was 14.6 % (range 11-21 %) and the mean soil temperature was 16.8 °C (range 16.0-17.6 °C). The mean PAR, recorded by the quantum sensor, during the daylight cultivation in the *S. nigrum* area, was 1,533 $\mu\text{mole/m}^2/\text{s}$ (range 1,170-1,790). The mean PAR, recorded by the quantum sensor during the daylight cultivation in the *S. physalifolium* area, was 979 and 987 $\mu\text{mole/m}^2/\text{s}$ (range 710-1,285) for the covered and un-covered plots, respectively. During the night cultivation for both experimental areas the PAR was 0 $\mu\text{mole/m}^2/\text{s}$.

Post November cultivation treatment emergence counts: Soil capping was a problem in the plots, as 66.9 mm of rain fell in the 10 days following the cultivation treatments. Repeated measures analysis indicated that seed sown was the only significant ($P < 0.001$) factor for the analysis (Table 4-15, a). Both time and cultivation had no significant effect (Table 4-15, b). Analysis of counts of resident *S. nigrum* seedlings from this area indicated there was no significant cultivation effect (data not presented). Analysis indicated that 12.6 % of the sown *S. physalifolium* seed had emerged 34 d after the 14 November 2002 cultivation and 17.1 %, in total, for the pre and post November cultivation. The repeated measures analysis for the *S. nigrum* area indicated that seed sowing had a significant ($P < 0.001$) effect, but that cultivation had no significant effect (Table 4-16). Time interacted ($P < 0.01$) with seed source and seed sown (Table 4-16). With significantly more seed present in plots sown with seed from black fruit at the second count date, this did not occur for plots sown with seed from green fruit. Analysis indicated that 1.7 % of sown *S. nigrum* seed had emerged.

Table 4-15. The number of *S. physalifolium* seedlings/m² from repeated measures analysis 27 and 34 d after cultivation a) seed sowing effects (s.e. = 2.51, d.f. = 76, Tukey HSD = 7.09), b) cultivation effects (s.e. = 2.51, d.f. = 76, Tukey HSD = 7.09). (Experiment 4B).

a) Seed sown	absent	present
	3.7	14.5
b) Cultivation	day	night
	9.8	8.4

Table 4-16. The number of *S. nigrum* seedlings/m² from repeated measures analysis 25 and 34 d after cultivation a) seed sowing effects (s.e. = 3.3, d.f. = 98, Tukey HSD = 9.3), b) cultivation effects (s.e. = 2.5, d.f. = 76, Tukey HSD = 7.1). Means followed by the same letter in rows are not significantly different. (Experiment 4B).

a) Seed sown	absent	present	
	17	34	
b) Cultivation	day-no cover	day-with cover	night
	26 a	27 a	23 a

Table 4-17. *Solanum nigrum* seedlings/m² from repeated measures analysis for the interaction of time (days after cultivation) seed source and seed sown (s.e. = 1.0, d.f. = 108, Tukey HSD = 2.8). Means followed by the same letter in rows and columns are not significantly different. (Experiment 4B).

Seed source	black fruit		green fruit	
	absent	present	absent	present
Time				
25	19 b	35 d	13 a	31 c
34	19 b	39 e	16 a	32 cd

Soil NO₃ levels (mg/kg dry soil) increased significantly ($P < 0.001$) between the first and fourth sample date (Table 4-18). Soil NO₃ levels dropped significantly following the second cultivation of the plots on 21 November 2002, for tests made on 28 November and 10 December 2002. However, by the final sample date in January 2003 soil NO₃ levels had increased significantly compared to the November and December tests.

Table 4-18. Soil NO₃ and NO₃-N levels (mg/kg dry soil) on eight sample dates in 2002 and 2003, as indicated by a KCL extractable nitrate method (s.e. = 62.2, d.f. = 47). Means followed by the same letter are not significantly different (Tukey HSD = 281.1). (Experiment 4B).

Sample date	NO ₃
13 Sept.	347 a
27 Sept.	693 ab
25 Oct.	1,154 abcd
8 Nov.	1,639 bcd
19 Nov.	1,945 cd
28 Nov.	518 ab
10 Dec.	900 abc
13 Jan.	2,298 d

4.6 Discussion

4.6.1 Dormancy of freshly collected *Solanum nigrum* seed

Collection site and fruit colour effects

Two seed lots collected in Lincoln and tested in 20/30 °C for 14 d in light had low levels of germination (< 10 %) (Figure 4-1). Results for *S. nigrum* seed collected from Lincoln and Gisborne, in 2001, and stored dry for 6-12 weeks at 5 °C before testing under the same conditions, for 21 d, also gave low germination levels of 12 and 23 %, respectively (Bithell *et al.*, 2002) (Appendix 1). However, the germination of a seed lot collected, in 2002, from Gisborne was ~ 70 % and both Manawatu seed lots had ~ 40 % germination, after 14 d (Figure 4-1). Furthermore, in the absence of light, pre-germination chilling and KNO₃ individual seed lots from the Manawatu and Gisborne had a ~30-35 % germination. It is reported that *S. nigrum* seed collected in New Zealand had primary dormancy (Bithell *et al.*, 2002) (Appendix 1). The results from this present work indicate that primary dormancy is present to different extents in different seed lots (Figure 4-1), providing no support for the null hypothesis that the germination

requirements of freshly harvested *S. nigrum* seed collected from different sites does not differ (hypothesis 1).

With regard to the effect of fruit colour, it was hypothesised that the germination requirements of seed from black and green fruit does not differ (hypothesis 1). However, there were significant differences in the germination between some seed lots of black and green fruit collected at the same site. For example, for seed from the Manawatu, seed from green fruit had higher germination than seed from black fruit in the presence of prechilling or nitrate only (Figure 4-1). This indicated some support for the report of Kazinczi and Hunyadi (1990), that seed from black *S. nigrum* fruit was more dormant than seed from green fruit. However, the germination responses of seed from black and green fruit differed when comparing the germination of seed of the same fruit colour from different sites. This indicates no consistent colour effect. In addition, the most dormant seed of the six seed lots, in this present work, was seed from a seed lot of green fruit (Lincoln collection, seed lot SN11B). The implication of the results from this present work is that for *S. nigrum* seed collected from different sites, and for *S. nigrum* seed from different coloured fruit, germination requirements will differ.

Ranking the germination requirements of seeds with primary dormancy

It is reported that there is no evidence to suggest that the mechanisms regulating primary and secondary dormancy differ for seed with physiological dormancy (Khan, 1996; Foley, 2001). Therefore, understanding the importance of different factors for the germination of seed with primary dormancy is also useful for understanding the effects of similar factors for seed with secondary dormancy. Comparisons of the factors, light, pre-germination chilling and KNO_3 when singularly present, indicated that pre-germination chilling caused significantly higher germination of seed lots, compared with the two other factors (Figure 4-1). There was no difference in seed lot germination between light and KNO_3 treated seed. This result agrees with the identification of temperature as the most important factor influencing the dormancy status of seed with non-deep physiological dormancy (Vleeshouwers *et al.*, 1995).

For the two factor combinations of pre-germination chilling and light, KNO_3 and pre-germination chilling, and KNO_3 and light (Figure 4-1). Pre-germination chilling and light had a significantly greater effect on seed lot germination than the other two, two factor combinations. All three factors were required to significantly increase the germination response of only one *Solanum nigrum* seed lot (SN11B). This indicates that only for the most dormant *S. nigrum* seed is light, nitrate and prechilling necessary to maximise germination for seed tested at 20/30 °C. It is reported that to achieve the

maximal germination of laboratory stored *Chenopodium album* and *Capsella bursa-pastoris* L. seed with primary dormancy, that prechilling, light, and nitrate and alternating temperatures were required (Roberts and Benjamin, 1979). However, this report was for work based on single seed lots. The results of this present work indicate that the importance of the above factors can differ for seed from different sites.

4.6.2 Dormancy and light

It was hypothesised that: field stored *Solanum nigrum* seed has a light requirement for germination that is constant due to a static seed dormancy status (hypothesis 2); that *S. physalifolium* seed does not have a light requirement (hypothesis 3); that the germination of *S. nigrum* seed following SDLE at different dates would indicate no change in light requirements, and no variation in SDLE responses between *S. nigrum* seed lots from green and black fruit, it was also hypothesised that *S. physalifolium* germination would not be affected by SDLE (hypothesis 4); that the dormancy of field stored *S. physalifolium* and *S. nigrum* seed does not differ as indicated by percentage germination at the sub-optimal temperature of 5/20 °C, and time to 50 % germination (hypothesis 5); and that there are no differences in the germination requirements of field stored *S. nigrum* seed from black and green fruit (hypothesis 6). This section addresses the evidence for these hypotheses.

Methodology

For studies of seed light responses it is reported that green light filters, as used in this present work, need to be used with caution, as significant germination responses to green light filters have been reported for some plant species (Baskin and Baskin, 1979). Germination responses to green filters are reported to be due to a VLFR status in the seed, where a positive germination response is observed to wavelengths and light intensities that do not normally stimulate germination (Pons, 1992; Hartman and Mollwo, 2002). Nevertheless, such filters are commonly used (Roberts and Lockett, 1978; Froud Williams *et al.*, 1984; Benvenuti and Macchia, 1998; Murdoch, 1998; Benvenuti *et al.*, 2001). Because of possible green light stimulated germination effects, it is appropriate to acknowledge that the observed germination responses, especially for the dark control treatments, could include very low fluence responses.

The use of green filters may confound the ability to detect the source of germination stimulation in dark treatments, i.e. whether germination in the dark results from a VLFR response or from an absence of light requirement. However, it can be argued that in terms of evaluating the suitability of dark cultivation, that determination of

the cause of dark germination is not as important as firstly identifying whether it occurs or not.

Germination requirements for light, and seed dormancy status

Solanum nigrum: The dormancy status of the *S. nigrum* seed changed during the test period in Experiment 2. Changes in dormancy status are identified by the percentage germination at different temperatures, germination rates, and germination responses to other factors such as light (Karssen and Vries, 1983; Karssen and Hilhorst, 1992; Murdoch, 1998). For seed lot SN7A there were significant differences in percentage germination, at both 5/20 and 20/30 °C, observed on a number of test dates (Figure 4-4). In addition, over the same period the d_{50} value at 5/20 °C exhibited significant variation between test dates, and also 50 % germination at two test dates was not achieved by 14 d (Table 4-5). Similar variability in d_{50} values at 5/20 °C were also observed for another *S. nigrum* seed lot, SN7C, when tested at eight dates (Table 4-3). These results are consistent with the classification of *S. nigrum* as a species with dormancy that cycles from non-dormancy to conditional dormancy (Baskin and Baskin, 1998).

The light requirements for the *S. nigrum* seed lot SN7A in Experiment 2 were not constant, nor was there any simple pattern of a progressive decline or increase in light requirements when tested at ten dates, from early spring through to summer (Figure 4-3). Rather, germination in darkness was low (< 20 %) at most test dates, but for two test dates significant peaks of germination (79 and 67 %) in darkness were observed for the interaction of light and test date (Figure 4-3). Changes in light requirements are identified as being linked to changes in seed dormancy status (Hilhorst *et al.*, 1996). The results of this work indicate that the time frame required to observe such shifts in dormancy status for *S. nigrum* may be quite short, i.e. significant variation is observable, in some cases, between tests made at two week intervals (Figure 4-4). One explanation may be that for short periods a VLFR dormancy status is present in this seed, but that this dormancy status is not sustained. It is reported, for seeds of *Lactuca sativa* L., that sensitivity to light can differ by a factor of 10^8 for seeds chilled for 7 d at 3.5 °C before germination at 22 °C, in comparison to non-chilled seed germinated at 22 °C (Hartman and Mollwo, 2002). Differences in imbibition temperatures of 4 °C are also reported to affect the VLFR sensitivity in *Echinochloa crus-galli* (L.) Beauv. (Taylorson and Dinola, 1989). Although the test temperatures used in these previous two reports differ from the temperatures experienced by the seed in the field (Figure 4-2), it is possible that soil temperature may interact with the seed dormancy to induce periods of VLFR sensitivity.

Results from Experiment 2 also indicated that temperature interacted with light, and test date. There was significantly higher levels of germination at 5/20 °C and 20/30 °C for seed lot SN7A tested in darkness (Appendix 6). This effect was also observed for another *S. nigrum* seed lot, SN7C, tested over eight test dates, but not for lot SN7A in comparison to lot SN7C (Figure 4-5). For both of these seed lots germination at 20/30 °C provided greater differences between light and dark germination than testing at 5/20 °C (Figure 4-6). A previous report for *Solanum nigrum* indicates that the amplitude of alternating test temperature can interact with light responses. For *S. nigrum* seed, stored for 15 weeks at 4 °C in moist sand, before testing in alternating temperatures (16/8 h) of 17/23, 15/25, 10/25, 15/30, 10/30 and 4/25 °C with respective amplitudes of 6, 10, 15, 15, 20 and 21 °C, germination in the dark was 4, 28, 51, 79, 90 and 90 %, respectively (Roberts and Lockett, 1978). Germination in the light at 17/23 °C was 85 %, while at all other temperatures the minimum germination in light was 99 %. Other *S. nigrum* seed, stored in moist sand at 17 or 30 °C or in the field for 15 weeks and tested at the same temperatures as above, had low levels of germination in the dark (4-30 %), only at 17/23 °C. At the other test temperatures in the light or dark and at 17/23 °C in the light, there were high germination levels (71-100 %). This report for *S. nigrum* seed indicates that a narrow temperature amplitude or differences in amplitude between temperatures of only 5 °C can limit germination in the dark regardless of seed storage temperature and for seed stored at low temperatures, the amplitude of test temperatures used in relation to mean temperature can have a large effect on germination in the dark. Evaluations of the usefulness of mean temperature, temperature amplitude and thermoperiod as predictors of germination of *Chenopodium album* seed exposed to diffuse laboratory light indicated that the mean temperature was as reliable a predictor as the other variates (Murdoch and Roberts, 1997). The results for *Solanum nigrum* in this present work and those of Roberts and Lockett (1978), however, indicate that mean temperature can be a poor predictor of germination of *S. nigrum* in the dark.

Light may inhibit the induction of conditional dormancy that can occur when testing seed at high temperatures (Roberts and Totterdell, 1981). For example, it was reported for *Rumex crispus* that light inhibited the induction of conditional dormancy when seeds were tested at temperatures of 15-25 °C (Roberts and Totterdell, 1981). This effect may be due to light counteracting the dark reversion of P_{fr} to P_r which is reported to occur at increased rates at high temperatures (Smith, 1995). Germination of *Solanum nigrum* in the dark at 20/30 °C (mean temperature 23.3°C) may have induced conditional dormancy except for retrievals when the seed was non dormant and substantial germination occurred, as observed, for example for the mid-August and early October test of seed lot SN7A (Figure 4-3). In comparison, 16 h at 5 °C in the

5/20 °C (mean temperature 10.0 °C) cycle may have prevented the induction of dormancy. Alternatively, or additionally, the 16 h at 5 °C could also have broken any dormancy present in the seed at retrieval through a stratification effect. Such dormancy would not have been broken by germination at 20/30 °C. However, further examination of amplitude effects, not confounded by mean temperature differences and vice versa, are required to clarify the relative importance of these factors on the dark germination of *S. nigrum*.

The light and dark treatments in Experiment 2 used a 24 h lighting regime, while the SDLE method in Experiment 3 used a brief light exposure followed by germination in darkness. The SDLE method has been used to assess the effects on weed seed germination by the hypothetical flash of light that seeds receive during daylight cultivation before they are reburied (Milberg *et al.*, 1996; Milberg, 1997; Gallagher and Cardina, 1998c). The results of the SDLE method in this present work provided different germination results from that using 24 h lighting, with 24 h lighting on a number of test dates providing significantly higher germination than that for SDLE treatments exposed to light (data not presented). This indicates that changes in dormancy status not evident with 24 h lighting, may be detected with SDLE testing (Figure 4-8). This result is consistent with a report for the germination of five of eight weed species tested monthly, for 14 months, by Milberg and Andersson (1997). In their experiment seed exposed to SDLE (5 s, 210 $\mu\text{mole}/\text{m}^2/\text{s}$) in comparison to seed exposed to light (10-40 $\mu\text{mole}/\text{m}^2/\text{s}$) 12 h/day, exhibited greater seasonal variability in percentage germination than the latter treatment.

In this present work, four different general relationships between the germination responses of the SDLE and nil exposure treatments were apparent (Figure 4-8). First, for the initial test date in July no significant differences in germination between the three treatments were apparent, and germination percentage was of mid range values (range 34-71 %). Secondly, for test dates in September, germination in light and darkness did not differ, with high levels of germination for all treatments (range 73-100 %). A third relationship was apparent for seed tested on 19 August and in October and November, where low levels of germination for the nil light exposure was observed (range 0-19 %) but for seed exposed to light, moderate to high levels of germination were apparent (range 65-100%). The fourth relationship was for the final test date there was no significant difference between the three treatments but germination was low (range 0-22 %). This final test indicated that dormancy had inhibited the germination of all treatments.

These SDLE results indicate that light may not be significantly important to the germination of *S. nigrum* seed in three situations: when the germination percentage

indicates the seed is neither fully non-dormant or fully conditionally dormant, as for the first July test date; when the seed is fully non dormant, as for the September test dates; and when the seed is most dormant, as observed for the December test date. The results indicated that light was most the important factor controlling the germination of *S. nigrum* seed when the dormancy status was in transition from non-dormancy to conditional dormancy, as observed for the October and November test dates. Previous reports of date effects for SDLE germination responses are made (Gallagher and Cardina, 1998c). For example, a report for work on *Alopecurus myosuroides* Huds. seed indicates that date of testing affects the SDLE response where testing in summer gave no percentage germination differences between a SDLE (R light 5 min 0.2 W/m²) and nil light exposure, for seed tested at (16/8 h) 10/20 °C (Froud Williams *et al.*, 1984). However, in spring, germination differed by ~60 % between these treatments (Froud Williams *et al.*, 1984). In addition, as with the December SDLE result in this present work, it is reported for a species that the largest difference between a SDLE treatment and a nil light treatment occurred due to the induction of conditional dormancy occurring for nil light treatments prior to SDLE treatments. Where the monthly testing of SDLE (described previously) and nil light exposures for *Chenopodium suecicum* J. Murr. indicated that the greatest difference in germination percentage between these two treatments occurred for the June test date, this test date preceded the induction of secondary dormancy for both treatments in July and August (Milberg and Andersson, 1997).

Implications of changes in light requirements for Solanum nigrum: Results from Experiment 2 did not support the null hypothesis that the light requirement for germination of *Solanum nigrum* seed is constant, and that seed dormancy status is static (hypothesis 2). The results of Experiment 3 did not support the null hypothesis that the SDLE responses of *S. nigrum* seed would not differ between test dates (hypothesis 4). Hypothesis 4 was supported partly in that no evidence was found to indicate that seed from different seed lots had different responses to SDLE, however, seed lots did have differing responses to nil light treatments at some test dates (Table 4-6). The findings have implications for the success of dark cultivation. Both the results from Experiments 2 and 3 indicated that the period in which dark cultivation for *S. nigrum* may be successful, is limited (Figure 4-3 and Figure 4-8).

Solanum physalifolium: At no test date did seed of *S. physalifolium* demonstrate a positive requirement for light in either Experiment 2 or 3 (Figure 4-7). In fact, at one test date of seed in September for Experiment 2, seed of *S. physalifolium* had a

significant negative response to light. The SDLE also did not enhance percentage germination. These results are consistent with a previous report for the germination requirements of *S. physalifolium* (del Monte and Tarquis, 1997). These results supported the hypotheses that *S. physalifolium* does not have a light requirement, and that germination will not be affected by SDLE (hypotheses 3 and 4). This indicates that a reduction of field germination by dark cultivation for this species is not to be expected.

Dormancy processes in *Solanum nigrum* and *S. physalifolium*

It was hypothesised that the dormancy of field stored *S. physalifolium* seed and *S. nigrum* seed does not differ, as indicated by percentage germination at the sub-optimal temperature of 5/20 °C, and time to 50 % germination (hypothesis 5). The results of Experiment 2 did not support this null hypothesis, with some evidence for different dormancy processes regulating the germination of *S. nigrum* and *S. physalifolium*. For seed stored for approximately a year, in the field, and germinated at 5/20 °C, *S. physalifolium* germination was strongly inhibited at the first retrieval in July (Figure 4-7). The percentage germination then progressively and significantly increased through to the November test date. No such pattern was evident for the germination of *S. nigrum* seed lot SN7A when tested at this temperature (Figure 4-3 and Figure 4-4). Comparison of the d_{50} values for *S. nigrum* and *S. physalifolium* also indicated that *S. nigrum* exhibited significantly variable counts during the test period at 5/20 °C (Table 4-5). In comparison, the d_{50} values for *S. physalifolium* from 31 July to 2 December 2002 did not vary.

Baskin and Baskin (1998) classified *Solanum sarrachoides* as having a dormancy that cycles from non-dormancy to dormancy, with no period of conditional dormancy. This classification was based on work on *S. sarrachoides* in 1983 (Roberts and Boddrell, 1983) which was prior to the splitting of *S. sarrachoides*, into *S. sarrachoides* and *S. physalifolium* (Edmonds 1986). Thus, the work of Roberts and Boddrell (1983) could have been on *S. physalifolium*, or the dormancy cycle of *S. sarrachoides* may be similar to that of *S. physalifolium*. Regardless of taxonomic difficulties, it is clear that the germination percentages and the d_{50} values of *S. physalifolium* (Figure 4-7 and Table 4-5) indicate a species in which seeds are fully non-dormant following the abatement of dormancy. While, for *S. nigrum*, the variable responses for the same germination parameters indicate apparent continual dormancy induction and abatement, probably in response to environmental stimuli. This regulation of *S. nigrum* germination may explain the lower emergence (1.7 %) of sown

seed following cultivation in Experiment 4B, in comparison with *S. physalifolium* (12.6 % emergence).

These differences in dormancy regulation between the two species have implications for crop weed interactions. The relative time of crop and weed emergence is reported to significantly affect the yield of *Zea mays* more than absolute weed density (Massinga *et al.*, 2001). There is a similar report for process peas (Nelson and Nylund, 1962). The dormancy regulation of germination for weeds such as *Polygonum persicaria* L. is reported to affect crop yield loss potential, by affecting the rate of weed emergence (Vleeshouwers, 1998). *Solanum physalifolium* seed had significantly faster d_{50} values than *S. nigrum* under non-optimal conditions of 5/20 °C (Table 4-5) but equivalent field emergence requirements following germination (data not presented). These results indicate that *S. physalifolium* may emerge ~ 3 days prior to *S. nigrum* under non-optimal temperatures, and so *S. physalifolium* has a competitive advantage over *S. nigrum*, and this increases the contaminant potential of *S. physalifolium*.

Seed lot effects on germination requirements, including light

There were significant differences in the germination requirements among the *S. nigrum* seed lots in relation to a number of factors. These results did not support the null hypotheses that: the germination requirements of freshly harvested seed from black and green fruit, and from fruit collected from different sites does not differ (hypothesis 1); and that the germination requirements of field stored seed from green and black fruit would not differ (hypothesis 6). In addition, seed from black and green fruit in the SDLE testing responded differently to the nil light exposure (Table 4-6). A number of factors may be responsible for these differences. The results for the freshly collected seed implied differences in primary dormancy status among seed lots (Figure 4-1). Differences in germination requirements among populations suggest that dormancy characteristics may be environmentally induced (Wulff, 1995). For example, the levels of endogenous nitrate in *Chenopodium album* seed influences dormancy. *Chenopodium album* seeds with low endogenous nitrate were more dormant, but were more responsive, to exogenous nitrate than seed with high endogenous nitrate levels (Saini *et al.*, 1986). The temperatures that seeds experience after-ripening can also affect their subsequent germination temperature optima (Baskin and Baskin, 1987).

In this present work, there were significant differences in germinating field stored *S. nigrum* seed lots collected from the same plants but processed separately from black and green fruit (Table 4-7). For example, for seed tested at 5/20 °C in darkness the seed from black fruit had significantly greater germination than seed from green fruit (Figure 4-5). This indicates that qualitative differences in the light

requirements of these seeds probably existed prior to their burial. The maintenance of differences between different seed lots of the same species following burial and testing is reported for a number of species (Milberg and Andersson, 1998b; Gallagher and Cardina, 1998c). Reports indicate that the R:FR quality of light a plant with developing seed receives, can significantly affect subsequent light responses of the seed (Haynes and Klein, 1974; Benvenuti and Lercari, 1994). Therefore, environmental factors could be responsible for the differences in levels of dark germination among *S. nigrum* seed lots from different sites and different coloured fruit. However, the temperature responses of seed from black and green fruit also differed at some test dates following burial (Appendix 7). This indicates additional differences in germination responses that may or may not be related to phytochrome.

Genotype differences can also affect germination requirements. For example, an intensive examination of variation among *Chenopodium album* populations after growth in a common environment provided evidence for genetic differences in temperature requirements for germination (Christal *et al.*, 1998). These differences were reported to have important implications for the speed of dormancy release and germination temperature optima. Givelberg *et al.* (1984) proposed that reported differences in the primary dormancy status of *Solanum nigrum* seed may be due to genotype effects. The significant variability observed in this work among seed lots of *S. nigrum* could be due to environmental and/or genotypic effects. The collection of seed from different sites and growth of the seed at a common site followed by seed collection and testing is required to substantiate the possible role of genotype versus environmental effects (Baskin and Baskin, 1998). Wulff (1995), however, observed that genotype can interact with environmental effects, so confusing the source of variation. Variability of *S. physalifolium* seed was not assessed as only seed from a single collection of *S. physalifolium* was tested. This was a weakness in this work, as conclusions about the germination requirements of a species should be based on multiple seed lot comparisons (Milberg and Andersson, 1998b).

The implications of these differences between seed lots are that it may be expected that the light requirements of *S. nigrum* seed from different coloured fruit will differ following burial. This may affect the success of night cultivation, as seed from different sources in the soil seed bank may be expected to have a range of light requirements. In addition, where the stimulation of germination is advantageous, such as with stale seed bed practices (Bond and Baker, 1990), the maximisation of germination will also be limited by the heterogeneous nature of the germination requirements for *S. nigrum* seed.

4.6.3 Assessment of dark cultivation effects

Experiment 4A indicated that the cultivation treatments had no significant effect on the number of emerged *S. nigrum* seedlings (Table 4-9). There was also no significant cultivation effect on *S. physalifolium* emergence (data not presented). Counts of two other commonly occurring weed species also indicated that there was no significant reduction in these species (Table 4-8). Of these species, *Chenopodium album* is reported to have reduced germination under dark cultivation (Jensen, 1995; Botto *et al.*, 2000). Experiment 4B had no significant cultivation effect for either *Solanum nigrum* or *S. physalifolium* (Table 4-15 and Table 4-16). These results indicate that the germination of *S. nigrum* and *S. physalifolium* was not reduced in any dark cultivation experiment. These results supported the hypothesis that dark cultivation would not reduce the germination of *S. nigrum* and *S. physalifolium* seed (hypothesis 7).

Possible causes of variability

Solanum nigrum germination had been reported to be reduced by dark cultivation (Scopel *et al.*, 1994). However the success of dark cultivation is reported to be variable, with this variability limiting the usefulness of the practice (Buhler, 1997; Fogelburg, 1998; Gallagher and Cardina, 1998d). A number of factors in the literature were identified as contributing to the variability in response to dark cultivation. Some of these factors may have contributed to the results observed for dark cultivation with *S. nigrum*.

Soil moisture: Soil moisture has been identified as a factor contributing to the success of dark cultivation practices for *Chenopodium album* and *Datura ferox* L. (Botto *et al.*, 2000). For *D. ferox*, it was identified that VLFR responses required soil moisture levels higher than -0.5 MPa for up to 6 days after cultivation. If this moisture level was maintained then germination following daylight cultivation significantly exceeded that following night cultivation. However, it was reported for *Amaranthus retroflexus* that water-limited photoinduction of seed germination only occurred in dry microsites or under extreme drought conditions (Gallagher and Cardina, 1998a). In this work, irrigation did not precede or immediately follow the cultivation trials as pea growers are not advised to irrigate pea crops until after pea emergence (A. White, Heinz Watties's Ltd, pers. comm.). In addition, it would be expected that wetter soil conditions would contribute to a greater total weed emergence (Roberts and Potter, 1980). The work of Botto *et al.* (2000) also indicated that the level of irrigation required to obtain a dark cultivation effect, contributed to significantly greater overall weed emergence compared with non-irrigated plots. In the two trials, the soil moistures at the cultivation dates were

low at 14.5-14.9 %. However, in the second trial, over 65 mm of rain fell in the 10 days following sowing, indicating that water limitation following cultivation should not have been a factor affecting the response.

Soil nitrate: In a laboratory study soil nitrate effects were hypothesised to be a factor contributing to variable dark cultivation success (Milberg, 1997). Analysis of soil nitrate levels in soil surrounding the seed burial bags (Table 4-4), did not give a significant relationship with germination in the dark of SN7A seed (data not presented). Application of 0.2 % KNO₃ to field stored *S. nigrum* seeds (SN7A and SN7C) caused significant increases in seed germination relative to dark treatments not receiving KNO₃ on approximately three of four test dates (data not presented). Testing of cultivated soil NO₃ levels in plots adjacent to the area used for the dark cultivation trials, in 2002, indicated that soil NO₃ levels could reach levels equivalent to that used in the laboratory KNO₃ testing (Table 4-18). However, these levels did not occur until six to eight weeks after cultivation. Levels ranged from 347-693 mg/kg dry soil at two and ten days after cultivation. These results indicate that seeds would have to interact with soil NO₃ levels that were considerably lower than those tested in this work, for NO₃ to be considered as a factor capable of negating the effects of dark cultivation. However, with species such as *Amaranthus retroflexus* low levels of NO₃ (75 ppm) can cause significant increases in dark germination, with dark control of ~5 % germination and ~45 % germination for seed receiving NO₃ (Gallagher and Cardina, 1998b).

Seed source: In the 2002 trial resident *S. nigrum* seed showed no significant response to dark cultivation. In this trial the sown *S. nigrum* seed was shown to have a light requirement for germination in laboratory testing prior to burial (data not presented), but this seed showed no significant response to dark cultivation. Seed age effects may have been one possible explanation for the reduction in *S. nigrum* seedlings, following dark cultivation, reported by Scopel *et al.* (1994). The area used by Scopel *et al.* (1994) was reported to have been in vegetable production prior to the trial, indicating that recent weed seed inputs to the soil seed bank may have been possible. It is reported that for laboratory stored seed, freshly harvested seed had a greater light requirement than seed stored in moist sand for 15 or 50 weeks (Roberts and Lockett, 1978; Kazinczi and Hunyadi, 1990). This suggests that 'recent' seed may have stronger light requirements than seed resident in the soil seed bank for longer periods. However the results of Experiment 4B did not demonstrate any light cultivation differential response between 'recent' and 'old' seed.

Very low fluence responses: The design of laboratory germination experiments in this work did not provide the ability to discriminate if VLFR were elicited by the use of a green filter. However, it is possible that high levels of germination in the dark observed at some test dates may be due to VLFR responses in *S. nigrum* seed (Figure 4-3 and Figure 4-8). If this effect occurs in the field then VLFR responses may have affected the levels of germination following cultivation in darkness. There is disagreement in the literature over the effect of VLFR for dark cultivation. A number of workers cite VLFR as a factor that contributes to the unreliability of dark cultivation because this effect can cause germination at very low light levels (Gallagher and Cardina, 1998a; Gallagher and Cardina, 1998b; Hartman and Mollwo, 2002). Botto *et al.* (2000) state that VLFR are necessary to reduce germination when cultivating in the dark to control *Datura ferox*. The VLFR was identified as being irreversible in *D. ferox*, thereby causing a greater relative germination following daylight cultivation than after dark cultivation. For other species such as *Amaranthus retroflexus* and *Rumex obtusifolius* it is not clear from the literature whether the VLFR can be reversed through dark reversion or other processes following seed reburial (Kendrick and Heeringa, 1986; Gallagher and Cardina, 1998b). Further work is required to demonstrate what the role of VLFR seed responses, such as for *Solanum nigrum*, are to the success of dark cultivation.

Light intensity: In all the daylight cultivation treatments, no cultivation took place at < 1,000 $\mu\text{mole/m}^2/\text{s}$. This is consistent with the methods used in a number of previous dark cultivation trials (Jensen, 1995; Buhler, 1997; Gallagher and Cardina, 1998d). In particular, the minimum PAR of >1,200 $\mu\text{mole/m}^2/\text{s}$ in the 2002 trials, meets with the minimum light intensity reported for two successful dark cultivation trials (Botto *et al.*, 1998; Botto *et al.*, 2000). Therefore, inadequate light probably does not explain the variability observed in the dark cultivation results.

Seed dormancy and light requirements: Differences in the light requirements for germination due to seasonal changes in dormancy status of a number of weed species, have been demonstrated. These differences in seasonal light requirements were proposed as a potential source of variation in the success of dark cultivation (Milberg and Andersson, 1997). Testing of *S. nigrum* SDLE and dark germination responses indicated that for the trial in November 2002, germination in the absence of SDLE would be significantly lower than in the presence of short term light exposures (Figure 4-8). However, the dark cultivation trial in November did not support these laboratory results. A possible explanation is that *S. nigrum* seed resident in the seed bank had a different dormancy status to the seed that had been tested.

Another explanation of the lack of dark cultivation success for *S. nigrum* provided by the seed burial and germination studies is that reports indicate that for some species germination following SDLE and seed reburial may be controlled. Dark cultivation is successful at reducing weed seed germination, due to the greater number of buried seeds germinating following daylight cultivation relative to dark cultivation (Jensen, 1995). This effect appears to rely on the germination stimulus effect being irreversible. If seed which received SDLE during cultivation does not germinate following reburial, then the differential effect of SDLE induced germination is not achieved. Hence, irreversible germination responses following reburial may be crucial for light cultivation to cause higher levels of germination than dark cultivation. However, for two species, *Rumex obtusifolius* and *Datura stramonium* L. the induction of dormancy is reported for seed exposed to SDLE and then buried (Benvenuti and Macchia, 1998; Benvenuti *et al.*, 2001). The gaseous environment of the soil was implicated as interacting with phytochrome processes to induce dormancy (Benvenuti and Macchia, 1998).

4.7 Conclusions

1. The germination requirements for *S. nigrum* seed from black and green fruit from the same collection can be significantly different. *Solanum nigrum* seed collected from different sites has different germination requirements, due to different levels of primary dormancy.
2. *Solanum physalifolium* seed did not have a positive light requirement. *Solanum physalifolium* had a progressive dormancy loss from July through to November. This dormancy significantly restricted germination in July and August at non-optimal temperatures (5/20 °C). The dormancy pattern of *S. nigrum* differed from that of *S. physalifolium* with the level of germination fluctuating at 5/20 °C throughout the process pea sowing season.
3. The light requirements of *S. nigrum* seed were greatest immediately following the induction of conditional dormancy. This may be the optimum time for dark cultivation.
4. Dark cultivation did not reduce the emergence of *S. nigrum* seedlings and this practice is not recommended. The germination of *S. physalifolium* will not be reduced by dark cultivation.

Chapter 5 Growth and dry matter production in *Solanum nigrum* and *S. physalifolium*

5.1 Introduction

The contamination of process pea crops by the fruit of *Solanum nigrum* and *S. physalifolium* occurs due to their successful flowering and fruiting prior to pea crop maturity. This occurs because these weeds are successful competitors. Factors identified as contributing to the competitive success of weeds and associated crop yield reductions are also relevant to the contamination potential of weeds.

Early emerging cohorts of weeds in a crop usually have the greatest reproductive output (Fernandez-Quintanilla *et al.*, 1986; Mohler and Callaway, 1995). For crops where the interval of time between sowing and crop harvest is not a phenological limitation to the development of later weed cohorts, it appears that the duration of growth and the growth environment affect the volume of weed reproductive output. This was demonstrated for *S. nigrum* seedlings transplanted into *Zea mays* at 3 and 38 days after crop emergence. By crop maturity the weed plants in the first cohort had ~ 800 fruit each, while plants in the second cohort had ~ 200 each (Kremer and Kropff, 1998c). There are similar reports for fruit production of different cohorts of *Solanum ptycanthum* growing in *Glycine max* (Quakenbush and Andersen, 1984; Stoller and Myers, 1989). These reports do not however, provide the ability to determine the cause of reductions in reproductive output between the effects of time from initial flowering to that of plant growth effects caused by competition with a crop at different growth stages. Both processes are probably taking place. Yield reduction studies, identify the time of weed emergence relative to the crop as an important influence on the proportion of crop to LA at crop harvest. The proportion of weed to crop LA was demonstrated to be linked to the degree of crop yield losses (Kropff *et al.*, 1992; Dieleman *et al.*, 1995). Thus, early emerging weeds have the potential to produce greater DM production than later emerging weeds, due to their early establishment that allows greater radiation interception.

As for weeds within a cohort, the largest weeds produce the most seed (Thompson *et al.*, 1991; Mohler and Callaway, 1995). Thus, if phenological requirements have been met, it may be assumed that factors that negatively affect weed DM production in a cohort, will negatively affect their reproductive output. This is demonstrated by the effect of limitations to radiation interception through shading on plants of the same age, which caused significant linear reductions in total DM production and numbers of fruit produced by *Solanum nigrum* (Singh, 1972; Fortuin and Omta, 1980) and *S. ptycanthum* (Stoller and Myers, 1989; Croster *et al.*, 2003).

The number of fruit produced by a nightshade plant in a process pea crop is important, as the more nightshade fruit produced, the more likely it is that some of this fruit will be harvested with the pea crop, and/or the greater the volume of peas that may be contaminated.

It is clear from reports for *S. nigrum* that factors affecting plant DM production affect the number of fruit produced per plant. However, there is no published work on plant or fruit DM production of *S. physalifolium*, or comparisons between these two species, in the literature. Anecdotal evidence indicates that *S. physalifolium* plants can be larger than *S. nigrum* (T.C. Chamberlain, pers. comm.). For comparisons between weed species, morphological traits such as a weed species height, total LA and vertical LA distribution are shown to give competitive advantages in radiation interception (Stoller and Wooley, 1985; Legere and Schreiber, 1989; Mosier and Oliver, 1995; Hirose *et al.*, 1996). Differences in morphology and growth rate were reported to be responsible for the differences in DM production between *S. ptycanthum* and *S. sarrachoides* when both were grown with and without process peas (maturity 738 °Cd, Tb = 4.4 °C). Their respective DM production was ~ 4 and 12 g/plant with peas, and ~ 55 and 130 g/plant without peas (Heider, 1996). The greater DM of *S. sarrachoides* in both environments was attributed to a faster initial growth rate and a denser leaf and stem morphology. Information on the morphological characteristics of *S. nigrum* and *S. physalifolium*, and the relationship between DM production and fruit production when they are grown with and without peas, is relevant to developing an understanding of these species relative competitiveness and, thus, their contamination potential. Such information may be useful in developing specific weed management practices.

For some plant species it has been identified that particular growth criteria must be met for the initiation of reproductive development to occur. For example, for an accession of *Lupinus angustifolius* L. flowering did not occur until 24 nodes had been produced regardless of sowing date (Gladstones and Hill, 1969). There is no report in the literature, to indicate that nightshade species have growth criteria that must be met before the initiation of reproductive development. For weed species, a greater degree of phenotypic plasticity may be exhibited than that of most agricultural crops (Ghersa and Holt, 1995).

This chapter addresses the third objective of this study, to describe the growth and DM production of *Solanum nigrum* and *S. physalifolium* when grown with and without peas. The hypothesis examined is that the production of DM between *S. nigrum* and *S. physalifolium* does not differ (hypothesis 1). The second hypothesis

is that for both of these species reproductive initiation is not linked to particular growth thresholds (hypothesis 2).

5.2 Methods

In two of the following three sections the estimation of a contamination index (CI) for *S. nigrum* and *S. physalifolium* plants was calculated. This was calculated as the number of fruit > 3 mm on a plant divided by the plant DM, excluding fruit DM.

5.2.1 Growth of *Solanum nigrum* and *S. physalifolium* in the absence of peas

Site description and experimental design: The site used was in field H19 (see sections 4.3.1 and 4.5.1). The trial design was a randomised complete block design with four blocks. Factors were species and sowing date. The species were *S. nigrum* or *S. physalifolium*, and there were five sowing dates for each species giving a total of 40 plots (1.2 x 5 m). In each plot six seed sowing sites were established centrally down the length of the plot with the first site at 0.5 m in from the plot edge. The remaining sites were spaced 0.8 m apart. Soil samples (20 x 150 mm depth cores) taken for soil tests on 2 February, 2003, gave test values of 16, 9, 17, 7 (MAF units) and 5.2 for Olsen P, K, Mg, Ca, and pH, respectively.

Temperature records: Hourly air temperature at 0.2 m was recorded with a Hobo[®] H8 4-Channel data-logger. Temperatures were recorded from 22 August 2002 to 1 May 2003. However due to a programming error the logger was non operational from 1 September 2002 to 26 September 2002. A regression between the 75 mm soil temperature records from the H19 site (Figure 4-2) and soil and air temperatures from Ashley Dene (14 km from the experimental site) provided an accurate predictor of the missing 0.20 m air ($r^2 = 0.939$) temperatures.

Sowing, seedling emergence and plant development: Seed of lots SN7C and SP3 were germinated at 20/30 °C in 24 h light, as described for Experiment 2. Germinated seeds were collected daily and following the methods of Kremer and Lotz (1998b), the seeds were stored in the dark at 5 °C, until a minimum of 48 seeds of both species had been collected. On the day the minimum number of seeds for sowing had been collected, the pre-germinated seeds were sown at a depth of 15 mm in the late

afternoon. Two to three seeds were sown together, in the same hole, at each site at each of the six plant sites per plot. Sown seeds were hand watered at 1-2 day intervals until emergence. Sowings were on 23 August 2002, 23 September 2002, 21 October 2002, 21 November 2002 and 21 December 2002. Hereafter, these sowings are referred to as the August, September, October, November and December sowings. For the August, September, October and November sowings following initial emergence number of emerged seedlings was recorded every two days until emergence ceased. For the December sowing, the emergence of seedlings was not recorded. Slug damage to seedlings was controlled with prills of Slugout (metaldehyde) at 3 g per plant site within a plot. Where more than one seedling emerged per plant site ($n = 6$) in a plot, seedlings were thinned to one seedling at the 2–3 leaf stage. The experimental area was weeded regularly throughout the experiment.

Sampling: For each plant from seedling emergence until flowering (observation of an open corolla) occurred, the leaf number was recorded weekly. Coloured wire (0.8 mm D) was used to tag buds and clusters (set of flower buds on a single peduncle) to assist with monitoring. Twice a week plants were inspected for flower bud appearance, and following bud appearance, for flowering and cessation of flowering, for each flower bud within the first two clusters of buds on a plant to flower. Following the flowering of each bud in the two monitored clusters, measurements were made twice a week of the maximum fruit diameter of each fruit using vernier calipers. When the calyx covered the young fruit, the calyx was included in the fruit diameter measurement. A value of 0.2 mm was subtracted from the fruit diameter measurement to account for the calyx.

Plants were harvested on the day after a single fruit from both of the two monitored clusters on a plant had a diameter of ≥ 8.0 mm. For a number of plants fruit growth had ceased prior to fruit reaching a diameter of ≥ 8.0 mm. For these plants, a secondary harvest criteria was used where plants were harvested when the mean fruit growth of the three largest fruits on each of the two monitored clusters had a growth rate of < 0.1 mm/d for three consecutive measurements. Plants were harvested by cutting the stem at ground level. The roots were collected using a garden fork (depth 200 mm).

Of the harvested plants on each harvest date, a single plant from each plot was sampled according to the following procedure. First, all fruits were removed from the plant and calyx. A sub-sampling procedure of the remaining leaf and stem material from each plant was used. For plants less than 600 g (wet weight) approximately 25 % of their plant mass was sub-sampled, and for plants > 600 g approximately 12.5 % of their mass was sub-sampled. The sub-samples were separated into: leaves; stems;

unopened buds, flowers, and calyces. The leaves and stems were assessed for leaf area by a planar area meter (Licor 3100). The fruit was size graded using sieves, with circular apertures, from a Kamas Westrup LA-LST seed cleaning machine. Grading was in the following order ≥ 9 mm, ≥ 7 mm, ≥ 5 mm, ≥ 3 mm. The remaining < 3 mm fruit was collected. The number of fruit in each of the four largest size grades was counted. The three components of each sub-sample and the five size grades of fruit and the non-sub-sample portion were then dried for 48 h at 65 °C in a forced air oven, and weighed. Plants that were not sub-sampled were dried as described above. Roots were dried for 72 h at 65 °C.

Post emergence irrigation: Percentage volumetric soil moisture was recorded weekly to a depth of 0.20 m, with a Time Domain Reflectometry probe (Hydrosense™, Campbell Scientific Australia Pty. Ltd.) from the base of a single plant in each of the eight plots for each monthly sowing date. The field capacity, was 22.5 %. Plots for each monthly sowing were irrigated when the mean soil moisture was 50 % of field capacity. Individual plants were irrigated with a dripper irrigation system that delivered 2.1 l/h. Irrigation was applied for three hours per application.

Analysis: Analysis of above ground DM including fruit at harvest and DM accumulation, was made on all harvested plants ($n = 192$). For this data set analysis with missing values was used in Genstat Sixth Edition® (2002). The standard error of these means was calculated using a procedure for unequal group sizes (Kramer, 1956). The Tukey HSD for comparison of means with unequal samples sizes was calculated as the mean Tukey HSD value for each pair of values in the comparison. The remaining analysis was made with the General Linear Model in Systat® Version 9.01 (1998). The rate of leaf appearance, in days and leaf number from emergence to bud appearance on 50 % of plants within a plot, was calculated ($n = 40$). For the plants sampled for fruit, leaf and stem components, two of these plants, from each plot, were randomly selected for analysis ($n = 80$). For all analyses, variates were analysed for the factors block, sowing date, species and the species by sowing date interaction. Linear regression was used for the analysis of fruit DM and plant DM relationships.

5.2.2 Growth of *Solanum nigrum* and *S. physalifolium* in the presence of peas

Growth in Experiment 4A

Trial site: These comparisons of the growth of the two nightshade species and peas were from the continuation of Experiment 4A (see Chapter 4). The experimental area, establishment procedures, sample areas and weed seedling counts are described in section 4.5.1.

Sampling: On the 31 October 2001 the number of emerged pea plants was counted in one fixed quadrat per sub-plot of Experiment 4A, described in Chapter 4. Destructive 0.1 m² samples from each plot were made on the 7 to 8 November, 16 November, 24 to 25 November, 30 November to 1 December and 17 December 2001. On 29 December 2001 two samples were taken, one from a fixed emergence quadrat area, and a yield sample was taken from between the two previous sample points. At this time the peas and large weeds were cut to ground level with shears. Small nightshade plants were cut to ground level with a scalpel and stored separately. Plant samples were stored at 5 °C prior to measuring their green area (GA). The GA is the planar area of leaves and stems of seedlings. Pea maturity was determined by sampling peas from the area sown outside the plots, and samples from 22 December, 24 December, 27 December, and 28 December 2001 were taken to the Heinz Wattie's Ltd, Hornby factory and tested with a tenderometer. The final harvest was taken on 29 December 2001, following a mean tenderometer reading of 99.4, on 28 December.

For the six pea and weed sample dates the number of nightshade seedlings in each leaf number class was counted for each species. The DM of peas, *S. nigrum*, *S. physalifolium* and the combined mass of the other weed species present was determined after drying for 48 h at 65 °C in a forced air oven. On all of the six dates except 24 to 25 November peas, the GA for the samples of *S. nigrum*, and *S. physalifolium* was determined using a planar area meter (Licor 3100). A sub-sampling procedure was used for the pea samples on the last sampling date. The GA of the other weed species was determined on four dates. For the pea yield harvest sample, pods were hand shelled, and the peas > 5 mm were weighed to determine their fresh and DM yield. On 19 November, 16 December and 30 December 2001 Sunfleck Ceptometer readings were taken, above and below the canopy, from 24-30 subplots.

Analysis: Statistical analysis indicated that the cultivation treatments in Emergence Experiment 1 had no significant effect ($P < 0.05$) on the number of *S. nigrum* seedlings following cultivation, as indicated by the Tukey HSD (Table 4-17). It also had no effect on the number of *S. physalifolium* seedlings. In the plots sown with *S. nigrum* and *S. physalifolium* there were no significant sowing effects for either species, with the exception of one count of *S. nigrum* seedlings at 46 DAS (Table 4-18). Because of the lack of cultivation and species sowing treatment effects, statistical analysis was made for the factors sample date and block only, using the General Linear Model (GLM) in Systat® Version 9.01 (1998). Means separation tests were made using the Tukey HSD test (Zar, 1984). Comparisons of growth parameters between *S. nigrum* and *S. physalifolium* were made using paired *T*-tests in Systat®. Logit regression of the number of nightshade plants with fruit from each species against the mean population density at the final sampling date was carried out using Genstat Sixth Edition® (2002).

Nightshade growth in an organic pea crop at harvest

Sampling: A commercial organic process pea field (field A6, Kowhai Farm, Lincoln University) was sampled for nightshade plants on the day of harvest (15 January 2003). The crop had been sown on 31 October 2002 and had a FE of 135 pea plants/m². Mature nightshade plants were collected by cutting at ground level from a 0.1 m² area. Twenty five *S. nigrum* and 19 *S. physalifolium* plants were collected and stored at 5 °C. Each plant was assessed for the number of leaves, fruit clusters per plant and the number of clusters that contained any fruit. The maximum length of each plant was also measured. Dry matter was determined after drying for 48 h at 65 °C. On the basis of their flower and fruiting development, the most mature 19 *S. nigrum* plants were selected for comparison with the 19 *S. physalifolium* plants.

Analysis: Analysis used the two sample *T*-tests in Systat® Version 9.01 (1998), pooled variances were used where the Bonferroni Adjusted Probability test indicated the variances did not differ significantly, and separate variances were used where the variances differed significantly. Linear regressions of plant DM exclusive of fruit DM and fruit number (> 3 mm) and fruit DM were made.

5.3 Results

5.3.1 Growth of *Solanum nigrum* and *S. physalifolium* in the absence of peas

There were progressive increases in mean air temperature over the sowing dates (Figure 5-1).

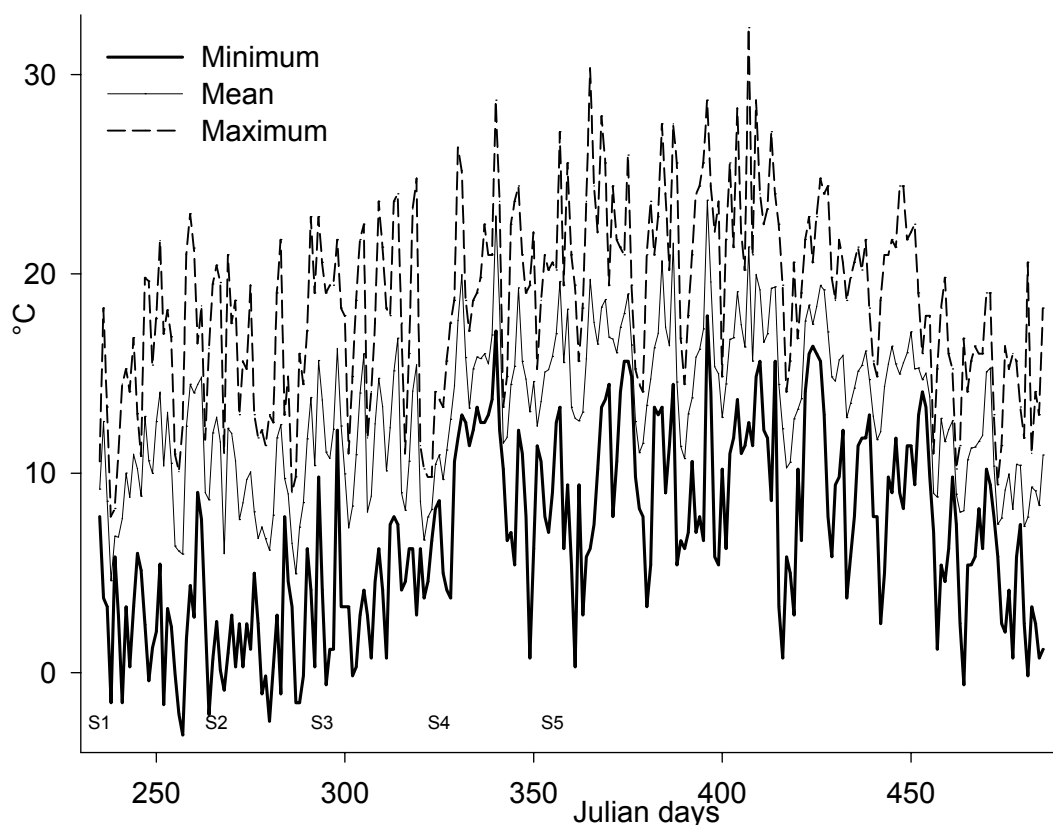


Figure 5-1. Minimum, mean and maximum daily air temperature recorded at the experimental site at a height of 0.20 m from 23 August 2002 to 30 March 2003. The five sowing dates (S1-S5) are indicated.

Plant establishment

Poor emergence in some months and some loss of seedlings to slugs resulted in reductions from the planned six plants per plot (Appendix 11). Rainfall for each month including August 2002 to April 2003 was 30, 43, 86, 32, 32, 15, 44, and 75 mm.

Dry matter at harvest

The harvest criteria used resulted in mean days to harvest for both species of 142, 113, 93, 87 and 85 for the August, September, October, November and December sowings, respectively. Both nightshade species grew into dense sprawling shrubs (Appendix

12). For plant DM there was a significant ($P < 0.001$) sowing date by species interaction (Table 5-1). Plant DM did not differ significantly over the sowing dates in *S. nigrum*, but for *S. physalifolium* the October sowings plants were significantly heavier than at the other sowing months, except September. For the August sowing plant DM did not differ significantly between the two species, but for the other four sowing months there were significant differences between the species. *Solanum physalifolium* plants (88.7 g/plant) were significantly ($P < 0.001$) heavier than *S. nigrum* plants (30.3 g/plant) at all sowing dates. For growth rate there was a significant ($P < 0.001$) sowing date by species interaction. Dry matter accumulation did not differ significantly in *S. nigrum*, but in *S. physalifolium* the August value (0.42 g/d/plant) was significantly less than at all other sowings. The October value in *S. physalifolium* was significantly greater than the November and December values. Overall, *S. physalifolium* (0.87 g/d/plant) had a significantly greater growth rate than *S. nigrum* (0.30 g/d/plant) (Table 5-1).

Table 5-1. Dry matter (DM) (g/plant), and growth rate (g/d/plant) from sowing for *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants. Interaction DM/plant (s.e. = 6.23, d.f. = 179, Tukey HSD = 27.89) and species effect (s.e. = 2.77, Tukey HSD = 7.7), growth rate interaction (s.e. = 0.061, d.f. = 179, Tukey HSD = 0.272) and species effect (s.e. = 0.027, Tukey HSD = 0.075). Means followed by the same letter within and between columns for the same factor are not significantly different. An * indicates a significant species main effect.

Sowing	DM excluding roots		Growth rate	
	SN	SP	SN	SP
August	32.3 a	59.9 bc	0.23 a	0.42 a
September	33.2 a	110.3 cd	0.30 a	0.96 bc
October	31.5 a	123.9 d	0.32 a	1.23 c
November	32.3 a	82.0 c	0.37 a	0.94 b
December	22.3 a	67.7 c	0.26 a	0.80 b
Mean	30.3*	88.7	0.30*	0.87

Plant leaf area and leaf:stem ratio

For plant leaf area (PLA) there was a significant ($P < 0.05$) species by sowing date interaction (Table 5-2). Sowing date did not affect *S. nigrum* PLA, but in *S. physalifolium* the September, October and December values were significantly higher than the August value. At all sowing dates except August, *S. physalifolium* (0.511 m²/plant) had a significantly higher PLA than *S. nigrum* (0.191 m²/plant). The *S. nigrum* plants (1.40) had a significantly ($P < 0.001$) higher leaf:stem DM ratio value than *S. physalifolium* plants (1.08) (Table 5-3). The leaf area ratios (LAR) of 61.9 and 63.3 mm²/g of *S. nigrum* and *S. physalifolium*, respectively, did not differ significantly, nor did they interact with sowing date (data not presented). There was also no

significant differences for any factors for the respective green area ratio (GAR) values of 69.8 and 74.4 mm²/g (data not presented). The leaf weight ratios of 0.362 and 0.350 g leaves/g plant of *S. nigrum* and *S. physalifolium* respectively, also did not differ significantly ($P < 0.05$) or interact with sowing date (data not presented). However, the stem weight ratio of 0.264 and 0.330 of *S. nigrum* and *S. physalifolium*, respectively, differed significantly ($P < 0.001$) (Table 5-3).

Table 5-2. The plant leaf area (PLA) m²/plant for *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants. Interaction of PLA (s.e. = 0.0505, d.f. = 67, Tukey HSD = 0.2245), species effect (s.e. = 0.0226, Tukey HSD = 0.0639). Means followed by the same letter within and between columns for the same factor are not significantly different. An * indicates a significant species main effect.

Sowing	PLA	
	SN	SP
August	0.251 abc	0.379 bc
September	0.217 ab	0.592 d
October	0.158 ab	0.631 d
November	0.195 ab	0.453 cd
December	0.134 a	0.499 d
Mean	0.191*	0.511

Table 5-3. Leaf:stem DM ratio and stem:plant DM ratio for *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants, Leaf:stem (s.e = .040, d.f. = 67, Tukey HSD = 0.113), stem:plant DM (s.e = .012, d.f. = 67, Tukey HSD = 0.052). An * indicates a significant species main effect.

Leaf:stem DM		Stem:plant DM	
SN	SP	SN	SP
1.40*	1.08	0.26*	0.33

Leaf appearance and number at bud appearance

For leaf appearance rate at bud appearance on 50 % of plants from emergence, there was a significant ($P < 0.05$) sowing date by species interaction (Table 5-4). Leaf appearance rate did not differ significantly in *S. nigrum*, but in *S. physalifolium* the August sowing had a significantly lower rate of appearance, 0.15 leaves/day, than the October and November values (0.90-0.92 leaves/day). There was no significant species effect on leaf appearance rate. For total leaf number at bud appearance there was a significant ($P < 0.01$) sowing date by species interaction. Leaf number did not differ significantly for *S. nigrum*, but in *S. physalifolium* the August sowing had significantly fewer leaves than the September and October sowings. Overall, *S. physalifolium* had significantly ($P < 0.05$) fewer leaves (30.5) than *S. nigrum* (37.3).

Table 5-4. Leaf appearance rate (leaves/d) to bud appearance on 50 % of plants, and total number of leaves at bud appearance on 50 % of plants for *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants. Leaf appearance interaction (s.e = 0.087, d.f. = 21, Tukey HSD = 0.415) and species (s.e = 0.043, Tukey HSD = 0.127), leaf number interaction (s.e = 4.38, d.f. = 27, Tukey HSD = 20.15) and species (s.e = 1.99, Tukey HSD = 5.66). Means followed by the same letter within and between columns for the same factor are not significantly different. An * indicates a significant species main effect.

Sowing	Leaf appearance rate		Leaf number	
	SN	SP	SN	SP
August	0.46 ab	0.15 a	40.0 b	9.9 a
September	0.76 bc	0.70 bc	46.9 b	40.8 b
October	0.67 bc	0.92 c	34.1 b	45.0 b
November	0.77 bc	0.90 c	29.3 ab	27.7 ab
December	-	-	36.2 b	29.0 ab
Mean	0.67	0.66	37.3*	30.5

Fruit production at harvest

The number of fruit ≥ 9 mm was low, with ten *S. nigrum* plants of the 40 selected *S. nigrum* plants in this data set producing a mean of 1.3 fruit ≥ 9 mm/plant. Of the 40 *S. physalifolium* plants sampled none produced fruit ≥ 9 mm. Due to the small numbers of fruit ≥ 9 mm the fruit were pooled to give a fruit size grade of ≥ 7 mm. The number of fruit in each of the 3-5, 5-7, and > 7 mm size classes differed significantly with species (Table 5-5). *Solanum physalifolium* produced ~140, 210 and 30 more fruit per plant in each of the respective size grades than *S. nigrum*. The species by sowing date interaction was not significant and sowing date had no effect on fruit size classes.

Table 5-5. Mean number of fruit per plant in three size grades: 3-5 mm, 5-7 mm, > 7 mm in *Solanum nigrum* (SN) and *S. physalifolium* (SP). Effects, all d.f. = 67, 3-5 mm (s.e = 10.01, Tukey HSD = 28.31), 5-7 mm (s.e = 14.20, Tukey HSD = 40.18), > 7 mm (s.e = 7.00, Tukey HSD = 19.80). An * indicates a significant species main effect.

	Fruit/plant	
	SN	SP
3-5 mm	56.2*	201.3
5-7 mm	41.4*	261.7
> 7 mm	34.4*	76.0

Solanum physalifolium produced significantly more total fruit DM, at 8.7 g, than *S. nigrum*, at 2.2 g (Table 5-6). Fruit DM was not affected by sowing date and there was no interaction between sowing date and species. The harvest index (HI) of *S. physalifolium* (0.125) was significantly ($P < 0.001$) higher than in *S. nigrum* (0.077). The sowing date by species interaction was not significant, but the sowing date was significant ($P < 0.01$) (data not presented). The August and September values of 0.080

and 0.078 were significantly lower than the December value of 0.137 (s.e. = 0.013, d.f. = 67, Tukey HSD = 0.054). *Solanum physalifolium* with a CI value of 7.8 had a significantly ($P < 0.001$) higher CI, than *S. nigrum* with a value of 4.8 (Table 5-6). For CI, sowing date did not significantly interact with species, but there was a significant ($P < 0.001$) sowing date response (data not presented). Values ranged from 4.9-5.3 for the first three sowings. However, in the December sowing the value had increased significantly to 8.8 (s.e. = 0.694, d.f. = 67, Tukey HSD = 2.89).

Table 5-6. Total fruit DM per plant (g), harvest index (HI) and contamination index (CI) for *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants. Effects, all d.f. = 67, DM fruit (s.e = 0.511, Tukey HSD = 1.45), HI (s.e = 0.008, Tukey HSD = 0.023), CI (s.e = 0.44, Tukey HSD = 1.24). An * indicates a significant species effect.

	SN	SP
DM fruit/plant	2.2*	8.7
HI	0.077*	0.125
CI	4.8*	7.8

5.3.2 Growth of *Solanum nigrum* and *S. physalifolium* in the presence of peas

Growth of *Solanum nigrum* and *S. physalifolium* from Experiment 4A

Monthly rainfall for October, November and December 2001 was 63, 70, and 33 mm, respectively. Pea emergence was observed on 11 October (10 DAS), when populations of nightshade weeds were estimated to be below 10/m². Pea density at 31 October was 152 plants/m². At 41 DAS the mean pea height was 203 mm. At 49 DAS transmission of PAR, at a height of 20 mm, was 29.0 % of the above canopy PAR. By 78 and 90 DAS these values were 1.1 and 1.8 %, respectively. The fresh weight yield of shelled peas > 5 mm was 1,110 g/m², giving a DM of 253.4 g/m².

Pea DM reached 874 g/m² at the final sample date (89 DAS), while weed DM reached 125 g/m² at this date (Figure 5-2). The number of nightshade seedlings for each species significantly declined over the sample period (Tables 4-18 and 4-19). There was a strong decline in small seedlings with four leaves or fewer (Appendix 13). Paired *T*-tests of the mean DM/plant for *S. nigrum* and *S. physalifolium* provided no significant differences between the two species at any of the six sample dates (Figure 5-3). Final DM for *S. nigrum* and *S. physalifolium* was 0.029 and 0.017 g/plant, respectively.

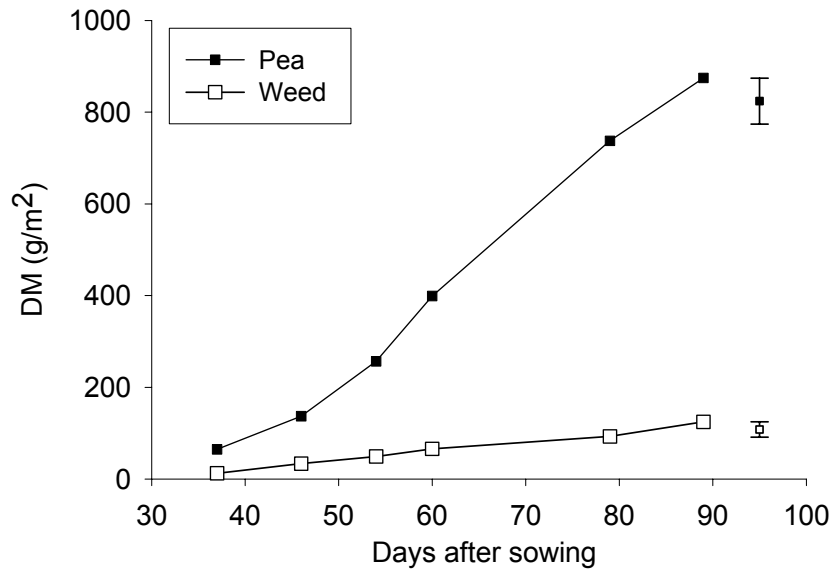


Figure 5-2. Pea and weed (excluding nightshade species) dry matter (DM) (g/m²) at six sample dates after the sowing of the peas. Error bar = Tukey HSD value.

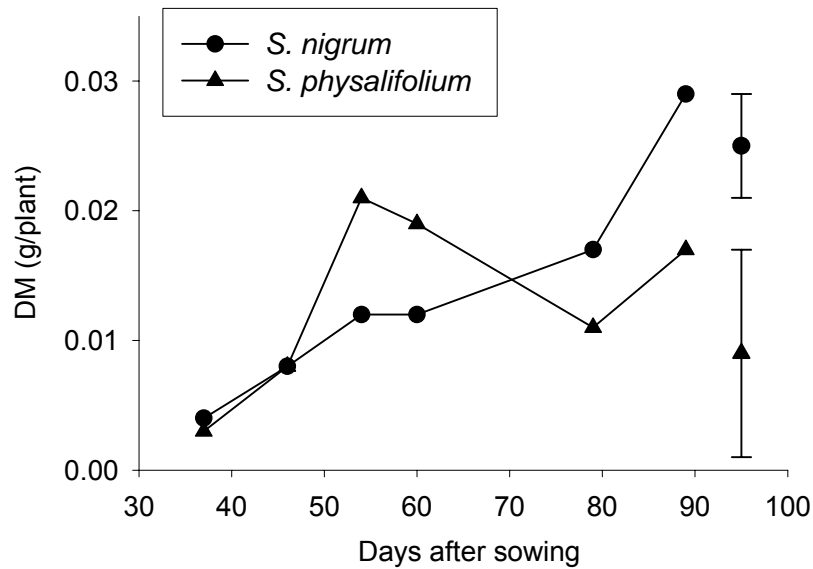


Figure 5-3. *Solanum nigrum* and *S. physalifolium* dry matter (DM) g/plant, for plants growing in a pea crop at six sample dates after the sowing of the peas. Error bar = Tukey HSD value.

The GA for peas and weeds, excluding the nightshade species, are presented in Figure 5-4. Pea GA had reached 4.87 GA/m² at 60 DAS, this had declined significantly to 3.75 GA/m² at 89 DAS. At 60 DAS the weed GA was 0.71 GA/m², there was no significant GA decline or increase for the weeds after this sample. The GA/plant values for *Solanum nigrum* and *S. physalifolium* indicated significant sample date variation (Figure 5-5). However, paired *T*-tests indicated that only at 79 DAS were there significant differences between the two species. At 79 DAS *S. nigrum* plants had significantly ($p = 0.05$) greater GA/plant than *S. physalifolium* plants, but overall there was no significant species main effect.

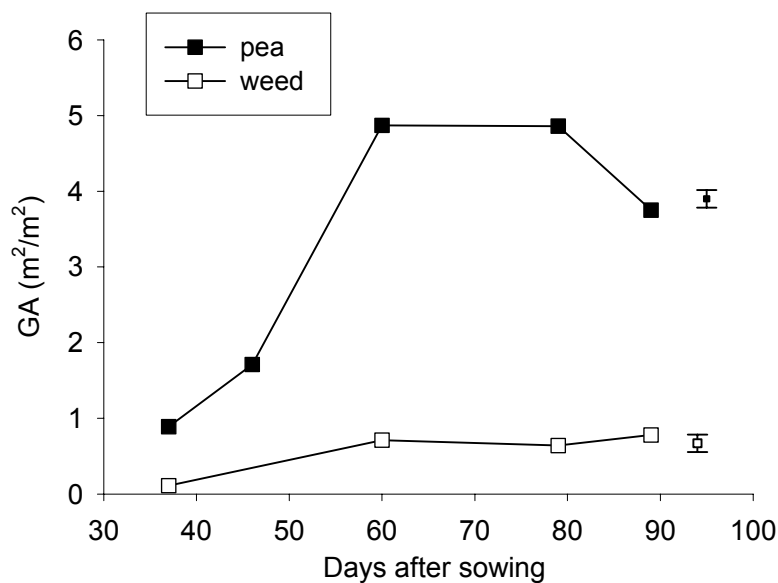


Figure 5-4. Pea and weed (excluding the two nightshade species) green area (GA) m²/m² (GA) at six and five sample dates respectively. Error bar = Tukey HSD value.

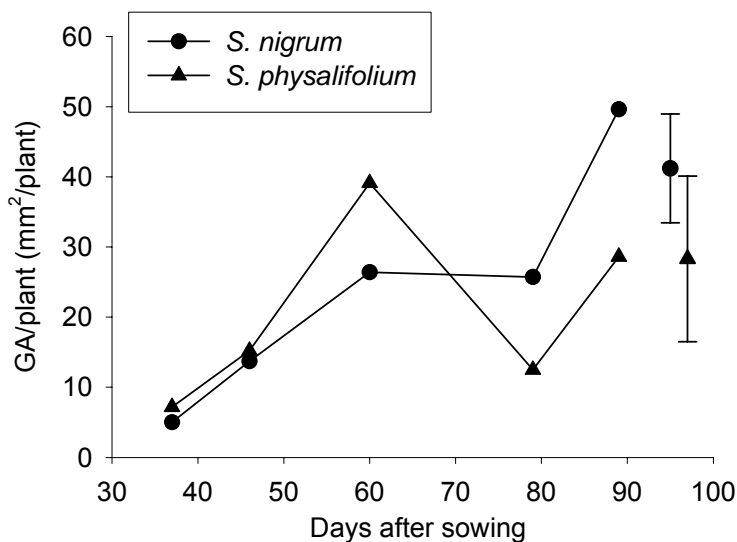


Figure 5-5. *Solanum nigrum* and *S. physalifolium* green area (GA) per plant (mm²/plant), for plants growing in a pea crop at five sample dates after the sowing of the peas. Error bar = Tukey HSD value.

The green area ratio (GAR) of the *S. nigrum* plants was not significantly affected by sample date, this differed from the effect of sample date indicated by the Tukey HSD value (Table 5-7). The GAR of *S. physalifolium* plants was significantly ($P < 0.01$) affected by sample date, with the value at 60 DAS being greater than at 37, 79 and 89 DAS. Paired *T*-tests for each sample date indicated that at 37, 79 and 89 DAS *S. nigrum* had a significantly ($P < 0.01$) higher GAR than *S. physalifolium*, and that over all dates there was a significant ($P < 0.001$) difference between the two species with values of 1,717 and 975 mm²/g, respectively.

Table 5-7. Green area ratio (GAR) (mm²/g) per plant *Solanum nigrum* (SN) and *S. physalifolium* (SP) growing in a pea crop for five samples at days after sowing (DAS). Effects: d.f. = 141, SN (s.e. = 338.2, Tukey HSD = 1,324.7), SP (s.e. = 207.2, Tukey HSD = 811.6). Values followed by a different letter within a column are significantly different. An * indicates a significant species effect.

DAS	GAR	
	SN	SP
37	1,122 a*	512 a
46	2,450 b	1,226 ab
60	2,045 ab	1,727 b
79	1,501 ab*	681 a
89	1,468 ab*	728 a
Mean	1,717*	975

Three *S. nigrum* and twelve *S. physalifolium* plants, at the final harvest, had fruit present from the 30 LA and the 30 pea yield quadrats. Nightshade plants with fruit were etiolated and lodged within the pea canopy (Appendix 12). Two sample *T*-tests, comparing a number of growth and fruit parameters, indicated there were no significant differences for any parameter between the species (Appendix 14). Logit regression indicated that there were significantly ($P < 0.001$, d.f. = 2) more *S. physalifolium* (density 13.7 plants/m²) plants with fruit than *S. nigrum* (density 107 plants/m²) plants with fruit (Table 5-8).

Table 5-8. Proportion of *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants bearing fruit at the final sample date. An * indicates a significant species effect.

	Plants with fruit	
	SN	SP
LA quadrats (3 m ²)	0.9 %	19.5 % *
Yield quadrats (3 m ²)	0 %	9.8 % *

Nightshade growth in an organic process pea crop

Comparisons between the two species of leaf number, plant DM and plant length did not indicate any significant differences between the species (Table 5-9). Only a small number (4) of *S. nigrum* plants had fruit, and for two of these plants total fruit DM was < 0.01 g, only one *S. physalifolium* plant did not have fruit. There were significant differences ($P < 0.05$) for the following fruiting parameters: the number of clusters with fruit, the number of fruit in each of the size classes, maximum fruit diameter, fruit DM, HI and CI. Plant DM, excluding fruit was a significant ($P < 0.001$) predictor for both nightshade species of total fruit DM, and the number of fruit > 3 mm on a plant (Table 5-10).

Table 5-9. Plant dry matter (DM), height and number of fruiting parameters including harvest index (HI) and contamination index (CI) for 19 *Solanum nigrum* (SN) and 19 *S. physalifolium* (SP) plants sampled from a commercial organic pea crop. (Significance indicated from Bonferroni Adjusted Probability test.)

	SN	SP	p
Leaf number	52.8	68.2	ns
Plant DM including fruit (g)	1.04	1.60	ns
Plant DM excluding fruit (g)	1.04	1.51	ns
Plant length (mm)	357	399	ns
Number of clusters with fruit	0.42	3.6	< 0.05
Total number of clusters	8.6	11.2	ns
Number of 0 - 2.9 mm fruit	0.53	7.8	< 0.05
Number of 3 - 4.9 mm fruit	0.32	3.6	< 0.05
Number of 5 - 6.9 mm fruit	0.05	3.3	< 0.05
Maximum fruit diameter (mm)	0.8	4.6	< 0.05
Fruit DM (g)	0.002	0.091	< 0.05
HI	0.001	0.041	< 0.05
CI	0.14	0.39	< 0.05

Table 5-10. Regression results (r^2 values) of plant dry matter (DM) excluding fruit against fruit DM and the number (No.) of fruit greater than 3 mm diameter, for 19 *Solanum nigrum* (SN) and 19 *S. physalifolium* (SP) plants.

	SN	SP
Fruit DM	0.648***	0.712***
No. fruit > 3mm	0.664***	0.865***

***P < 0.001

5.4 Discussion

Differences in dry matter production

The DM production of *S. physalifolium*, when grown under non-competitive conditions, was significantly greater than *S. nigrum* at all sowing dates except August (Table 5-1). The differences in DM production between *S. nigrum* and *S. physalifolium* were supported by the growth rate values, where for all except the August sowing, of *S. physalifolium* (0.87 g/d/plant) had significantly higher growth rates than *S. nigrum* (0.30 g/d/plant) (Table 5-1).

When *S. nigrum* and *S. physalifolium* plants were grown in a field trial with peas or collected from a commercial pea crop there were no significant differences in DM between the two species (Table 5-9 and Appendix 14). Heider (1996) reported that *S. sarrachoides* plants produced greater DM than *S. ptycanthum* plants by pea harvest, for both seedlings growing in bare ground, and within process peas where weeds were controlled with herbicide.

Heider (1996) also reported that by the time of pea harvest *S. sarrachoides* and *S. ptycanthum* seedlings transplanted into peas had approximately 10 % of the DM of plants transplanted into bare ground on the same date. The DM production of *S. sarrachoides* and *S. ptycanthum* plants in the pea crops was 3.3 % and 1.2 %, respectively, of the DM of the non-competitively grown plants (Table 5-1 and Table 5-9). For fruiting plants from the pea trial sown in October 2001 the mean DM/plant values were only 1.0 and 0.1 % for *S. nigrum* and *S. physalifolium*, respectively, at 89 DAS (Appendix 14).

A number of possible explanations exist for these differences. In the pea trial and in the commercial pea crop, the nightshade plants had naturally germinated in the field. The counts of seedling classes from the pea trial indicated that there may have been a range of emergence times in both species (Appendix 13). Such differences may have confounded comparisons and also affected weed growth responses through differential competition.

In addition, the pea trial had a high density of peas (151 plants/m²) giving a final crop DM of 874 g/m², and the area was not weeded, which contributed to a high final non-nightshade weed DM at harvest (125 g/m²) (Figure 5-2). A previous study of non-weeded process pea plots in Canterbury, reported weed and crop DM values at pea maturity of only 9.3 and 521 g/m², respectively (Reddix *et al.*, 2001). Weed density was not assessed in the commercial pea crop. The use of transplants by Heider (1996) and the control of additional weed pressure may have provided competitive advantages for the nightshade seedlings in relation to the crop and other weed species in his work, thereby giving a greater total DM production relative to nightshade plants grown in bare ground. Thus, the reason for the DM production values of *S. nigrum* and *S. physalifolium* being 0.1-3.3% of the non-competitively grown plants when grown with peas, is probably due to the effects of weed pressure and the time of establishment. Similarly, the lack of differences between *S. nigrum* and *S. physalifolium* may be the result of the competitive effects identified above. This has the implication that in a competitive growing environment the greater potential growth of *S. physalifolium* may not be realised.

Further work using controlled emergence times or transplants and crop and weed density studies are required to substantiate the finding that the growth of *S. physalifolium* is not superior to that of *S. nigrum* when growing in a pea crop. However, these results suggest that current pea crop management may be adequate to limit the potentially greater DM accumulation of *S. physalifolium*.

Morphological differences and plasticity

One of the greatest differences for the non-competitively grown nightshade plants was the difference in PLA between the species. The PLA of *S. physalifolium* was significantly higher than that of *S. nigrum* at all dates, except August, and there was, overall, a significantly higher species mean for *S. physalifolium* (0.511 m²/plant) than for *S. nigrum* (0.191 m²/plant) (Table 5-2). However, at no date in the pea trial was the mean PLA of *S. physalifolium* significantly higher than that of *S. nigrum*. In fact, at 79 DAS the opposite occurred (Figure 5-5). This indicates that different growth responses may occur for these two species when they are grown with peas. Initial LA development is identified as being an important growth parameter in identifying competitive species particularly in conjunction with a high relative growth rate (Seibert and Pearce, 1993; Wall, 1995). However, the non-competitive growth trial only investigated PLA values at harvest, thus it was not possible to determine if the greater PLA of *S. physalifolium* was the result of initial differences in PLA at the seedling stage.

The GAR values for *S. nigrum* when grown with peas were greater than those for *S. physalifolium* (Table 5-7). *Solanum nigrum* is reported to have significant increases in LAR and decreased leaf mass per unit area in response to increased shade levels (Fortuin and Omta, 1980). In the non-competitive trial the GAR, LAR and leaf weight ratios values between the two species did not differ significantly. The greater ability of *S. nigrum* than *S. physalifolium* to either regulate leaf mass per unit area or non-leaf DM investments in environments where competition for radiation affects plant growth, indicates that *S. physalifolium* may be a poorer competitor than *S. nigrum* in such environments. *Solanum nigrum* and *S. physalifolium* values for DM/plant, GAR/plant and GA/plant, at each sample date, were calculated from all the seedlings in each sample. Seedling counts on the basis of leaf number indicated that for each successive sample date an increasingly greater range of seedlings with different leaf numbers were present (Appendix 13). Therefore the interpretation of these values may require some caution, as the differing contributions of leaf number seedling classes at different sample dates may have affected comparisons.

Physiological factors may have also affected the observed growth responses in both the non-competitive and competitive trials. There is some evidence in the literature that *S. nigrum* may have superior photosynthetic efficiencies when grown in shade. Differences in the quantum yield of *S. nigrum* for plants in full sun or shade (80 %), were better maintained than that of *Amaranthus cruentus* L. (Sattin *et al.*, 1992). Unfortunately, there is no similar information apparent in the literature for *S. physalifolium* but there was some evidence that growth processes may differ between the two species. For example, in the non-competitive trial, the growth rate and PLA of *S. physalifolium* did not differ from that of *S. nigrum* in August, but did for all

later months (Table 5-1). In addition, the leaf appearance rate for *S. physalifolium* at bud appearance, was significantly less than for the later months (Table 5-4). These results could be due to deviations from the optimum temperature range for photosynthesis of this species, which would result in a decline in radiation use efficiency. This has been reported for the effect of temperature on *Zea mays* growth (Andrade *et al.*, 1993). Mean temperature differences during growth were identified as contributing to the differential competitive ability of *Solanum ptycanthum* when grown with peas, with *S. ptycanthum* competing more successfully during periods of high mean temperatures (23.9 °C) (Croster and Masiunas, 1998). The August sowing of this work experienced the lowest temperatures, with mean temperatures in both August and September at some dates below 10 °C (Figure 5-1). This may indicate that *S. physalifolium* has a higher minimum optimum temperature range than *S. nigrum*, as the August DM accumulation rate for *S. nigrum* did not differ significantly from those observed in later months. If this is correct, the implication is that the greater growth potential of *S. physalifolium* relative to *S. nigrum* may not be evident when low temperatures are limiting. However, this work did not identify a threshold value.

Using growth to identify reproductive initiation

An evaluation of leaf number at flower bud appearance was made to test if a readily identifiable growth threshold, such as leaf number, could be used to identify the initiation of reproductive development. The results of leaf number for *S. nigrum* indicated no significant variation by sowing date (Table 5-4). However, plants had from ~30 to ~47 leaves/plant at bud appearance. A number of *S. nigrum* plants from the pea trial were observed to have buds on plants with seven leaves (data not presented). This suggests that a growth threshold of leaf numbers prior to bud appearance may not exist in *S. nigrum*. For *S. physalifolium*, sowing month produced significant variation in the number of leaves/plant at bud appearance. This indicates that for this species there is no evidence to suggest that bud appearance was linked to a leaf number threshold. These results confirm a plasticity of growth response capacity present in many weed species (Elmore *et al.*, 1994).

Fruit production

Non-competitive growth comparisons between the two species indicated that *S. physalifolium* produced a greater number of fruit (Table 5-5), had a greater HI and importantly, in terms of contaminant potential, a greater CI than *S. nigrum* (Table 5-6). When these species were grown with peas, a significantly greater proportion of *S. physalifolium* than *S. nigrum* plants produced fruit by the time of pea harvest (Table

5-8). For plants of a similar DM from a pea crop, *S. physalifolium* plants produced more fruit and had a higher CI than *S. nigrum* (Table 5-9). This indicates that the timing of initiation of reproductive development, between the two species, probably differed. Differences in the time of flowering are investigated in more detail in Chapter 6.

Independent of phenology, in both species, contamination potential was linked to growth effects, as plant size was positively associated with fruit DM and the number of fruit per plant > 3mm (Table 5-10). Nightshade fruit of 3 mm is estimated to be a minimum fruit diameter for the contamination of process peas (data not presented). Thus plant size can effect potential contamination. Similar results are reported for *Amaranthus retroflexus* and *Chenopodium album* where it was reported that for a given tillage or mulch treatment, the majority of weed seed was produced by the largest plants in both species (Mohler and Callaway, 1995). Therefore, factors that limit the growth of these species can be used to manage the risk of nightshade contamination. Although competition between plants occurs for nutrients, water and radiation, a number of studies identify the success of competitive interactions between crops and weeds as being linked with factors which affect radiation interception (Stoller and Wooley, 1985; Akey *et al.*, 1990; Kropff *et al.*, 1992; Barbour and Bridges, 1995; Blackshaw *et al.*, 2000). If the growth limitation to *S. physalifolium* in pea crops is similarly caused by radiation limitation, then attention to factors that limit radiation interception for weeds are required.

Increases in crop density were reported to affect weed PAR interception, so negatively affecting weed DM production including weed seed production in *Triticum aestivum* L. crops (Blackshaw *et al.*, 2000). Increased pea density has also been reported to negatively affect weed growth in process peas (Lawson, 1982; Lawson and Topham, 1985). However, pea yields ceased to increase above pea densities of 140 and 180 plants/m² in two separate years of trials (Lawson, 1982). Current recommendations to organic pea growers are for a FE of 120 plants/m². It seems possible that that pea sowing density could be further increased to help control nightshade. This question requires further research. However, in terms of limiting the growth of weeds, it is clear that minimising events that cause low pea populations such as drilling errors, or poor seed quality, or soil capping reducing FE densities are also important.

5.5 Conclusions

1. *Solanum physalifolium* grown in the absence of competition produced more DM, and grew faster than *S. nigrum*. However, the DM production of *S. physalifolium* and *S. nigrum* did not differ when they were grown in a process pea crop.
2. Growth parameters for both nightshade species exhibited plasticity in relation to the growth environment. *Solanum nigrum* exhibited a greater GAR than *S. physalifolium* when grown with peas, but not without peas. This growth response to the environment may make *S. nigrum* more competitive in radiation limited environments than *S. physalifolium*.
3. Leaf counts did not provide a useful means of identifying bud appearance in either nightshade species due to their growth plasticity.
4. Differences in fruit production between the two species were due to differences in their phenology with regard to reproductive initiation. However, for plants of either species that had initiated flowering, plant size was positively related to the amount of fruit produced.

Chapter 6 Flowering and fruit phenology

6.1 Introduction

The analysis of seasonal records of nightshade fruit contamination of process peas in Canterbury, indicate a trend that nightshade contamination occurs in the second half of the sowing season (Section 3.3.3). Fruits of both *Solanum nigrum* and *S. physalifolium* can cause this contamination, although it is not known if either species predominately causes the contamination or if seasonal factors affect the potential of either species to contaminate process peas differentially.

Time to flowering of *S. nigrum* is strongly influenced by seasonal field temperatures (Keeley and Thullen, 1983). A study of the thermal time requirements of *S. nigrum* and *S. ptycanthum* reported that flowering occurred at a value of approximately 600 °Cd (T_b 6°C) in both species. However, it was not reported if this value was related to the initiation of flowering or a proportional flowering value such as 50 % flowering (McGiffen and Masiunas, 1992). There is no information on the flowering phenology of *S. physalifolium* in the literature, but it would be useful for evaluating the risk of contamination for this species. Similarly, there is no published information on fruit growth rates for either species. The rate of fruit growth is important, as this will affect the ability of the nightshade fruit to reach a diameter that will contaminate process peas.

Crop competition with *Glycine max* is reported to slow the flowering of *S. ptycanthum* (Quakenbush and Andersen, 1984). However, other studies of this and other nightshade species reported that crop competition does not appear to affect the timing of development. For example, in *S. ptycanthum* and *S. sarrachoides* it was reported that competition with pea crops did not significantly affect the time of bud appearance or flowering (Heider, 1996). There is a similar report for *S. nigrum* and *S. sarrachoides* growing with and without *Lycopersicon esculentum* Mill. (Hinckley, 1981). Further, light quality did not affect the time of flowering of *S. ptycanthum* or *S. sarrachoides* (Croster *et al.*, 2003). Crop competition is also reported not to affect the flowering time in *Raphanus raphanistrum* L. (Cousens *et al.*, 2001).

This chapter addresses the fourth objective of this study, to quantify the phenology of both nightshade species in relation to flower bud appearance, flowering and fruit growth. To study these factors an experiment was conducted using the two nightshade species when grown under non-competitive conditions. The first hypothesis investigated was that flower bud appearance and flowering of *S. physalifolium* does not differ from that of *S. nigrum* (hypothesis 1). *Solanum sarrachoides* and *S. physalifolium* are morphologically reported to be very similar

(Edmonds, 1986). While *S. sarrachoides* is reported to flower ~ 80 °Cd before *S. ptycanthum* (Heider, 1996). A comparison of *S. ptycanthum* and *S. nigrum* reported that the flowering requirements of these two species did not differ (McGiffen and Masiunas, 1992). The hypothesis investigated for fruit growth, was that fruit growth rates for the two species do not differ (hypothesis 2).

Comparisons indicated that pea cultivars sown during the contamination period had significantly greater thermal time requirements than cultivars sown prior to contamination occurring in the growing season (see section 3.3.3). Mean temperatures also increase over the pea sowing season. From these observations it was hypothesised that nightshade contamination for late sown peas and pea cultivars with mid to high thermal time maturity requirements would not differ from early sown peas or peas with short thermal time maturity requirements (hypothesis 3).

6.2 Methods

6.2.1 Nightshade development experiment

The site description, sowing dates, bud appearance, flowering and fruit growth data collection methods, and temperature data for this experiment are described in Chapter 5 (see section 5.2.1).

Analysis: Hourly thermal time values were calculated by:

$$^{\circ}\text{Ch} = (\text{hourly temperature} - T_b)/24$$

If the hourly temperature value was < T_b no value was calculated. Calculation of phenological intervals used a T_b of 6 °C (Alm *et al.*, 1988). The hourly thermal time values were summed to provide thermal time values for various plant phenological periods.

Thermal time accumulation from sowing was initiated one hour after the last pre-germinated seed was sown. The thermal time intervals for the various phenological stages were calculated by ceasing thermal time accumulation at midday (12:00) on the day of bud appearance or flowering. Bud appearance and flowering of plants was considered to be on the date when one visible bud or flower was present on 50 % of plants in a plot.

Two methods of fruit growth analysis were used. Both were based on the selection of one fruit from each of two monitored clusters on a plant. Fruit were

selected on the basis that flowering of the bud was observed and that the fruit from this bud reached a minimum diameter of 7 mm prior to plant harvest (n = 353). The first growth analysis method was based on the calculation of 95 % of maximum fruit diameter for fruit in the data set. Both days from flowering and the thermal time to 95 % of maximum diameter were calculated.

The second method of fruit growth analysis used was based on the calculation of mean fruit growth in relation to thermal time values at each measurement date after flowering for each fruit for the set of 353 fruit. A Gompertz curve of the form:

$$y = A + C \exp(-\exp(-b(x-m)))$$

was then fitted using Genstat 6th Edition (2002) for the thermal time (x, °Cd) values versus fruit diameter (y, maximum fruit diameter mm) for observations from flowering for each fruit. Subsequent analysis was made of maximum diameter (C), maximum growth rate (Cb/e) and average growth rate (C/duration of growth (4/b)).

Fruit diameter (y, mm) was predicted using the equation with thermal time (x, °Cd):

$$x = (\text{LOG}((\text{EXP}(b*m))/(\text{LOG}(C/y))))/b$$

The analysis used was the GLM in Systat[®] Version 9.01 (1998). For all analyses, variates were analysed for the factors block, sowing date, species and the species by sowing date interaction. Means separation tests were made using the Tukey HSD test (Zar, 1984). For the comparisons of fruit growth means, which had unequal sample numbers, the standard error of these means was calculated using a procedure for unequal group sizes (Kramer, 1956). The Tukey HSD value for comparison of means with unequal sample sizes was calculated as the mean Tukey HSD value for each pair of values in the comparison. Linear regression was used to examine the relationship between mean fruit DM versus fruit diameter. For regressions of the mean temperature, mean temperature was calculated from the mean number of days of each species for each planting date for two periods: 50 % emergence to 50 % flowering; and sowing date to 50 % flowering. Analysis of phenological intervals from 50 % emergence was limited to the first four sowing dates, as 50 % emergence for the December sowing was not observed (see Section 5.2.1).

6.2.2 The effect of sowing date and cultivar on the potential for nightshade contamination

Four process pea cultivars with varying thermal time requirements were selected from the literature (Table 6-1). Using the same hourly air temperature data as cited in Figure 5-1, the date of pea maturity, using a T_b of 4.5 °C, was predicted from sowing for six dates (from 23 August to 6 December) at three weekly intervals. The thermal time

accumulation, using a T_b of 6.0 °C, was also calculated for the predicted date of pea maturity from the pea sowing date. For sowing dates where it was predicted that *S. nigrum* or *S. physalifolium* preceded pea maturity, the thermal time weed free period with a T_b of 6.0 °C between the predicted nightshade flowering and that of the cessation of accumulation at pea maturity was calculated. Using the temperature data, the thermal time weed free period was then calculated in terms of the number of days from the pea sowing date.

Table 6-1. Thermal time values (°Cd) for four pea cultivars from sowing to crop maturity (T_b of 4.5 °C) (Mikkelsen, 1981; Friis *et al.*, 1987). (Values in brackets are the CV %).

Pea cultivar	Maturity °Cd
Avola	692 (6.0)
Freezer 69	729 (8.6)
Visto	777 (5.7)
DSP	839 (5.0)

6.3 Results

6.3.1 Nightshade development experiment

Flower bud appearance (BA)

The days from seedling emergence to BA on 50 % of plants were significantly ($P < 0.001$) affected by the interaction between species and sowing date (Table 6-2). In both species there were significant decreases in the day counts to BA for each later sowing date, but in the August and November sowings *S. physalifolium* took significantly fewer days to BA than *S. nigrum*. *Solanum physalifolium*, overall required 51 d to BA, this was significantly ($P < 0.001$) fewer than for *S. nigrum* (60 d). The thermal time to BA was also significantly ($P < 0.01$) affected by the species by sowing date interaction, with *S. nigrum* requiring more thermal time for BA in the August sowing than in the October and November sowings. Values for *S. physalifolium* were not significantly affected by sowing date, and were significantly lower than those for *S. nigrum* in August and November. Overall, *S. nigrum* had a significantly ($P < 0.001$) higher value of 434 °Cd than that of *S. physalifolium* (352 °Cd). For the period from sowing to BA only *S. nigrum* was significantly ($P < 0.01$) affected by sowing date. The August and November values were greater than those observed for October and December. Values for *S. physalifolium* were significantly greater those for *S. nigrum* in the August and November sowings. The overall species means also differed

significantly ($P < 0.001$). *Solanum physalifolium* had a significantly lower value (401 °Cd) than *S. nigrum*, (482 °Cd).

Table 6-2. Days (d) and thermal time (°Cd) values from emergence to bud appearance (BA), and °Cd from sowing to BA for *S. nigrum* (SN) and *S. physalifolium* (SP) plants. Effects: day count interaction (s.e. = 1.6, d.f. = 21, Tukey HSD = 7.8) and day species effect (s.e. = 0.8, Tukey HSD = 2.4), BA °Cd from emergence interaction (s.e. = 14.4, d.f. = 21, Tukey HSD = 68.6) and BA °Cd species effect (s.e. = 7.2, Tukey HSD = 21.2), BA °Cd from sowing interaction (s.e. = 16.0, d.f. = 27, Tukey HSD = 77.4) and BA °Cd species effect (s.e. = 7.2, Tukey HSD = 20.7). Means followed by the same letter within and between columns for the same factor are not significantly different. An * indicates a significant species main effect.

Sowing	d from emergence		°Cd from emergence		°Cd from sowing	
	SN	SP	SN	SP	SN	SP
August	87 g	67 f	496 c	337 a	537 d	377 a
September	62 ef	58 de	428 bc	362 ab	480 cd	403 abc
October	51 cd	49 c	407 b	383 ab	453 abc	429 abc
November	39 b	31 a	407 b	323 a	480 d	401 ab
December	-	-	-	-	457 bc	395 ab
Mean	60*	51	434*	352	482*	401

Flowering

Days from emergence to flowering of 50 % of plants were significantly affected by sowing date and species. *Solanum physalifolium* required significantly ($P < 0.001$) fewer days (79 d) to flower than *S. nigrum* (67 d) (Table 6-3). There was a significant ($P < 0.001$) decline in the number of days to flowering from emergence, with ~42 d difference between the August and November sowing (Table 6-4). The thermal time values from emergence to flowering on 50 % of plants also gave no significant interaction, but there were significant species ($P < 0.001$) and sowing date ($P < 0.05$) effects. *Solanum physalifolium* had a lower thermal time requirement (509 °Cd) than *S. nigrum* (633 °Cd) (Table 6-3). Sowing date had no effect on thermal time to flowering. There was a significant ($P < 0.001$) species effect on the thermal time to flower. *Solanum physalifolium* (562 °Cd) required fewer thermal units than *S. nigrum* (686 °Cd) (Table 6-3).

Solanum physalifolium plants required significantly fewer days (13 d) to progress from BA to flowering than did *S. nigrum* (18 d) (Table 6-3). For this interval, there was no significant interaction between species and sowing date, or was there a significant sowing date effect. For thermal time values for the interval from BA to flowering, there was a species effect, with *S. nigrum* (188 °Cd) having a significantly ($P < 0.001$) higher value than *S. physalifolium* (138 °Cd) (Table 6-3). Sowing date had a significant effect ($P < 0.001$) on the thermal time required for BA to flowering (Table

6-4). The August value was significantly lower than the November and December values. There was no significant interaction for thermal time values for this interval. Comparison of the days between the flowering on the first and second of the two monitored clusters indicated a significant ($P < 0.001$) species effect, with *S. nigrum* requiring fewer days (3 d) than *S. physalifolium* (5 d) (Table 6-3). Sowing date had no significant effect for this variate, and there was no significant interaction of sowing date by species.

Table 6-3. The flowering intervals of *S. nigrum* (SN) and *S. physalifolium* plants in days and thermal time ($^{\circ}\text{Cd}$). Effects: days from emergence (s.e. = 1.0, Tukey HSD = 2.8), $^{\circ}\text{Cd}$ from emergence (s.e. = 9.3, Tukey HSD = 27.3), $^{\circ}\text{Cd}$ from sowing (s.e. = 9.2, d.f. = 27, Tukey HSD = 26.5), days from bud appearance (BA) (s.e. = 0.4, Tukey HSD = 1.1), $^{\circ}\text{Cd}$ from BA (s.e. = 3.6, Tukey HSD = 17.4), days from cluster one to cluster two (s.e. = 0.3, d.f. = 27, Tukey HSD = 0.9). An * within a row indicates a significant difference between species.

Interval	SN	SP
From emergence (days)	79*	67
From emergence ($^{\circ}\text{Cd}$)	633*	509
From sowing ($^{\circ}\text{Cd}$)	686*	562
From BA (days)	18*	13
From BA ($^{\circ}\text{Cd}$)	188*	138
Cluster one to cluster two (days)	3*	5

Table 6-4. Sowing dates effects on flowering from emergence in days (s.e. = 1.4, d.f. = 21, Tukey HSD = 5.87), thermal time ($^{\circ}\text{Cd}$) from emergence (s.e. = 13.1, d.f. = 21, Tukey HSD = 51.9), $^{\circ}\text{Cd}$ from bud appearance (BA) (s.e. = 8.1, d.f. = 27, Tukey HSD = 39.0) for *S. nigrum* (SN) and *S. physalifolium* (SP) plants. Means followed by the same letter in single columns are not significantly different.

Sowing	Days from emergence	$^{\circ}\text{Cd}$ from emergence	$^{\circ}\text{Cd}$ BA to flowering
August	99 d	587 a	148 a
September	76 c	563 a	152 ab
October	65 b	570 a	155 ab
November	52 a	565 a	174 b
December	-	-	188 b

Base temperatures

There was a significant relationship between rate to flower from emergence and mean air temperature for both species (Figure 6-1). From the regressions, a T_b of 6.24 $^{\circ}\text{C}$ for *S. nigrum* and 5.66 $^{\circ}\text{C}$ for *S. physalifolium*, were calculated. The thermal time requirements for flowering from emergence were 583 $^{\circ}\text{Cd}$ and 499 $^{\circ}\text{Cd}$ for *S. nigrum* and *S. physalifolium*, respectively. Similarly, analysis of the rate to flowering for the period between sowing and 50 % flowering also gave significant ($P < 0.001$) linear relationships in both nightshade species. There was a T_b of 5.42 $^{\circ}\text{C}$ and thermal time

requirement of 707 °Cd, and T_b of 4.70 °C and thermal time requirement of 628 °Cd, respectively, for *S. nigrum* and *S. physalifolium* (data not presented).

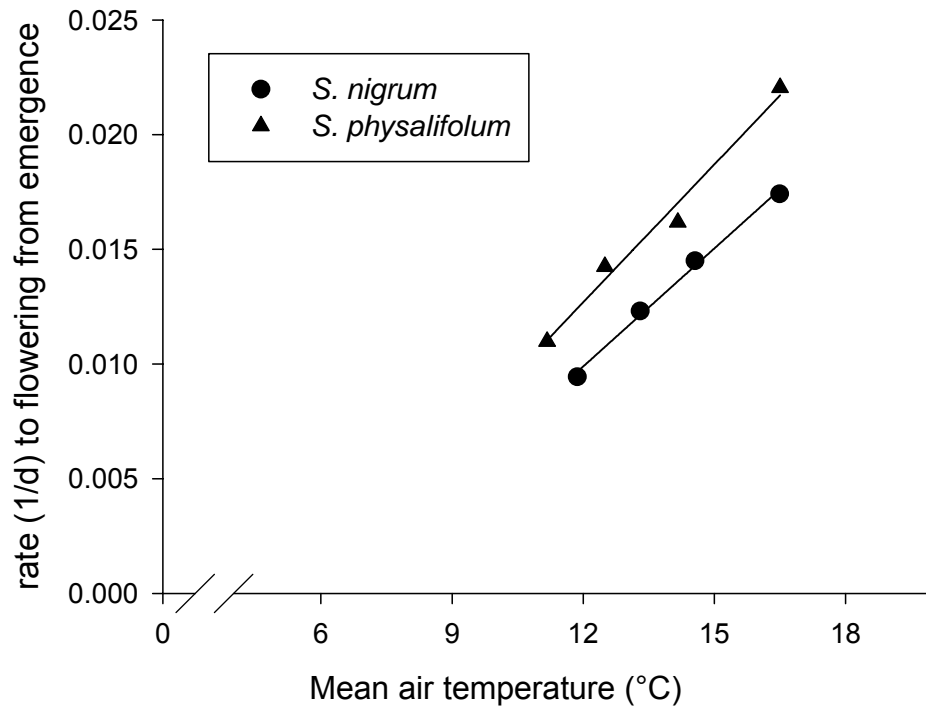


Figure 6-1. The mean temperature from 50 % emergence to 50 % flowering, for four sowings of *Solanum nigrum* ($y = -0.01070 + 0.0017159 (x)$, $r^2 = 0.993$, $P < 0.01$) and *S. physalifolium* ($y = -0.01134 + 0.002003 (x)$, $r^2 = 0.973$, $P < 0.01$).

Fruit growth

In the set of 353 fruit that were analysed, 192 fruit were from *S. nigrum* and 161 were from *S. physalifolium*. The thermal requirement from flowering to 95 % maximum fruit diameter was affected by species ($P < 0.001$). *Solanum nigrum* required less thermal units (154 °Cd) than *S. physalifolium* (239 °Cd) (Table 6-5). Sowing date had a significant effect ($P < 0.001$). The August (191 °Cd) sowing required significantly less thermal time to reach 95 % maximum fruit diameter than the December sowing (207 °Cd) (Table 6-6). The daily growth rate was significantly ($P < 0.001$) affected by species with *S. nigrum* having a faster growth rate (0.62 mm/d) than *S. physalifolium* (0.36 mm/d) (Table 6-5). Species effects on daily growth rate did not interact with sowing date. However, sowing date did have a significant ($P < 0.001$) effect (Table 6-6). Fruit growth rates in December were significantly lower than in the previous four months. The thermal time growth rate was significantly ($P < 0.01$) affected by species and *S. nigrum* had a greater thermal growth rate (0.056 mm/°Cd) than *S. physalifolium* (0.033 mm/°Cd) (Table 6-5). For this thermal growth rate there was no species by sowing date interaction, but sowing date was significant ($P < 0.001$). The December

sowing value (0.0041 mm/°Cd) was significantly lower than values for the previous four months (Table 6-6).

Table 6-5. Species effects for thermal time (°Cd) from flowering to 95 % of final maximum fruit diameter (s.e. = 2.6, Tukey HSD = 7.3), and growth rates from flowering to 95 % of final maximum fruit diameter for mm/d (s.e. = .007, Tukey HSD = 0.020), and mm/°Cd (s.e. = 0.0020, Tukey HSD = 0.0056) for *S. nigrum* (SN) and *S. physalifolium* (SP). All d.f. = 340. An * indicates a significant species effect.

	SN	SP
°Cd 95 %	154*	239
mm/d	0.62*	0.36
mm/°Cd	0.056*	0.033

Table 6-6. Sowing dates effects for thermal time (°Cd) from flowering to 95 % of final maximum fruit diameter (s.e. = 4.1, Tukey HSD = 16.0) and growth rates from flowering to 95 % of final maximum fruit diameter for mm/d (s.e. = 0.0116, Tukey HSD = 0.0449), and mm/°Cd (s.e. = 0.0010, Tukey HSD = 0.0039). All d.f. = 340. Means followed by the same letter in single columns are not significantly different.

Sowing	°Cd for 95 % growth	mm/d from flowering	mm/°Cd from flowering
August	191 a	0.52 b	0.046 b
September	192 ab	0.52 b	0.045 b
October	194 ab	0.49 b	0.046 b
November	199 ab	0.49 b	0.043 b
December	207 b	0.43 a	0.041 a

The growth curve values indicated that maximum fruit diameter (C) was significantly ($P < 0.001$) affected by species. *Solanum nigrum* (8.8 mm) had a greater C value than *S. physalifolium* (8.1 mm) (Table 6-7). Maximum fruit diameter was not significantly affected by sowing date and sowing did not interact with species. The maximum growth rate (GR) was significantly ($P < 0.001$) faster in *S. nigrum* (0.068 mm/°Cd) than in *S. physalifolium* (0.044 mm/°Cd) (Table 6-8). Maximum GR was significantly ($P < 0.001$) affected by sowing date, but there was no sowing date by species interaction. Sowing date values for August (0.066 mm/°Cd) and September (0.060 mm/°Cd) were greater than those in December (0.048 mm/°Cd). The maximum GR for the August sowing was also significantly greater than values for the October and November sowings.

The mean GR was significantly ($P < 0.001$) affected by species, with the values for *S. nigrum* (0.037 mm/°Cd) greater than that for *S. physalifolium* (0.024 mm/°Cd) (Table 6-7). The mean GR was also significantly ($P < 0.001$) affected by sowing date, with the mean GR in August greater than that in December (Table 6-8). The August

sowing mean GR was also significantly greater than values for the October and November sowings. Sowing date did not interact with species for this variate.

Table 6-7. Species effects for maximum fruit diameter (C) (mm) (s.e. = 0.05, Tukey HSD = 0.14), maximum growth rate (GR) (mm/°Cd) (s.e. = 0.0014, Tukey HSD = 0.0037) and mean GR (mm/°Cd) (s.e. = 0.0007, Tukey HSD = 0.0020) for *S. nigrum* (SN) and *S. physalifolium* (SP) fruit. All d.f. = 340. An * indicates a significant species effect.

	SN	SP
C	8.8*	8.1
Max GR	0.068*	0.044
Mean GR	0.037*	0.024

Table 6-8. Sowing date effects on maximum growth rate (GR) (mm/°Cd) (s.e. = 0.0021, Tukey HSD = 0.0082) and mean GR (mm/°Cd) (s.e. = 0.0012, Tukey HSD = 0.0045) for *S. nigrum* (SN) and *S. physalifolium* (SP) fruit from flowering. All d.f. = 340. Means followed by the same letter in single columns are not significantly different.

	Maximum GR	Mean GR
August	0.066 c	0.036 c
September	0.060 bc	0.032 bc
October	0.055 ab	0.030 ab
November	0.053 ab	0.029 ab
December	0.048 a	0.026 a

Prediction of fruit diameter

The predicted thermal time values, to a maximum fruit diameter of 3 mm, were affected by the species by sowing date interaction ($P < 0.001$) (Table 6-9). Values for *S. nigrum*, were not affected by sowing date but values for *S. physalifolium* for the October, November and December sowings were lower than for the August and September sowings. Overall, *S. nigrum* (75 °Cd) took significantly ($P < 0.001$) less thermal time to reach 3 mm diameter than *S. physalifolium* (140 °Cd).

For thermal time to 5 mm fruit diameter, species and sowing date interacted significantly ($P < 0.001$). Sowing date did not significantly affect the values for *S. nigrum*. However, in *S. physalifolium* values for the October, November and December sowings were significantly greater than that in the August sowing. Overall, *S. nigrum* (120 °Cd) had a significantly ($P < 0.001$) lower 5 mm value than *S. physalifolium* (209 °Cd).

Table 6-9. The predicted thermal time ($^{\circ}\text{Cd}$) values for the period from flowering to a maximum fruit diameter of 3 and 5 mm for *S. nigrum* (SN) and *S. physalifolium* (SP). Effects: all d.f. = 340, 3 mm interaction (s.e. = 4.7, Tukey HSD = 21.2) and species effect (s.e. = 2.1, Tukey HSD = 5.8), 5 mm interaction (s.e. = 9.7, Tukey HSD = 43.5) and species effect (s.e. = 4.3, Tukey HSD = 12.0). Means followed by the same letter within and between columns for the same factor are not significantly different. An * indicates a significant species effect.

Sowing	3 mm $^{\circ}\text{Cd}$		5 mm $^{\circ}\text{Cd}$	
	SN	SP	SN	SP
August	75 a	109 b	112 a	163 b
September	74 a	124 b	118 a	187 bc
October	73 a	159 c	114 a	244 c
November	70 a	147 c	113 a	214 c
December	85 a	161 c	141 ab	235 c
Mean	75*	140	120 *	209

6.3.2 Results of estimated pea sowing date and pea cultivar effects on nightshade development

Figure 6-2 shows that in all pea cultivars examined *S. physalifolium* would flower prior to pea maturity. However, *S. nigrum* can only flower prior to pea maturity in sowings, which are later than September for the cv. Freezer 69, and for all sowings of cv.s Visto and DSP.

Estimation of the thermal time weed free period between nightshade flowering and pea maturity indicated that the thermal time weed free period increased with later sowing dates of the same cultivar (Table 6-10). However, the estimation of this same weed free period interval in days indicated a decline in day count duration with later sowings for the same pea cultivar (Table 6-10).

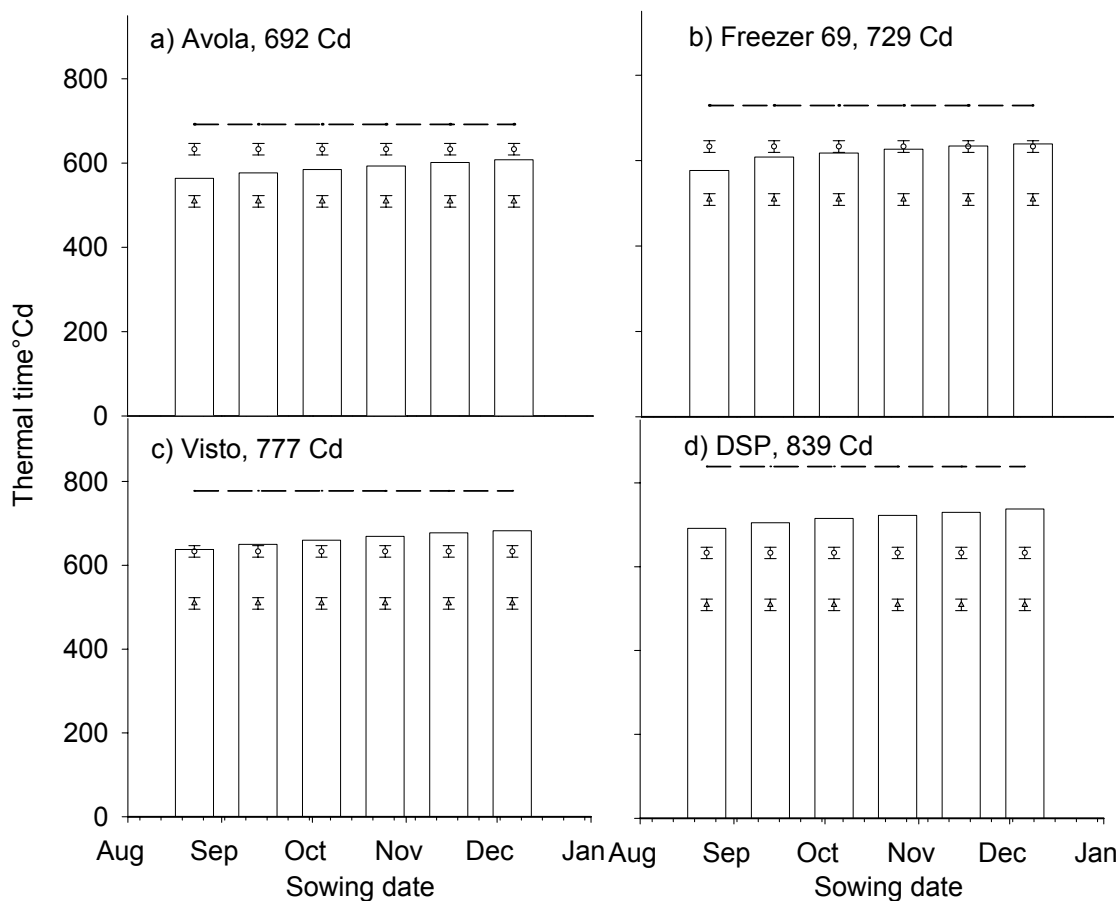


Figure 6-2. Plot of six sowing dates of four pea cultivars a) Avola, b) Freezer 69, c) Visto, d) DSP against thermal time. Dashed line (— — —) indicates the thermal time of pea maturity (T_b of 4.5 °C) from sowing, the bars indicate the accumulation of thermal time with a T_b of 6.0 °C at pea maturity for each sowing. The error bars (Tukey HSD 27.3 °Cd) indicate the predicted flowering date of *Solanum nigrum* (○) (633 °Cd) and *S. physalifolium* (Δ) (509 °Cd) from emergence at the day of pea sowing. Error bars below the 6.0 °C T_b thermal time accumulation bar, indicate that predicted nightshade flowering occurs prior to pea maturity.

Table 6-10. a) Predicted thermal time ($^{\circ}\text{Cd}$, T_b 6.0 $^{\circ}\text{C}$) weed free periods to prevent flowering of *S. nigrum* (SN) and *S. physalifolium* (SP) plants from the day of pea sowing, for four pea cultivars ($^{\circ}\text{Cd}$ maturity values in brackets, T_b of 4.5 $^{\circ}\text{C}$): Avola (692 $^{\circ}\text{Cd}$), Freezer 69 (Fr. 69) (729 $^{\circ}\text{Cd}$), Visto (777 $^{\circ}\text{Cd}$) and DSP (839 $^{\circ}\text{Cd}$). b) Predicted duration of weed free period in days from sowing, values in brackets are the Tukey HSD value (27.3 $^{\circ}\text{Cd}$) in days.

a)		SN				SP			
Pea cultivar	Avola	Fr. 69	Visto	DSP	Avola	Fr. 69	Visto	DSP	
Sowing date									
23 Aug.	-	-	18	72	68	102	142	196	
13 Sept.	-	-	30	86	81	113	154	210	
4 Oct.	-	-	40	96	90	123	164	220	
25 Oct.	-	7	50	103	98	131	173	227	
15 Nov.	-	15	58	110	106	139	182	234	
6 Dec.	-	20	63	118	113	144	187	242	

b)		SN				SP			
Pea cultivar	Avola	Fr. 69	Visto	DSP	Avola	Fr. 69	Visto	DSP	
Sowing date									
23 Aug.	-	-	7 (10)	23 (8)	20 (8)	28 (6)	38 (6)	52 (7)	
13 Sept.	-	-	6 (6)	19 (8)	19 (8)	27 (6)	36 (3)	43 (5)	
4 Oct.	-	-	11 (7)	19 (4)	18 (4)	22 (4)	30 (5)	37 (4)	
25 Oct.	-	1 (5)	7 (4)	16 (3)	15 (3)	20 (4)	29 (4)	34 (3)	
15 Nov.	-	4 (7)	11 (3)	16 (3)	15 (2)	18 (3)	22 (2)	27 (3)	
6 Dec.	-	1 (4)	6 (3)	13 (3)	12 (3)	15 (2)	19 (3)	24 (3)	

6.4 Discussion

6.4.1 Nightshade development

Thermal time or day counts?

The results indicate that days from 50 % emergence to BA was the phenological interval associated with flowering most affected by sowing date (Table 6-2), but the number of days from BA to flowering did not differ with sowing date in either species (Table 6-3). However, for the interval of BA to flowering thermal time values significantly increased in both species (Table 6-4).

The variation in thermal time values appear to have been caused by the duration of this interval in days not changing for any of the five sowings, while mean temperatures increased with later sowings (Figure 5-1 and Figure 6-1). This made thermal time estimates a less useful predictor of flowering from BA compared with day counts because of the significant monthly variation. For the interval from sowing to

flowering and emergence to flowering, thermal time was a useful predictor, as values for this interval were not affected by sowing date.

For fruit growth, day counts and thermal time values both indicated significant declines from early to late sowings (Table 6-6 and Table 6-8). If fruit growth was not positively related to temperature then this may be a partial explanation for the decline in growth rates based on thermal time, and the increase in the thermal time requirement to 95 % of the final maximum fruit diameter (Table 6-6 and Table 6-8), but not for the decline in growth rate in terms of days (Table 6-6). This indicates that other and/or additional factors may have been responsible for the declining growth rates. For example, it is reported that, independent of water availability, fruit growth rates for *Lycopersicon esculentum* increase with temperatures from 15 to 20 °C, but do not increase from 26 to 31 °C (Thompson *et al.*, 1999).

The effect of nightshade species on bud appearance, flowering, and fruit growth

There were significant differences between the two nightshade species. *Solanum physalifolium* plants produced reproductive buds and flowered significantly earlier than *S. nigrum* plants (Table 6-2 and Table 6-3). There were differences of ~ 120 °Cd between the species in time to 50 % flowering (Table 6-3). The earlier reproductive phenology of *S. physalifolium* may explain why a significantly greater proportion of *S. physalifolium* plants than *S. nigrum* plants bore fruit in the trial with peas (Table 4-10), and why greater numbers of *S. physalifolium* than *S. nigrum* plants from a commercial pea crop on the day of pea harvest had fruit (Table 4-11).

Differences in the thermal time requirement for the flowering of related *Carduus* species have been used to assist with the identification of morphologically similar species (McCarty, 1985). That differences in flowering requirements between the two nightshade species were observed was not unexpected, as differences in thermal time requirements for BA and flowering were reported for *S. nigrum* and *S. sarrachoides* (Hinckley, 1981) and for *S. sarrachoides* and *S. ptycanthum* (Heider, 1996). Values of 640 and 655 °Cd were reported for BA of *S. sarrachoides* and *S. ptycanthum*, and 777 and 862 °Cd for flowering of *S. sarrachoides* and *S. ptycanthum* by Heider (1996). A ~ 600 °Cd flowering value was previously reported for both *S. ptycanthum* and *S. nigrum* by McGiffen and Masiunas (1992). The figure of ~ 600 °Cd cited by McGiffen and Masiunas (1992) is substantially lower than that cited by Heider (1996) for *S. ptycanthum*, but the figure cited by McGiffen and Masiunas (1992) for *S. nigrum* agrees closely to that observed for the flowering of *S. nigrum* (633 °Cd) from emergence in this work (Table 6-4).

Differences in thermal time values between studies may be caused by different methods being used to calculate thermal time, for the study of Heider (1996), a T_m of 40 °C and no T_{opt} was used. However, it is important to note in this present work that the maximum hourly temperature was never > 34 °C (Figure 5-1). This indicates the inclusion of a T_m function with a value of 40 °C would not have altered the reported values. One cause of differences in the values between trials may be the lack of a T_{opt} function, where thermal time accumulation may overestimate actual growth or development when the temperature exceed the T_{opt} . Hinckley (1981) estimated T_{opt} values of 24 and 28 °C for *S. nigrum* and *S. sarrachoides* growth, respectively, from a T_b of 10 °C and a T_m of 40 °C. Interestingly, these T_{opt} values are lower than the T_{opt} of 28.2 °C reported for process peas (Olivier and Annadale, 1998). The T_{opt} values cited by Hinckley (1981) do not agree with reports of the growth of another nightshade species in peas, where *S. ptycanthum* growth was observed to be more competitive with peas during periods of high mean temperatures (23.9 °C) (Croster and Masiunas, 1998). This report and anecdotal accounts indicate that nightshade species may have a higher T_{opt} value than peas. More work is required in this area to improve the methods used to calculate thermal time of nightshade species.

Other factors may also have affected the thermal time estimates. Studies of other weed species report differences in flowering requirements for different populations of the same species (Andersen *et al.*, 1985; Warwick *et al.*, 1987). This indicates that there may be a genetic basis to differences in the flowering requirements among some weed populations.

Although the time of flowering differed between *S. nigrum* and *S. physalifolium* comparison of mean fruit growth rates indicated that *S. physalifolium* fruit grew slower than *S. nigrum* fruit (Table 6-5 and Table 6-6). This indicates that for *S. physalifolium* and *S. nigrum* plants, which flower at the same time, *S. nigrum* fruit will reach a greater diameter more quickly. There is a nil tolerance for the presence of any nightshade fruit in process peas. However, there is a minimum size nightshade fruit that can contaminate peas. This minimum size is determined by the pea viner used to harvest the peas, which causes some selection of pea and nightshade fruit sizes. A small survey of pea sizes from the weighbridge at a processing factory (Heinz Wattie's Ltd, Hornby), indicated that the minimum diameter of nightshade fruit capable of causing contamination was about 3 mm. *Solanum physalifolium* fruit will reach a diameter of 3 mm, at about 140 °Cd and for *S. nigrum* about 75 °Cd after flowering (Table 6-9). However, from the mean thermal time flowering values for the two species *S. physalifolium* could produce fruit of 3 mm diameter at ~ 60 °Cd prior to *S. nigrum* plants. Thus, despite the slower fruit growth rates of *S. physalifolium* this species has

the potential to contaminate process peas after less thermal time than for *S. nigrum*. This is due to the earlier flowering of *S. physalifolium*.

Base temperatures, evaluation and implications

A T_b of 6 °C was used for the thermal time analysis, this value was calculated in a growth cabinet trial as the T_b for mainstem leaf production of *S. ptycanthum* (Alm *et al.*, 1988). This value has been used in other studies of nightshade phenology and growth (McGiffen and Masiunas, 1992; Heider, 1996). The range of planting dates used in this present work provided the ability to evaluate T_b values for flowering in both species. The evaluation of a T_b for flowering of *S. nigrum* does not appear to have been reported in the literature, and the only previous report of a T_b for *S. physalifolium*, based on germination studies, gave a value of 21.0 °C (del Monte and Tarquis, 1997). This value was acknowledged by the workers to have been affected by the presence of primary dormancy. Work with other species indicates that different T_b 's can occur for different phenological stages. For example, *Triticum aestivum* L. is reported to have a T_b of 3.3 °C for the interval from emergence to floral initiation, but the interval from anthesis to maturity had a significantly different T_b , of 8.9 °C (Angus *et al.*, 1981). However, this present work did not provide evidence that the T_b of either species differed greatly from that based on the T_b for the leaf expansion rate of *S. ptycanthum* (Alm *et al.*, 1988). Although the degree of variability of this T_b , for either species, was not investigated in further detail, the results indicate that using a T_b of 6 °C gives an accurate calculation of thermal time. These results indicate that the earlier flowering of *S. physalifolium* compared to *S. nigrum* probably occurs because of a lower total thermal requirement and a slightly lower T_b .

6.4.2 Pea sowing date and cultivar effects on the potential for nightshade contamination

The comparisons of nightshade flowering and pea maturity adopted the approach that the flowering of the nightshade plants would not be slowed by growth in a crop. As a number of studies had indicated this to be the case (Hinckley, 1981; Heider, 1996). However, one study indicated that flowering could be delayed in a crop (Quakenbush and Andersen, 1984). Basing comparisons on the assumption that nightshade development would not be slowed in pea crops, provided a risk averse approach to the development of nightshades in pea crops.

The mean flowering values for *S. nigrum* and *S. physalifolium* (Table 6-2) were compared to their predicted thermal time accumulation for a T_b of 6 °C, and to that of the predicted maturity date, using a T_b of 4.5 °C, for four pea cultivars sown on six

dates (Figure 6-2). This indicated that *S. physalifolium* could flower prior to or at pea maturity for all pea cultivars regardless of their sowing date. For *S. nigrum* flowering prior to pea maturity occurred in three pea cultivars (Freezer 69, Visto and DSP). These comparisons indicate that *S. physalifolium* has a greater potential to contaminate process peas than *S. nigrum*. This is because this species flowers earlier than *S. nigrum* and earlier relative to pea crop maturity. This implies that for fields where *S. physalifolium* is present, additional attention to weed management may be required to grow uncontaminated process peas.

It has been reported, for a North American study, for April and May sowings of eight weed species and the process pea cv. Perfection Freezer, that the growth rates of most of the weeds relative to the peas was greater in the May sowing (Nelson and Nylund, 1962). Most of the weed species would be more serious competitors for late sown rather than early sown peas. These results indicate that sowing date can affect competitive interactions between weeds and peas, and this was probably due to differences in the optimum temperature requirements between the weed species and peas. Similarly, it was reported, in Canterbury, that *Cirsium arvense* another contaminant species of process peas had a faster growth rate in late sown (9 November) peas versus peas sown on October 6 (McGill, 1999). Comparisons of the thermal time flowering requirements of *S. nigrum* and *S. physalifolium* indicated that different sowing dates can cause differential nightshade development relative to pea maturity (Figure 6-2), due to the faster development of the nightshades at the later sowing dates.

For August sown cv. Freezer 69 predicted crop maturity preceded the flowering of *S. nigrum* (Figure 6-2), but for later sowings (November and December) of the same cultivar the predicted flowering time of *S. nigrum* shifted to precede pea maturity. Thus, the risk of contamination due to nightshade flowering preceding that of pea maturity increased with the later sowing of some cultivars. These seasonal effects have implications for the predicted duration of the weed free period required to prevent flowering of nightshades prior to pea crop maturity.

The thermal time flowering values of *S. nigrum* and *S. physalifolium* give the ability to predict the thermal time weed free period required to prevent flowering of these nightshade species in peas (Table 6-10). Studies of the duration of the weed free period necessary to prevent weed seed production in *Zea mays*, *Glycine max* and *Brassica oleracea* var. *capitata* L. crops have been made (Miller and Hopen, 1991; Swanton *et al.*, 1999; Swanton *et al.*, 2001). The prevention of weed seed production is, in methodology, comparable to the goal of preventing nightshade fruit production in process peas. While the effect of different sowing dates of crop weed competitive

interactions is reported in the literature (Oliver, 1979; Khan *et al.*, 1996; Spandl *et al.*, 1998; Anderson, 2000), the effect of crop sowing date on weed reproductive phenology appears to have been largely overlooked. Heider (1996) reported that for weekly transplantings of *Solanum sarrachoides* and *S. ptycanthum* into trials of two process pea cultivars, seedlings of neither species transplanted 21 d or later after pea sowing produced fruit by pea maturity. This indicates that a weed free period of approximately three weeks could prevent *S. sarrachoides* and *S. ptycanthum* fruit contaminating pea crops for the single sowing date used by Heider (1996).

However, analysis of the effects of sowing date on the predicted weed free period of cv. Freezer 69 (729 °Cd) that had a comparable maturity value to one to the cultivars (738 °Cd) used by Heider (1996) (Table 6-10) indicated that the thermal time weed free period required to prevent, for example, *S. physalifolium* from flowering from the date of pea sowing changed with sowing date (Table 6-10). Calculation of the length of the required weed free period for different sowing dates of this cultivar, indicated that the number of days required for the weed free period changed from 28 ± 3 d for an August sowing to 15 ± 1 d for a December sowing (Table 6-10). Thus, day value weed free period prescriptions, such as that proposed by Heider (1996), based on trials with a single sowing date may need to be viewed with caution. Such prescriptions could be either too short for early pea sowings, or too long for late sowings. This is particularly true if the pea seedlings are past the 2.5 leaf stage (A. White, Heinz Wattie's Ltd, pers. comm.) as mechanical weeding of peas when carried out at inappropriate pea growth stages causes yield reductions (Rasmussen, 1993).

Comparisons of the thermal time requirements for flowering and fruit growth of *S. nigrum* and *S. physalifolium* for hypothetical sowings of pea cultivars indicated that weed free periods based on these thermal time requirements may offer a useful route to assist with the management of nightshade contamination, including limiting nightshade weed seed inputs into the seed bank. However, field trials will be required to validate this approach. This method appears especially relevant to crops such as process peas, as the short duration of process pea crops mean that the success of weeding operations made over a relatively short period following crop emergence should be effective through to crop harvest. In crops with longer durations to harvest, such as *Zea mays*, the success of weeding during early crop growth does not necessarily prevent fruit production by weeds such as *S. nigrum*. *Solanum nigrum* seedlings established 38 days after crop emergence can still produce a substantial number of fruit prior to *Zea mays* harvest for ensilage (Kremer and Kropff, 1998c). Thus, this approach appears limited to use for short duration crops, or crops that can be weeded at a later crop growth stage.

6.5 Conclusions

1. Plants of *S. physalifolium* initiate reproductive buds and flower before *S. nigrum*. The fruit growth rate of *S. nigrum* is greater than in *S. physalifolium*. However, *S. physalifolium* can produce fruit of 3 mm diameter before *S. nigrum*. Therefore, *S. physalifolium* has a greater potential to contaminate process peas than *S. nigrum*.
2. Late pea sowings had a greater risk of contamination by both *S. nigrum* and *S. physalifolium*. This risk of contamination was also greater for late sowings of mid range to late maturity pea cultivars. Cultivars with short maturity values could be used for late sowings to reduce the risk of nightshade contamination.
3. The current cultivars could continue to be used and the risk of nightshade contamination managed by basing the duration of weed free periods on the thermal time requirements for flowering of the nightshade species in relation to pea crop cultivar maturity and sowing date.

Chapter 7 General discussion and recommendations for nightshade management in process pea crops

7.1 Introduction

Aspects of the biology of *Solanum nigrum* and *S. physalifolium* were examined to identify factors relevant to the management of these two weed species in pea crops. A knowledge of the biology of problem weeds is required for the development of management strategies (Mortensen *et al.*, 2000). Both nightshade species had different seed dormancy characteristics. The germination of *S. nigrum* seed under non-optimal conditions (5/20 °C) exhibited cyclical shifts between non-dormancy and conditional dormancy throughout the pea sowing season. The germination of *S. physalifolium* seed under these non-optimal conditions was only restricted early in the pea sowing season (July to August). These results indicate that the field germination of *S. nigrum* will be affected by its dormancy status throughout the pea sowing season. For *S. physalifolium*, dormancy will not affect germination from October to early December as the seed is fully non-dormant (Figure 4-7). In the absence of peas the DM production of *S. physalifolium* was superior to that of *S. nigrum*. When grown with peas the DM production of both nightshade species was reduced to a low level. However, relative reductions in the DM production of *S. physalifolium* were greater than for *S. nigrum*, indicating that a competitive growth environment may limit the growth of *S. physalifolium* more than *S. nigrum*.

The flowering phenology of the two species differed. *Solanum nigrum* flowered later than *S. physalifolium* (Table 6-3), but the fruit growth rate of *S. nigrum* was greater than that of *S. physalifolium* (Table 6-5). Comparisons indicated that because of the earlier flowering of *S. physalifolium* that this species could produce fruit of a diameter capable of contaminating process peas approximately 60 °Cd prior to *S. nigrum*. These differences have implications for the prevention of nightshade contamination in process pea crops. To prevent *S. physalifolium* flowering in pea crops required longer weed free periods than for *S. nigrum* (Table 6-10). *Solanum physalifolium* was capable of flowering prior to pea crop maturity in short maturity pea cultivars in which *S. nigrum* would not have flowered.

7.2 Possible nightshade control techniques

7.2.1 Cultivation

It has been reported that germination of *S. nigrum* seed is stimulated by cultivation (Roberts and Lockett, 1978; Roberts and Boddrell, 1983; Ogg and Dawson, 1984; Hartley, 1991b; Popay *et al.*, 1995). It is this stimulatory effect that dark cultivation practices attempt to inhibit. However, in this work dark cultivation did not restrict the germination of *S. nigrum* (Table 4-7 and Table 4-16), even though laboratory tests had demonstrated a positive light requirement for germination. In contrast, there was no positive light requirement for the germination of *S. physalifolium* in laboratory tests and, as with *S. nigrum*, dark cultivation had no effect on the field germination of this species. Therefore, the use of dark cultivation to control these two nightshade species in pea crops is not recommended. In *S. nigrum* laboratory germination studies indicated that light requirements varied with the test date, test temperature and seed lot (Appendix 7). Thus, the possible usefulness of dark cultivation is compromised by the lack of a consistent light requirement for *S. nigrum* germination. In addition, there was some evidence that factors in the soil environment, such as nitrate, can supplant the light requirement for germination. Knowledge about methods to ensure *S. nigrum* has a light requirement for germination is required for the success of dark cultivation for the control of *S. nigrum* seed germination.

Comparisons between the two species showed a much higher proportion of the seed bank for *S. physalifolium* (13 %) could be stimulated to emerge than for *S. nigrum* (2 %) in response to cultivation, in November 2002. The differences in proportional emergence appear to be related to the difference in dormancy types between *S. nigrum* and *S. physalifolium*, with *S. physalifolium* seed having periods of non-dormancy. This period of non-dormancy occurred from October to early December (Figure 4-7), indicating that a germination maximisation strategy for seed of *S. physalifolium* during these months, by the use of stale or false seed beds (Mohler, 2001b), may provide a way to deplete the seed bank of this species prior to pea sowing.

Stale seed beds utilise the stimulation of weed germination and emergence caused by cultivation. The emerged seedlings are then destroyed with methods intended to stimulate minimal additional weed germination (Mohler, 2001b). Herbicides are applied prior to or at the time of crop sowing in conventional systems (Oliver *et al.*, 1993; Johnson and Mullinix, 1995). In organic systems an equivalent practice would be flame weeding prior to sowing (Balsari *et al.*, 1994). The false seed bed system

uses additional shallow cultivations following the initial seed bed preparation to destroy seedlings and to further stimulate weed emergence (Mohler, 2001b). The success of these seed bed methods can be improved by rolling and by irrigation to improve weed seed germination (Roberts and Hewson, 1971; Bond and Baker, 1990; Hutcheon *et al.*, 1998).

7.2.2 Site selection

Comparisons of the thermal time requirement for flowering of *S. nigrum* and *S. physalifolium* with the maturity time of a number of process pea cultivars indicated that *S. physalifolium* poses a greater threat to the contamination of process peas than *S. nigrum* (Figure 6-2). It is, therefore, recommended that fields of process peas with *S. physalifolium* require additional attention to prevent contamination. *Solanum physalifolium* is a relatively new weed to New Zealand (Webb *et al.*, 1988). Interviews with farmers growing process peas indicated that *S. physalifolium* was not recognised by most farmers (data not presented). Therefore, it is recommended that extension services for farmer identification of this weed species are required. Inspections of spring or summer crops in fields to be used for process peas may be useful to establish the historical presence or absence of *S. physalifolium*. Where it is not known if *S. physalifolium* is present in a process pea crop or not, it would appear appropriate to adopt a risk averse based method of weed management. This would be for weed management practices to eliminate the risk of nightshade fruit contamination being based on assumption that *S. physalifolium* is present, although this could increase the costs of weed management.

7.2.3 Sowing date

Analysis of processing factory nightshade contamination records indicated that nightshade contamination was more common in mid and late season sown peas. For example, mean contamination was 4.8 % and 5.6 % for crops sown in October and November, while the figures for August and September were 0 % and 0.6 % (Table 3-1). Comparison of the thermal time values for flowering of the two nightshade species with crop maturity values for a number of pea cultivars, indicated that flowering of nightshades could occur earlier relative to crop maturity for late sowings, due to increases in the mean temperature during the pea sowing season (Figure 6-2). Manipulation of sowing date is identified as a useful means to assist with the management of crop weed interactions (Khan *et al.*, 1996; Anderson, 2000). However, currently, altering the sowing dates of process peas does not appear to be a viable

option for the management of nightshade contamination, as approximately 65 % of the total number of pea crops are sown in October and November each year (Table 3-1). Avoiding of mid and late sowings would lead to large scale production losses in order to avoid what can be a costly problem, but which is usually limited to a small number of crops in each season (Table 3-1). Other factors also limit the manipulation of the sowing date of process peas as a strategy to reduce nightshade contamination, as sowing dates, within a region, are also related to factors such as soil type (Ottoson, 1973). Farms in areas with light soils are usually sown prior to farms in areas with heavy soils (A. White, Heinz Wattie's Ltd, pers. comm.).

7.2.4 Weed free periods to prevent nightshade flowering

Processing factory staff use predicted thermal time maturity values for peas to schedule pea planting and factory processing (A. White, Heinz Wattie's Ltd, pers. comm.). The crop yield reduction effect of weeds that emerge prior to the emergence of process peas is reported to be greater than that of weeds emerging after crop emergence (Nelson and Nylund, 1962). Comparison of predicted pea maturity thermal time values from the date of sowing and date of pea emergence, to the thermal time flowering values for *S. nigrum* and *S. physalifolium*, indicated that nightshades emerging prior to crop emergence have a greater potential to flower prior to crop maturity (data not presented). Therefore, for these reasons the calculation of weed free periods should be made from the crop sowing date.

Comparison of the thermal time requirement for the two nightshade species with that of a number of process pea cultivars indicated that the thermal time between nightshade flowering and pea maturity would be the weed free period necessary to prevent flowering of the nightshade species, and so potential crop contamination. However, the comparisons indicated that the necessary duration of the weed free period differed with the thermal time maturity value of the pea cultivar, and also differs with different pea sowing dates for the same pea cultivar (Table 6-10). This implies that specific weed free periods need to be calculated for each different maturity value of a pea cultivar, for different sowing dates. For example, the predicted weed free period, in days, to prevent *S. physalifolium* flowering for the cv. Freezer 69 was 28 d for a 23 August sowing and 15 d for a 6 December sowing, while for the cv. Visto the respective values for the same two sowing dates were 38 and 19 d (Table 6-10).

It is not recommended that farmers calculate the necessary thermal time weed free period. Rather, that the necessary weed free period be calculated in day values for cultivars and sowing dates by the use of mean seasonal temperature records, as are currently used by the factory staff to schedule pea sowing and maturity dates.

Field officers from the processing factory could then advise farmers of the necessary weed free period in days for the cultivar and sowing date they are using. Farmers have little choice over the cultivar and sowing date of peas grown under contract for processing. Therefore, it is necessary that processing factory field officers provide farmers with information on the implications for nightshade management for the pea cultivars they are sowing.

7.2.5 Pea cultivar effects

The planting of cultivars with lower thermal time requirements early in the season is used as a method to extend the length of the processing season by providing early season harvests (Cawood, 1987). Factory contamination records show that the pea cultivars, which are contaminated with nightshade, are of long maturity types. On average, these cultivars have a ~ 100 °Cd ($T_b = 5$ °Cd) greater thermal time requirement than pea cultivars sown prior to contamination occurring (see Section 3.3.3). Thus, pea cultivars with a greater thermal time maturity value are associated with nightshade contamination.

Using long duration pea cultivars increases the ability of nightshades to flower before the peas are mature. Therefore, for farms or fields identified as having a high risk of nightshade contamination, it is recommended that early maturing pea cultivars should be sown to reduce the contamination risk. However, the effect of using such cultivars with lower thermal time values on crop yield will need to be assessed. This is because the earlier flowering of early pea cultivars is associated with a reduced LA (Aitken, 1978) and, thus reduced radiation interception capabilities, with reduced photosynthate which is also associated with reduced pea yields (Meadley and Milbourn, 1970; Munier-Jolain *et al.*, 1998). Sowing date also affects the yield of process peas, for example, the yield of the long maturity cv. DSP is reported to be reduced at late sowings (Milbourn and Hardwick, 1968; Hardwick *et al.*, 1979). This effect could be more pronounced in short maturity pea cultivars if sown late in the season when there are higher mean temperatures.

7.3 Recommendations for further work

1. The concept of weed free periods to prevent nightshade flowering requires validation by field trials. In addition, this method of using the thermal time for development of other contaminants of peas, such as *Cirsium arvense* buds, may be a useful tool for the management of these species.

2. During the period of low seed dormancy in *Solanum physalifolium*, the use of germination maximisation cultivation strategies to deplete seeds in the soil seed bank may be a useful way to reduce the density of this weed. It is recommended that the use of stale and false seed beds be investigated as management practices suitable for seed bank depletion of these weeds.
3. The literature for some species report that germination can be controlled following SDLE if the seed is buried (Benvenuti and Macchia, 1998; Benvenuti *et al.*, 2001). Investigations into the usefulness of dark cultivation should investigate this as a potential factor contributing to the reported variable success of dark cultivation.
4. The presence or absence of VLFR responses in *S. nigrum* requires further investigation.
5. Work is required to investigate what the response of *S. physalifolium* is to shade, and if factors such as crop density or pea leaf type can be used to assist with the management of this nightshade species.
6. Improvements in the use of thermal time to predict *S. nigrum* and *S. physalifolium* flowering could be made by the determination of T_{opt} and T_m values for the period from emergence to flowering for both of these species.
7. Evaluation of the requirements of *S. nigrum* and *S. physalifolium* for bud appearance and flowering when grown with peas is required.
8. Evaluation of seed production of *S. nigrum* and *S. physalifolium* plants growing in a range of crops that typically form part of a rotation with process peas, would provide information relevant to the crop selection compatible with process peas in a rotation.
9. Reports from New Zealand indicate that weed seed losses are higher after a period of nil cultivation following a crop, than for cultivated ground. (Hartley, 1991b; Rahman *et al.*, 2001). A comparison of the effect of direct drilling with conventional cultivation following seed production in crops infested by *S. nigrum* and *S. physalifolium*, on the weed seed bank of populations and subsequent densities in pea crops of these species, is required.

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Appendices

Appendix 1

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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 (Mortenson 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 were 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% KNO₃), gibberellic acid (0.05% GA₃) 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: Mean¹ germination (%) of three black nightshade seed collections at constant temperatures after 14 days in experiment 1, and at 7 days after transfer to 20/30°C.

	Temperature	Lincoln		Gisborne
		Green fruit	Black fruit	Black fruit
Days prior to transfer to 20/30°C				
14	20°C	0	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

¹Mean of 3 replicates.

²Means 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 14d 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.

TABLE 2: Mean¹ 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 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	8.0c	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 $P < 0.01$

¹Mean of 3 replicates.

²Means followed by the same letter within columns are not significantly ($P < 0.05$) different using Fischer's Least Significant Difference test.

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 GA₃ 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 KNO₃ increased ($P < 0.05$) germination compared to the 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.

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.

TABLE 3: Mean¹ 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	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			

¹Mean of two experiments

²Means followed by the same letter within columns are not significantly ($P < 0.05$) different using Fischer's Least Significant Difference test.

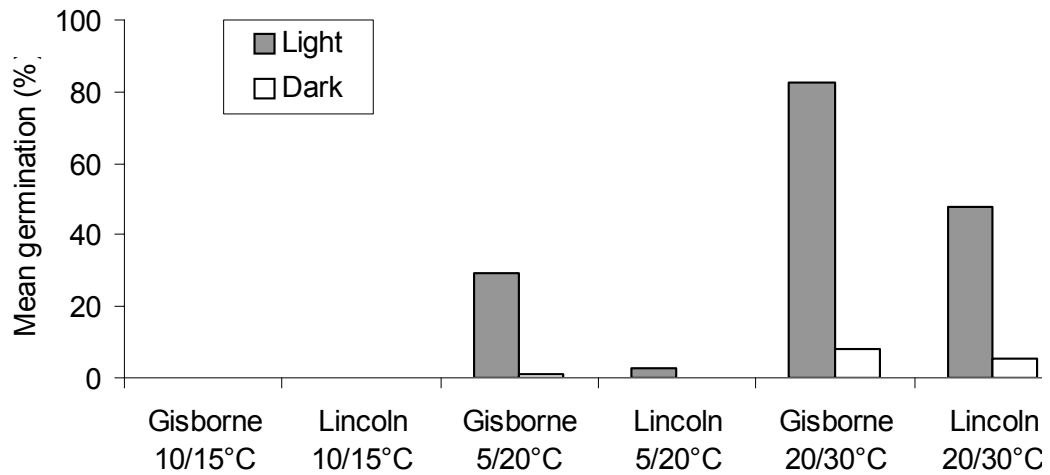


FIGURE 1: Mean germination after 14 days for black nightshade from two seed sources, green Lincoln fruit and black Gisborne fruit at 10/15, 5/20 and 20/30°C under 24 h light or dark conditions in Experiment 4.

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 and 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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Appendix 2

Table A2-1. Summary of factory nightshade contamination records, number (No.) of crops sown, No. and % of crops contaminated for the month the crop was sown for the 2000/01, 2001/02 and 2002/03 seasons. Data from Heinz Wattie's Ltd, Hornby factory.

	August	September	October	November	December
2000/01					
No. sown	26	101	158	186	4
No. contaminated	0	0	12*	2	0
% contaminated	0	0	7.6	1.1	0
2001/02					
No. sown	79	106	179	120	-
No. contaminated	0	2	7	17*	-
% contaminated	0	1.9	3.9	14.2	-
2002/03					
No. sown	100	70	181	129	28
No. contaminated	0	0	5	2	2
% contaminated	0	0	2.8	1.6	7.1

*indicates an organic crop included in total. - = no crops sown

Appendix 3

Table A3-1. Seed lot code, collection date, region, site, and number (No.) of *Solanum nigrum* (SN) and *S. physalifolium* (SP) plants seed was collected from. For each species collection codes with the same numeral e.g. 6A and 6B are separate seed lots from the same collection.

Seed lot code	Collection date	Region	Collection site	SN fruit colour	No. plants per collection
SN 2	4/2/01	Lincoln	Kowhai A3	green & black	15
SP 1	4/2/01	Lincoln	Kowhai A3		15
SP 2	24/2/01	Lincoln	Kowhai A3		>100
SP 3	12/3/01	Lincoln	FSC		53
SN 3	10/3/01	Lincoln	Kowhai A4	green	81
SN 6A	30/3/01	Gisborne	Laudon	black	39
SN 6B	30/3/01	Gisborne	Laudon	green	
SN 7A	30/3/01	Lincoln	Kowhai A2	green	184
SN 7C	30/3/01	Lincoln	Kowhai A2	black	
SN 8A	9/4/01	Gisborne	Holmes	black	73
SN8B	9/4/01	Gisborne	Holmes	green	
SN 9A	9/4/01	Lincoln	Kowhai A4	black	93
SN9B	9/4/01	Lincoln	Kowhai A4	green	
SP4	9/4/01	Lincoln	FSC		35
SP6	21/2/02	Lincoln	D2/FSC		63
SN10A	21/2/02	Lincoln	D2/FSC	black	53
SN10B	21/2/02	Lincoln	D2/FSC	green	
SN11A	20/3/02	Lincoln	D2/FSC	black	111
SN11B	20/3/02	Lincoln	D2/FSC	green	
SN12A	20/3/02	Gisborne	Holmes	black	151
SN12B	20/3/02	Gisborne	Holmes	green	
SN13A	20/3/02	Manawatu	Hogg	black	37
SN13B	20/3/02	Manawatu	Hogg	green	

Kowhai = The Heinz-Wattie's Organic farm, Lincoln University, FSC = Field Service Centre, Lincoln University, HWA = a Heinz Wattie's Australasia contract growers property, Laudon = G. Laudon's property, Gisborne, Holmes = A. Holme's property, Gisborne, Hogg = R. Hogg's property, Manawatu.

Appendix 4

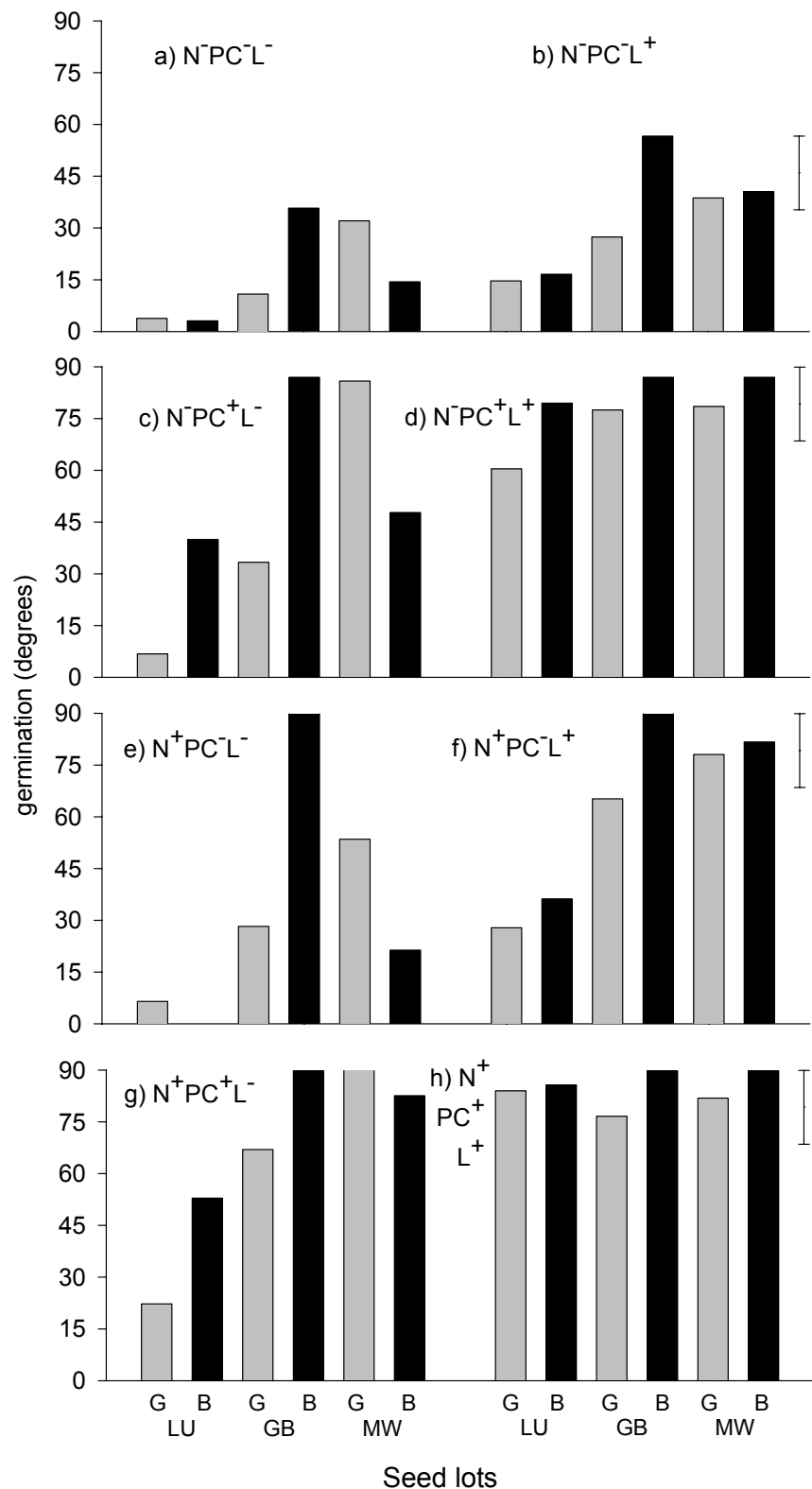


Figure A4-1. Arcsine transformed percent germination of viable green (G) and black (B) *S. nigrum* fruit collected from Lincoln (LU), Gisborne (GB) and the Manawatu (MW). Seed was germinated at 20/30 °C in the presence (+) or absence (-) of light (L), and/or prechilling (PC) and/or nitrate (N) (s.e. = 4.23, d.f. = 96). The error bar is the Tukey HSD value (21.37). (Experiment 1).

Table A4-1. Arcsine transformed percent of non viable seeds from six *S. nigrum* seed lots (A = seed of black fruit, B = seed of green fruit) of forty seeds (s.e. = 1.40, d.f = 138). Means followed by the same letter are not significantly different (Tukey HSD = 5.66) (Experiment 1).

Seed lot	No. non-viable
SN11A	3.2 a
SN11B	28.0 c
SN12A	3.7 a
SN12B	13.8 b
SN13A	6.1 a
SN13B	36.2 d

Appendix 5

Table A5-1. TTC viability and TSW values for *S. nigrum* (SN) and *S. physalifolium* (SP) seed lots, and values for seed collections for seeds/plant, TTC results, TSW and ratio of seed from green to black fruit (G:B seed) for SN collections where coloured fruit was processed separately.

Seed lot code	Values for seed lots			Values for collections		
	TTC	TSW	Seeds/plant	TTC	TSW	G:B seed
SN 2	93	0.727	322	93	0.727	
SN 3	69	0.687	556	69	0.687	
SN 6A	67	0.828	306	51	-	.89/.11
SN 6B	49	-*				
SN 7A	79	0.690	163	83	0.704	.78/.22
SN 7C	95	0.754				
SN 8A	95	0.753	323	83	0.732	.40/.60
SN8B	65	0.699				
SN 9A	100	0.875	353	66	0.782	.86/.14
SN9B	60	0.766				
SN10A	87	0.860	143	83	0.852	.28/.72
SN10B	71	0.832				
SN11A	94	0.755	1154	78	0.681	.58/.42
SN11B	66	0.628				
SN12A	91	0.761	67	78	0.696	.79/.21
SN12B	74	0.680				
SN13A	81	0.712	893	75	0.711	.46/.54
SN13B	68	0.711				
SP1	99	0.932	182			
SP2	100	0.932	-			
SP3	98	0.978	492			
SP4	-**	0.814	593			
SP6	83	0.881	101			

* SN6B was discarded due to fungal infection prior to TSW analysis, SP4 TTC is a missing value.

Appendix 6

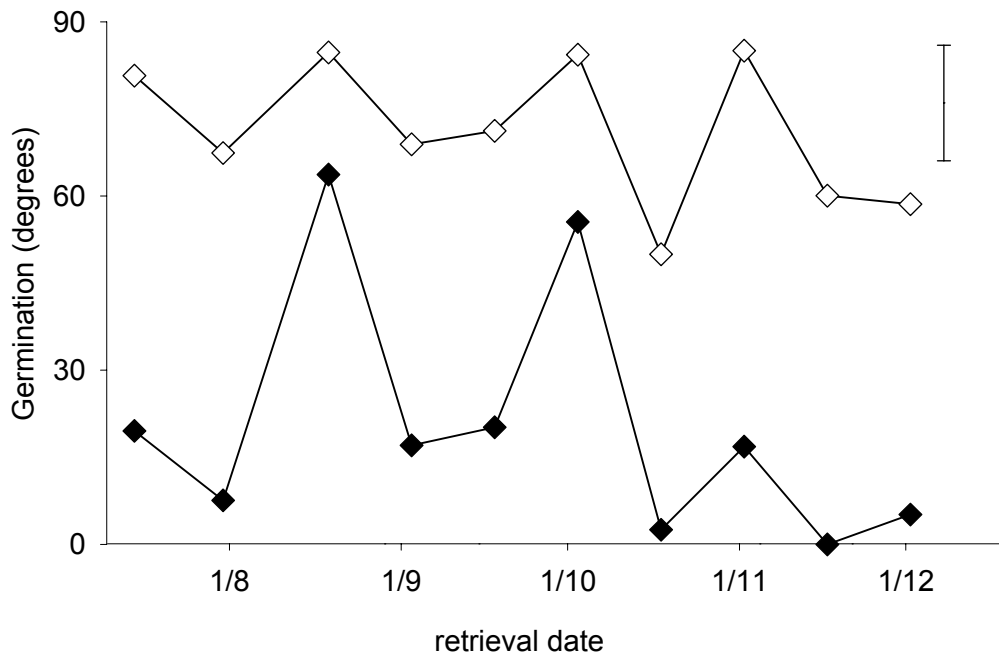


Figure A6-1. The arcsine transformed percent germination of viable seed of *S. nigrum* seed lot SN7A. Seeds were germinated in light \diamond and dark \blacklozenge for 14 d (s.e. = 3.8, d.f. = 72) at ten test dates. The error bar is the Tukey HSD value (19.8). (Experiment 2).

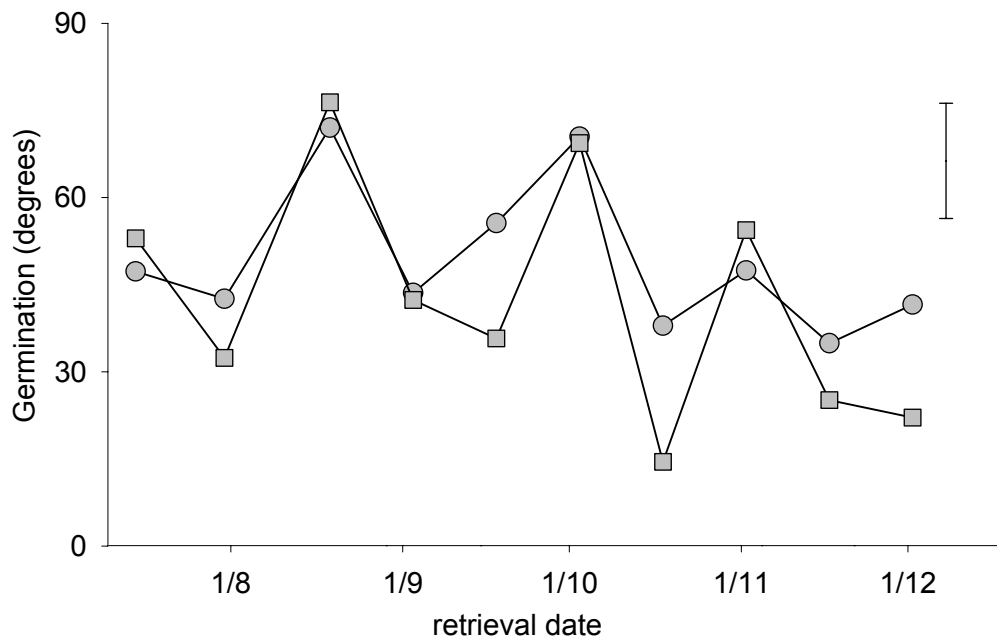


Figure A6-2. The arcsine transformed percent germination of *S. nigrum* seed lot SN7A at 20/30 °C (\circ), 5/20 °C (\square) for 14 d (s.e. = 3.8, d.f. = 80) at ten test dates. The error bar is the Tukey HSD value (19.9). (Experiment 2).

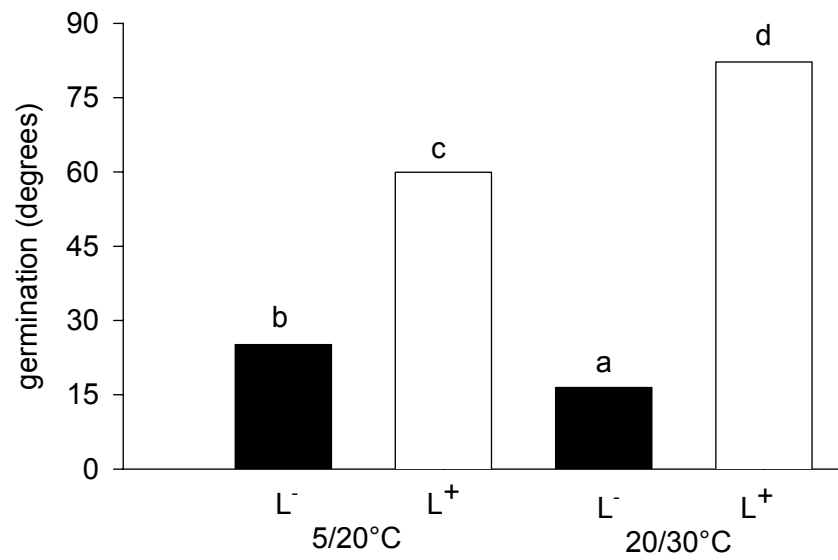


Figure A6-3. The arcsine transformed percent germination of viable seed of *S. nigrum* seed lot SN7A. Seeds were germinated at at 20/30 °C, 5/20 °C in light (L⁺) and in the dark (L⁻) for 14 d, (s.e. = 1.7, d.f. = 80). Bars with different letters are significantly different (Tukey HSD = 6.3). (Experiment 2).

Appendix 7

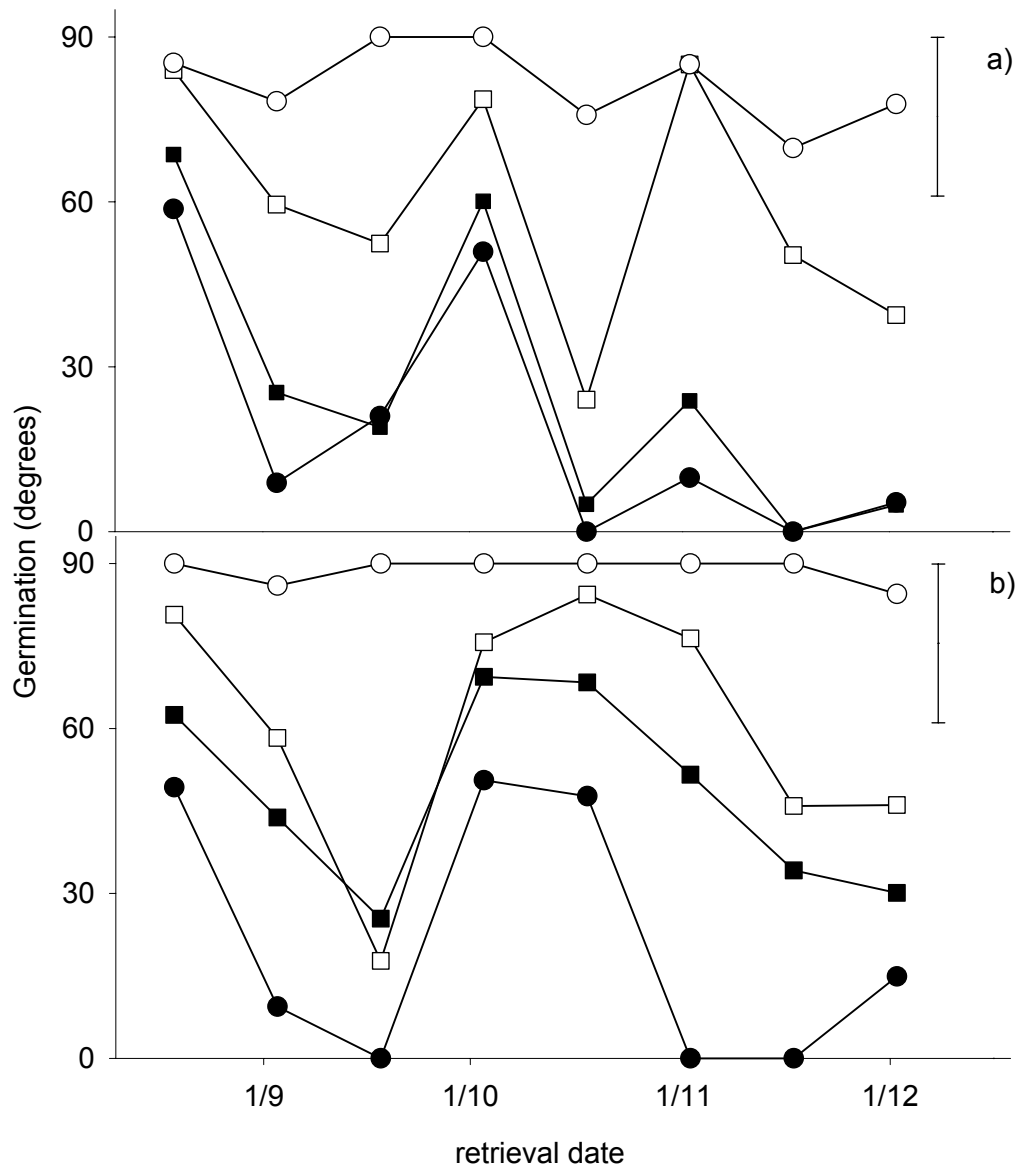


Figure A7-1. Arcsine transformed percent germination of viable seed of *Solanum nigrum* seed lots a) SN7A, seed of green fruit and b) SN7C, seed of black fruit. Seeds were germinated at 20/30 °C (in light ○ and dark ●) or 5/20 °C (in light □ and dark ■) for 14 d at eight test dates (s.e. = 4.9, d.f. = 128). The error bar is the Tukey HSD value (28.9). (Experiment 2).

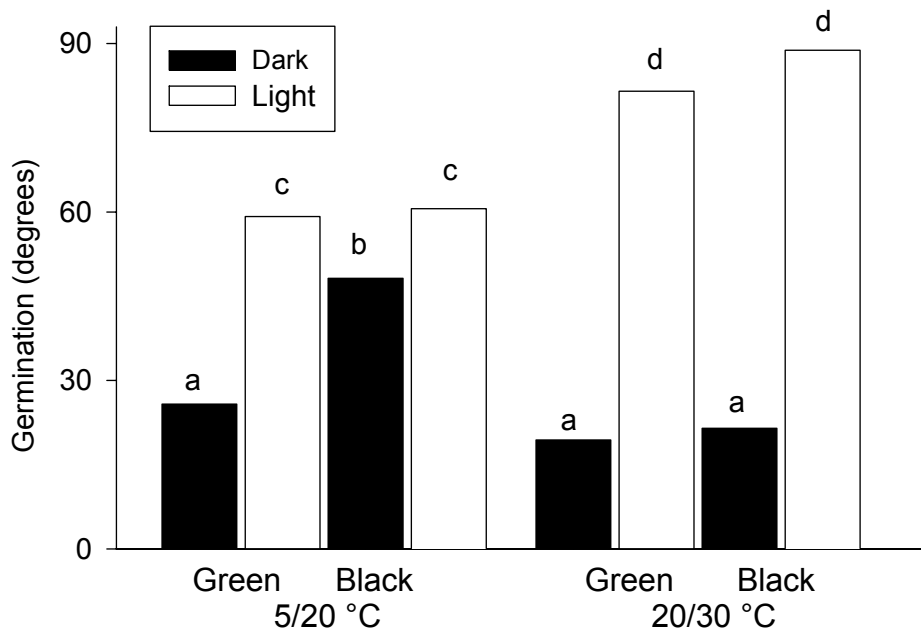


Figure A7-2. The arcsine transformed percent germination of viable seed of two *S. nigrum* seed lots from green fruit (SN7A) and black fruit (SN7C) in light (open bars) and dark (filled bars) at 5/20 °C and 20/30 °C (s.e. = 1.7, d.f. = 128). Bars with different letters are significantly different (Tukey HSD = 7.4). (Experiment 2).

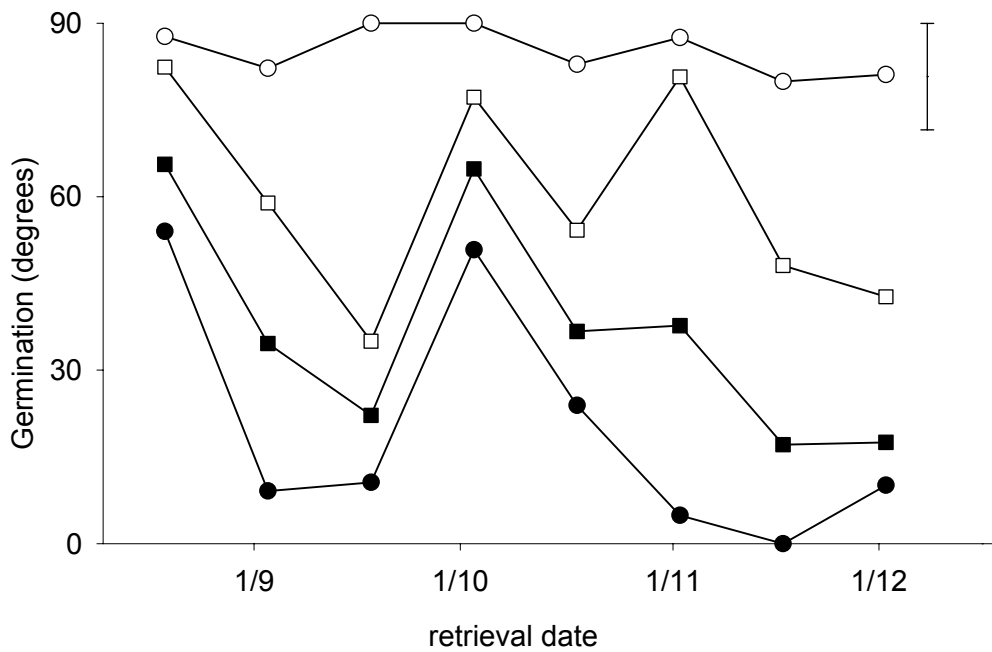


Figure A7-3. The arcsine transformed percent germination of viable seed of two *S. nigrum* seed lots in light (open symbols) and dark (filled symbols) on eight test dates. Seeds were germinated at 20/30 °C (in light ○ and dark ●), 5/20 °C (in light □ and dark ■) for 14 d (s.e. = 3.4, d.f. = 128). The error bar is the Tukey HSD value (18.4). (Experiment 2).

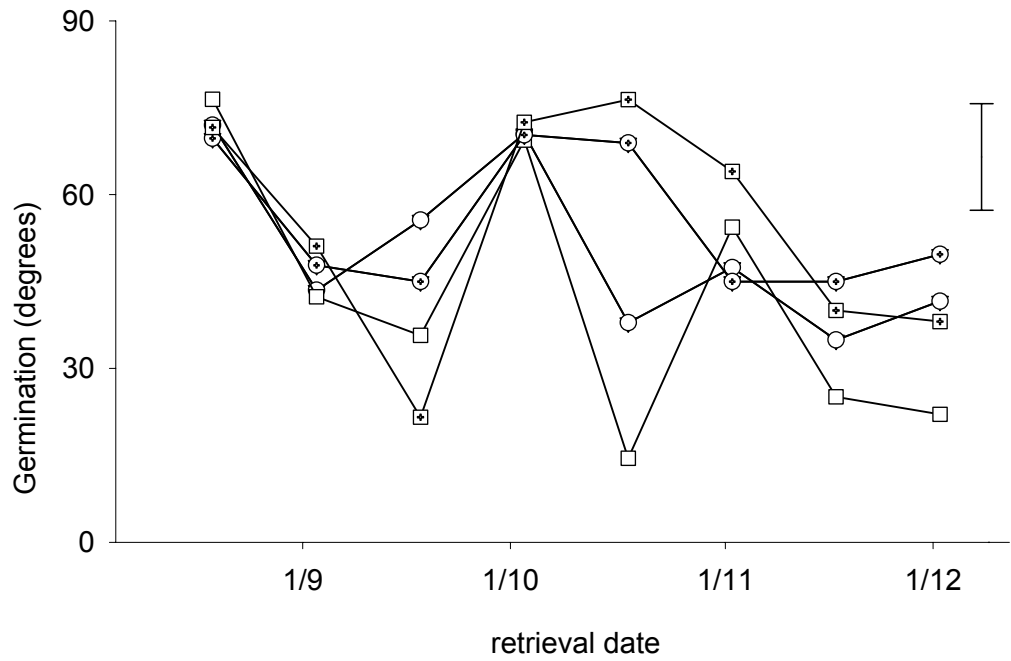


Figure A7-4. The percent germination of arcsine transformed viable seed of two *S. nigrum* seed lots from green fruit (SN7A, open symbols) and black fruit (SN7C, symbols with cross hair, +). Seeds were germinated at 20/30 °C (○), 5/20 °C (□) on eight dates for 14 d (s.e. = 3.5, d.f. = 128). The error bar is the Tukey HSD value (18.4). (Experiment 2).

Appendix 8

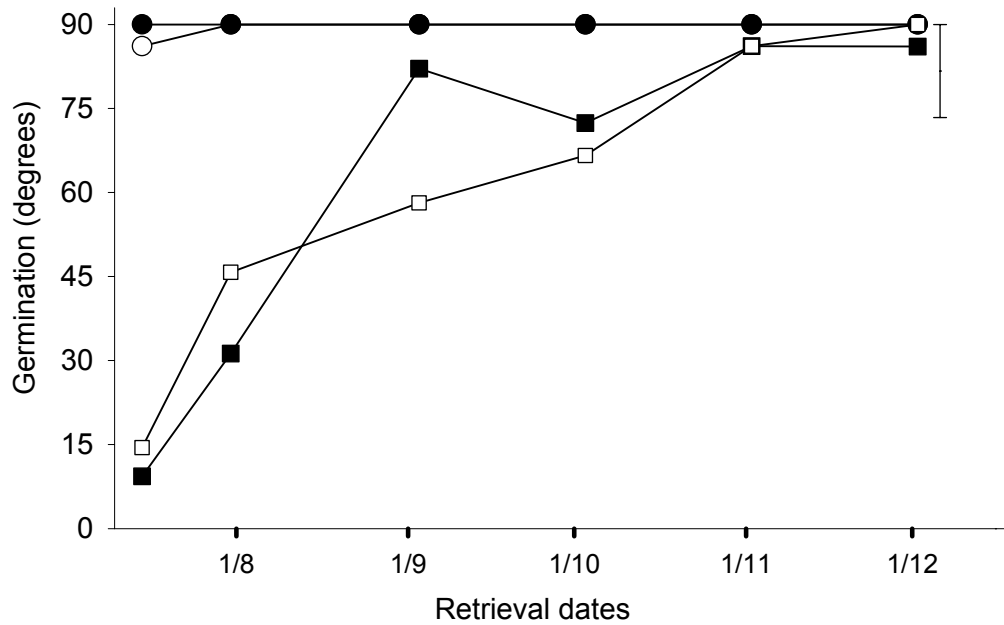


Figure A8-1. Arcsine transformed percent germination of viable seed of field stored *S. physalifolium* seed lot SP3. Seeds were germinated at 20/30 °C (in light ○ and dark ●) or 5/20 °C (in light □ and dark ■) for 14 d on six test dates (s.e. = 3.0, d.f. = 48). The error bar is the Tukey HSD value (16.6). (Experiment 2).

Appendix 9

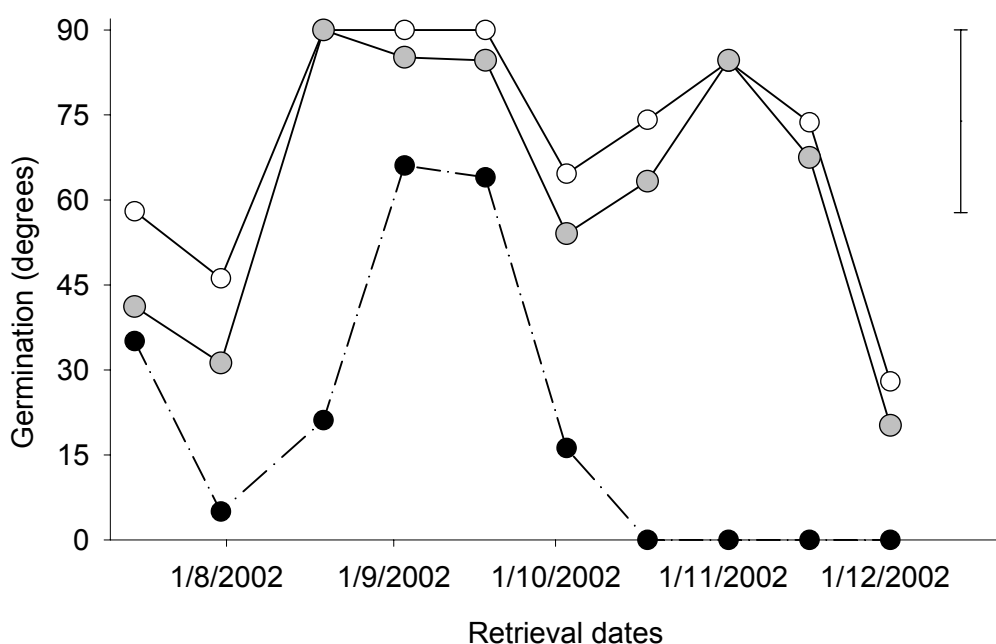


Figure A9-1. The arcsine transformed percent germination of viable *S. nigrum* seed (SN7A) at 14 d. Seeds germinated at 20/30 °C, following exposure to 25.8 s of light (○), 4.6 s of light (grey filled circle), and 0 s light exposure (—●—) on ten test dates (s.e. = 6.0, d.f. = 60). The error bars are the Tukey HSD value (32.2). (Experiment 3).

Table A9-1. The arcsine transformed percent germination of viable seed after 14 d at 20/30 °C for two seed lots (SN7A and SN7C) of *S. nigrum* seed retrieved on four dates and exposed to 25.8, 4.6 or 0 s of light prior to germination testing in the dark. a) Test date and light duration interaction (s.e. = 3.6, d.f. = 48). Means followed by the same letter in columns are not significantly different (Tukey HSD = 17.7). b) Seed lot and light duration interaction (s.e. = 2.6, d.f. = 48). Means followed by the same letter in columns and rows are not significantly different (Tukey HSD = 10.6). (Experiment 3).

a) Light duration (s)	0 s	4.6 s	25.8 s
Test date			
19 Aug	32 b	88 a	90 a
3 Sept	68 c	88 a	85 a
18 Oct	28 b	73 a	83 a
2 Nov	3 a	81 a	85 a
b) Light duration (s)	0 s	4.6 s	25.8 s
Seed lot			
SN7A	22 a	81 c	85 c
SN7C	44 b	84 c	86 c

Appendix 10



Plate A10-1. Duncan cultivator with cover on, behind the tractor.



Plate A10-2. Covered seed drill behind the tractor.



Plate A10-3. S.E.P. rotary hoe with Perspex shield covered during cultivation.

Appendix 11

Table A11-1. Number of *Solanum nigrum* and *S. physalifolium* seedlings to emerge, and the number of mature plants harvested per plot (6 m²) for five sowing dates in 2002.

Sowing	Plot	<i>S. nigrum</i>		<i>S. physalifolium</i>	
		Seedlings	Plants	Seedlings	Plants
August	1	14	5	10	5
	2	12	6	11	5
	3	16	6	11	5
	4	15	6	8	5
September	1	17	6	10	4
	2	15	4	6	3
	3	15	5	6	5
	4	13	6	5	4
October	1	13	4	10	4
	2	14	6	15	6
	3	11	5	15	5
	4	16	4	11	4
November	1	9	4	7	3
	2	9	4	11	4
	3	10	5	11	5
	4	17	5	8	4
December	1	9	5	17	6
	2	14	5	12	5
	3	15	4	16	6
	4	16	6	16	6

Appendix 12



Plate A12-1. A *Solanum physalifolium* plant prior to harvest in the non-competitive growth trial.



Plate A12-2. A *Solanum nigrum* plant prior to harvest in the non-competitive growth trial.



Plate A12-3. A *Solanum physalifolium* plant with fruit at pea harvest in the pea trial.

Appendix 13

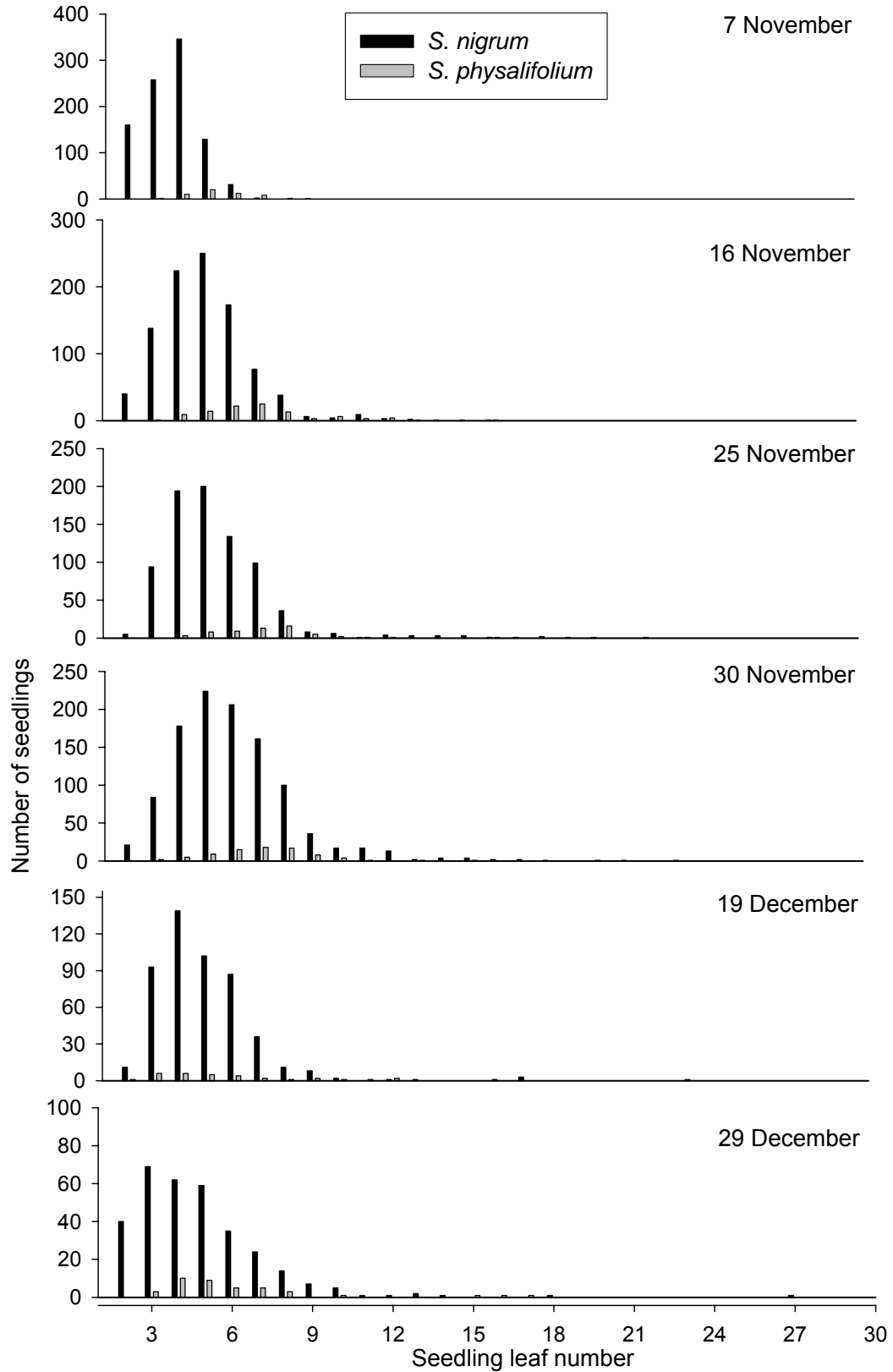


Figure A13-1. Counts of *Solanum nigrum* (SN) and *S. physalifolium* (SP) seedlings grown with peas, presented in relation to seedling leaf number, from 30 0.1m² quadrats on six sample dates from Experiment 4A.

Appendix 14

Table A14-1. Plant dry matter (DM), plant height and number of fruiting parameters including harvest index (HI) and contamination index (CI) for three *Solanum nigrum* (SN) and eight *S. physalifolium* (SP) plants with fruit at pea harvest from Experiment 4A. (Significance indicated from Bonferroni Adjusted Probability test).

	SN	SP	p
Leaf number	13.6	10.0	ns
Plant DM including fruit (g)	0.32	0.13	ns
Plant DM excluding fruit (g)	1.04	1.51	ns
Maximum length (mm)	281	156	ns
GA mm ²	323	152	ns
GAR mm ² /g	1657	1450	ns
Number of fruit < 5 mm	3.0	3.0	ns
Number of fruit > 5 mm	0.67	0.88	ns