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GROWER SUMMARY

Headline

- **Micropropagation set to boost quality and availability of snowdrops**
  Chris Selby and Ioanna Staikidou of Queen's University, Belfast, have developed successful micropropagation techniques for snowdrops including *Galanthus nivalis*, *G. elwesii* and *G. plicatus*. Recent research has increased bulblet multiplication rates, as well as improving bulblet growth and rooting in preparation for transfer to *ex vitro* conditions.

- **Commercial field-scale production of snowdrops in the UK may be possible**
  Snowdrop bulbs have been considered difficult or impractical to grow in the field under commercial conditions. Now field trials at HRI in Lincolnshire have shown that simple shading and mulching treatments can enhance snowdrop bulb yields.

Background and expected deliverables

There is a demand for snowdrop (*Galanthus*) bulbs that, in the past, has been met from bulbs collected from the wild, which is no longer acceptable. However, the crop is difficult to exploit commercially: there are difficulties in obtaining good stocks, in growing snowdrop bulbs satisfactorily in the field, and in storing bulbs. This project addresses these three aims:

- *Micropropagation* - to develop *in vitro* systems that sustain high vegetative propagation rates and yield superior quality, uniform bulb stocks;
- *Agronomy* – to develop novel growing systems for effective commercial production of snowdrop bulbs;
- *Storage* – to investigate more suitable bulb storage regimes for snowdrops.

Success with these objectives should enable the industry to meet and further stimulate the demand for snowdrops. This would apply to sales of (a) bulk sales of dry bulbs of commonplace material (*G. nivalis*), (b) choice material sold as pot-grown plants, and (c) less usual species and cultivars for the specialist market.

Summary of the project and main conclusions

Micropropagation

Experiments were carried out on the bulblet initiation and bulblet growth phases of micropropagation using tissues from *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*. A mineral analysis of bulb tissues was performed and this information used to design a basal medium better suited to snowdrop tissues grown *in vitro*. Factors regulating shoot/leaf initiation as opposed to bulblet initiation were investigated, as were factors controlling hyperhydration in *G. elwesii* tissues. The main findings were:

- Division of bulb chip explants into three single scale leaf explants increased the

productivity of bulblet initiation with *G. nivalis* and *G. elwesii* compared with initiation on intact chips. With these species scale leaf position within the bulb had little effect on their ability to regenerate bulblets. With *G. nivalis* Flore Pleno there was no advantage in dividing chips into separate scales owing to reduced bulblet production by the inner two scales, compared with the outer scale.

- A mineral analysis of snowdrop bulbs (*G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*) indicated that Murashige and Skoog (MS) medium, normally used to culture snowdrops, was under-supplying phosphorus and copper and over-supplying potassium, zinc and manganese. A redesigned basal medium (G) was developed that more closely reflected the ratios of minerals found in the bulbs. Comparison of MS and G basal media at various dilutions (full-strength to one-eighth-strength) showed that:
    - Greater numbers of bulblets were initiated throughout the dilution range on G medium with *G. nivalis* and *G. nivalis* Flore Pleno, whereas MS medium was superior with the full- and half-strength media with *G. elwesii*.
    - G and MS media supported similar bulblet multiplication rates throughout the dilution range, although dilution down to one-eighth significantly reduced bulblet multiplication.
    - Spontaneous root initiation was greater on G medium.
    - Use of G medium greatly reduced hyperhydration in *G. elwesii*.
    - Dilution of MS and G media reduced hyperhydration in *G. elwesii*.

- A bulblet initiation experiment was established with *G. plicatus* and a cultivar of this species, Wendy's Gold. Preliminary observations indicated that these tissues are producing bulblets.

- Bulblet initiation experiments were also performed with *G. elwesii* in attempts to restrict hyperhydration in this species. The main factors investigated were agar concentration, use of G basal medium and an alternative cytokinin (kinetin).

- Attempts to initiate rapidly multiplying shoot cultures rather than bulblets were unsuccessful, in that bulblets continued to be initiated regardless of the treatments imposed. Treatments tested, and main effects found, were:
    - Reducing the incubation temperature to 6ºC: this yielded no *de novo* bulblet or shoot initiation.
    - Reducing the photoperiod from 16h to 8h or a large enhancement of blue light in growth chambers: reduction in the photoperiod marginally increased bulblet initiation rates by bulb chips.
    - Fluridone treatments to inhibit abscisic acid (ABA) synthesis: fluridone (10μM) stimulated bulblet initiation, indicating strongly that ABA is not an important bulb initiation trigger in snowdrops.

- Several experiments are in progress exploring ways to stimulate bulblet growth to a stage where bulbs can be successfully transferred to *in vivo* conditions. All experiments involved the removal of plant growth regulator compounds from the culture medium, and increasing either the carbohydrate (sucrose) supply and/or incorporating activated charcoal in the medium. The main findings were:
    - Addition of activated charcoal greatly stimulated bulblet growth with *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii* on a full-strength MS basal medium.
    - Doubling the sucrose concentration to 60g/l had a lesser effect on bulblet growth

and stimulated growth only when used in combination with 5g/l charcoal.
- Longitudinal sections showed that bulbs, stimulated to enlarge by the addition of extra sucrose and charcoal, formed up to three normal looking scale leaves but developed no flowers.
- Activated charcoal stimulated initiation and elongation of roots.

Other factors currently being investigated with multiplying bulblets of *G. nivalis* and *G. nivalis* Flore Pleno include the use of G basal medium, and applying cold treatments prior to inoculating tissues in to bulb growth conditions.

Agronomy

Experiments were set up with *G. nivalis* to study the effects of shading, shelter, irrigation and mulching on plant, seed and bulb production. With two years' results available, the main findings from the first experiment were:

- Shading plots resulted in higher bulb yields after one and two years of treatment.
- Bulb yields were also better where plots were mulched; after two years there were particularly high yields in plots with mulch but neither shade nor windbreak.
- Plots that had neither mulch, shading nor windbreak produced the poorest yields. In such plots, bulb yields declined from year one to year two.
- Using a windbreak protected new crops from leaf loss in adverse winter weather, but did not have a major effect on bulb yields.
- Plants in shaded plots produced more stems, seed pods and seeds.
- Both shading and mulching delayed the onset of leaf senescence.
- There appeared to be prospects for improving snowdrop growth by using simple shading materials and (or) a straw mulch.
- The experiment is being continued for a third year.

In a second experiment, *G. nivalis* bulbs were grown in shaded or non-shaded plots, in plots inter-cropped with narcissus or cereal, or in plots over-sown with perennial rye-grass:

- In the first and second years, the highest numbers of shoots, stems, seed pods and seeds, and the highest bulb yields, were obtained in shaded plots. Yields were much poorer in inter-cropped plots and especially in plots sown with rye-grass. However, in the second year bulb yields in the rye-grass plots had started to recover.
- Inter-planted crops (cereal or narcissus) and, particularly, over-sown rye-grass, appeared too competitive to snowdrops to be used as alternatives to artificial shading, however the effects of rye-grass are still being tested.

Bulb storage

The effects of storage conditions on bulb quality were examined in an experiment with *G. nivalis*. Bulbs were lifted from the field in March (foliage still green), April (foliage beginning to senesce) and May (foliage fully died-down). Small batches of bulbs were stored in controlled temperature stores running at 10, 13, 17 and 20°C and 65 – 75% relative humidity. Bulbs were held in polythene bags of silver sand, in open trays, in loosely closed

polythene bags and in Perspex 'seed propagators', for 4, 8 or 12 weeks before being recovered and weighed and selecting healthy bulbs for growing-on.

- Storage in silver sand gave good protection from desiccation without resulting in mould growth, as did storage in loosely closed polythene bags, and storage at 13°C was satisfactory, irrespective of the dates bulbs were lifted from the field or the duration of storage.
- Storage in open trays resulted in desiccation, and storage in Perspex propagators resulted in excessive mould growth.
- When healthy bulbs were planted and grown-on, the best performance was from those stored in polythene bags (at 13 or 17°C) or in silver sand stored at 20°C.
- Further storage experiments are under way in 2003.

Financial benefits

An assessment of the benefits deriving from the project must await its completion. The success of micropropagation, however, suggests there is every prospect of establishing good, sustainable bulb stocks that would stimulate demand, not only of the 'ordinary' *G. nivalis*, but also of *G. elwesii* and other species and cultivars. Additional benefits would accrue from better bulb husbandry and storage. Assuming an annual import of 20 million 'ordinary' single snowdrop bulbs, half to be sold retail and half to be sold wholesale, at current prices sales would be worth about £2 million annually, or considerably more for double-flowered or choice types.

Action points for growers

- The results from micropropagation experiments are encouraging, and reliable methodology is now available for the sterilisation, bulblet initiation, multiplication and growth phases of propagation. Thus growers interested in taking up snowdrop cultivation in due course might find it worthwhile to survey sources of good stocks or superior species or cultivars of snowdrops and how they would be marketed.
- The findings to date suggest that the erection of simple shading materials over snowdrop crops would provide some protection and would delay leaf senescence, giving better bulb yields. Mulching with straw also improves yields (and would also control weeds and conserve water).

SCIENCE SECTION

Introduction

The background to this project, and results obtained up to 2001, were fully described in the first and second Annual Reports. Briefly, there is in the UK a demand for snowdrop bulbs that was in the past met from bulbs collected from the wild, a practice no longer acceptable. The crop is difficult to exploit commercially because of difficulties in obtaining good stocks, in growing snowdrop bulbs satisfactorily in the field, and in storing bulbs. This project addresses these three aims:

- *Micropropagation* - to develop *in vitro* systems that sustain high vegetative propagation rates and yield superior quality, uniform bulb stock;
- *Agronomy* – to develop novel growing systems for effective commercial production of snowdrop bulbs;
- *Storage* – to investigate more suitable bulb storage regimes for snowdrops.

Success with these objectives should enable the industry to increase snowdrop bulb sales, both of dry bulbs sold in bulk, and of choice species sold in growth. The following is a summary of the results already presented in the first annual report:

Micropropagation

Experiments focused on the initiation and multiplication phases, using bulb chip explants of *Galanthus nivalis* and *G. elwesii*.

- Explants initiated bulblets, but not shoots, after about 8 weeks in all culture conditions tested.
- Bulblets formed on the abaxial surface of scale leaves with both species.
- *G. nivalis* behaved quite differently to *G. elwesii* in culture:
  - Bulblets formed basally in *G. nivalis* but more randomly in *G. elwesii*.
  - Fungicides in the culture medium reduced bulblet numbers with *G. elwesii* but not *G. nivalis*.
  - *G. elwesii* tissues were more prone to the physiological disorder hyperhydration (vitrification) than those of *G. nivalis*.
- Fungicides, particularly imazalil, increased hyperhydration.
- The commercial product 'Plant Preservation Mixture' (PPM) used for surface sterilisation reduced bulblet numbers. This effect was worsened if fungicides were included in the medium.
- Steeping in a mixture of 20mg/l of both carbendazim and imazalil stimulated bulblet production, whereas use of either compound alone had no effect.
- Plant growth regulator (PGR) effects on bulblet induction were small:
  - PGRs were not essential.
  - Sole use of the cytokinin BA inhibited bulblet production.
  - A combination of BA and the auxin NAA induced more bulblets than either used alone.
- Multiplication of bulblets was relatively slow, but could be maintained even without removal or splitting of larger bulblets.

- New bulblets formed on scales of *in vitro* formed bulblets and tissues derived from explant

scales.
- Multiplication was increased by splitting larger bulblets of *G. elwesii*, but splitting was less effective with *G. nivalis*.
- Root initiation of bulblets was spontaneous, particularly on PGR-free medium.

Agronomy

Agronomy experiments were set up with *G. nivalis* to study the effects of shading, shelter, irrigation and mulching on plant, seed and bulb production:
- Using a windbreak protected crops from leaf loss in adverse winter weather.
- More seeds were produced in shaded than non-shaded plots.
- Both shading and mulching (but not using a windbreak) delayed the onset of leaf senescence
- Bulb yields (both numbers and weights) were consistently higher in shaded than in non-shaded plots. This effect applied to yields of both small and large bulbs.
- Mulching increased the yield of small bulbs, presumably by protection from desiccation.
- Inter-planted crops (cereal or narcissus) and, particularly, over-sown rye-grass, were too competitive to snowdrops to be used as alternatives to artificial shading.

This report describes further results from micropropagation, agronomy and storage experiments.

Micropropagation

Materials and methods

Plant material

Bulbs for bulblet initiation experiments
Galanthus nivalis (5-6cm circumference), *G. nivalis* Flore Pleno (5+cm) and *G. elwesii* (7+cm) bulbs were supplied by Jacques Amand International in October 2001 and 2002, and were stored at room temperature. In autumn 2002 both John Shipton Bulbs (Carmarthenshire) and Monksilver Nursery (Cambridgeshire) supplied *G. plicatus* bulbs. The latter nursery also supplied *G. plicatus* cv Wendy's Gold, a variety that is in short supply and commands a premium price.

Bulblets for growth experiments
Bulblets multiplying *in vitro* were used to inoculate experiments aimed at finding the culture conditions needed to stimulate their growth to a size adequate for planting out *in vivo*. Cultures derived from bulbs purchased in 2000 and 2001 were used for *G. nivalis* and *G. elwesii*. However, with *G. nivalis* Flore Pleno only bulblets produced from bulbs purchased in 2001 were used, thus avoiding use of bulbs bought in 2000 that were found to have single, rather than double, flowers (see Annual Report for 2001).

Bulb preparation for micropropagation

Healthy bulbs were selected and their tunics and any scale leaves showing discoloration or brown markings were removed by hand. Basal bulb tissues were cut away with a scalpel down to healthy white tissues, care being taken not to remove more base plate tissues than was necessary. Apical bulb tissues were also cut away 1mm below the region of scale leaf senescence so that only healthy white tissues remained. The bulbs were then ready for surface sterilisation. Throughout bulbs were given a preliminary surface sterilisation by shaking in 70% ethanol for 1min. Thereafter bulbs were surface sterilised singly in 100ml Erlenmeyer flasks (*G. nivalis*, *G. nivalis* Flore Pleno and *G. plicatus*) or in wide-form 100ml beakers (*G. elwesii*) capped in aluminium foil and shaken at 130 r.p.m. on a reciprocating shaker. Whole bulbs were treated with 50% Domestos for 20min, rinsed five times in sterile de-ionised water, and trimmed top and bottom to remove tissues damaged by the sterilant. Trimmed bulbs were cut aseptically into chip explants and the chips treated with 4% Plant Preservative Mixture (PPM) (in 50 mg/l $MgSO_4$) for 9h.

Bulb chips were approximately 8mm in height for *G. nivalis*, *G. nivalis* Flore Pleno and *G. plicatus* (all species yielding four chips per bulb) or 10mm in height for *G. elwesii* (yielding six chips per bulb). Four chips in 20ml of PPM was adequate to sterilise the smaller chips. Larger ones produced from *G. elwesii* bulbs required three chips in 30ml of PPM to improve surface sterilisation. Following sterilisation, explants were inoculated with their bases about 3mm into the agar-solidified culture medium. One explant was inoculated per culture vessel.

Media preparation and culture conditions

Pre-prepared Murashige and Skoog (1962) (MS) basal macronutrients, micronutrients and vitamins (Sigma Aldrich Co. Ltd.) were used throughout, dissolved in water purified with an Elga Prima reverse osmosis apparatus (Elga Ltd). Unless otherwise stated this was supplemented with 30g/l sucrose, 1mg/l 6-benzyladenine (BA) and 0.1mg/l naththaleneacetic acid (NAA). Media were adjusted to pH 5.6 with dilute KOH or HCl before adding 7g/l Oxoid purified agar. The agar was dissolved by heating, then 20ml aliquots of media were dispensed into boiling tubes. Tubes were enclosed with cotton wool bungs and autoclaved at 121ºC for 15 min.

Cultures were incubated in an air-conditioned growth room at a constant temperature of 18ºC. A photoperiod of 16h was provided by cool white fluorescent tubes giving a PAR of 100µ mol/m^2/s at bench height.

Bulblet initiation

Bulb scale leaf explants

It was previously reported that new bulblets form on the outer surface of scale leaves within chip explants and scales dissected from chips were still capable of forming bulblets independently under *in vitro* conditions. An experiment was therefore designed to examine if scale leaf position within the mother bulb influenced the ability of bulb scale explants to initiate bulblets.

Four chip explants were prepared from each of 17 bulbs of *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*. After surface sterilisation two chip explants from each bulb were inoculated directly to the culture medium. The remaining two explants were cut into single scale explants each with a section of bulb basal plate still attached. Normally three scale explants were prepared from each chip and these were numbered from one to three starting from the innermost bulb scale. Scales were individually inoculated base plates down onto the culture medium. The full experimental design was four explant types x three snowdrop types, 12 treatments in all. Each treatment was replicated with two chip explants and two scale leaf explants, from each bulb position, with each of 17 bulbs (408 cultures in total). For each explant type, data from the two explants was averaged for each bulb before the data was subjected to analysis of variance (ANOVA), i.e. there were 17 independent replications.

Basal medium composition

Methods similar to those described by Fulcheri, Morard and Henry (1998), Morard and Henry (1998), Bouman, Morris and Tiekstra (2001) and Bouman and Tiekstra (2001) were used in an attempt to optimise the mineral composition of the basal culture medium for snowdrops. This involved designing a medium of mineral composition that closely matched mineral analyses of snowdrop bulb tissues.

Mineral composition of snowdrop bulbs

In November 2001 bulbs of *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii* were cleaned by removal of tunics, roots and base plate contaminated by soil, apical necrotic tissues and shoots before drying down to constant weight at 70ºC. These dried tissues were then ground to a fine powder in a pestle and mortar. Three dried samples were prepared and analysed for mineral composition for each snowdrop type. Bulb samples were analysed for N by dry combustion using a LECO P-2000 elemental analyser, and for P, K, S, Ca, Mg, Na, Fe, Mn, Cu and Zn by inductively-coupled plasma emission spectrometry (ICP) after digestion in a 4:1 mixture of nitric and perchloric acids (Ministry of Agriculture, Fisheries and Food, 1986).

Table 1.1. Mineral composition of snowdrop bulbs.

Mineral	Species			Significance	LSD (5%)
	G. nivalis	*G. nivalis* Flore Pleno	*G. elwesii*		
(g/100gDW)					
N	1.00	1.28	0.69	***	0.202
P	0.18	0.18	0.13	**	0.028
K	0.70	0.65	0.44	***	0.061
Ca	0.09	0.12	0.29	***	0.077
Mg	0.05	0.05	0.05	NS	0.010
S	0.08	0.09	0.08	NS	0.016
(mg/KgDW)					
Cu	3.72	1.23	1.83	**	1.392
Zn	11.53	12.43	16.58	*	3.617
Fe	9.80	10.63	9.53	NS	3.754
Mn	4.88	3.60	5.23	NS	2.827

Calculation of Galanthus *medium composition*

Results of the mineral analysis are shown in Table 1.1. Since it was not practical in the first instance to formulate a medium for each species, mean concentration values were calculated for each mineral across the three snowdrop types. These data were used to calculate an 'ideal' composition for a *Galanthus* medium (G) that had a set nitrogen composition of 40mM NO_3^- and 20mM NH_4^+ (as in MS medium) but which matched the ratios of other minerals based on the bulb analysis (see Table 1.2 for comparisons of mean mineral compositions of bulbs with that of MS and G media). It was not possible to achieve an exact match with the bulb composition without allowing excesses of other unwanted ions. The composition of the G medium was achieved using the following components (all in mg/l): KNO_3 (861.1), NH_4NO_3 (1600.9), KH_2PO_4 (603.9), $MgSO_4.7H_2O$ (485.1), $Ca(NO_3)_2.4H_2O$ (1355.2), $FeSO_4.7H_2O$ (27.8), EDTA (37.2), $CuSO_4.5H_2O$ (0.76), $ZnSO_4.7H_2O$ (5.05), $MnSO_4.H_2O$ (1.21), $CoCl_2.6H_2O$ (0.025), H_3BO_3 (6.2), $Na_2MoO_4.2H_2O$ (0.25) and KI (0.83). Vitamins and inositol were set at the levels of MS. FeEDTA concentrations were also retained at those in MS medium, owing to known difficulties in keeping iron available to plants *in vitro*.

Table 1.2. Comparison of Galanthus (G) and Murashige and Skoog (MS) mineral compositions with that of the mean mineral composition of snowdrop bulbs.

Mineral	Mean concentration in bulbs	Mean bulb concentration converted by ratio to the MS nitrogen level	Culture medium	
			G	MS
	(mmoles/gDW)	(mM)	(mM)	(mM)
N	0.706	60.07	60	60
P	0.052	4.442	4.44	1.25
K	0.152	12.968	12.95	20.00
Ca	0.042	3.585	5.74	3.00
Mg	0.020	1.742	1.97	1.50
S	0.026	2.205	1.97	1.70
	(μmoles/gDW)	(μM)	(μM)	(μM)
Cu	0.036	3.028	3.04	0.100
Zn	0.207	17.585	17.56	30.00
Fe	0.179	15.212	100	100
Mn	0.083	7.074	7.15	100

Bulblet initiation on G medium
Media dilutions of 0 (full-strength), 0.5, 0.25 and 0.125 were tested with both MS (controls) and the new medium G for their ability to support bulblet initiation in bulb chip explants. Vitamins, inositol, sucrose, BA and NAA were kept constant at full-strength medium concentrations in all media. Each medium was inoculated with bulb chips of *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*. The full experimental design was two basal media (G and MS) x four dilutions x three snowdrop types, 24 treatments in all. Each treatment was replicated 15 times (360 cultures in total).

Bulblet multiplication on G medium
The same eight media described above were also tested for their ability to support the multiplication of bulblet clumps of *G. nivalis* and *G. elwesii*. Mean bulblet numbers on inoculation were 3.5 and 2.0 for *G. nivalis* and *G. elwesii* respectively. The full experimental design was two basal media (G and MS) x four dilutions x two snowdrop types, 16 treatments in all. Each treatment was replicated 20 times (320 cultures in total).

Bulblet initiation in G. plicatus
The ability of *G. plicatus* bulb chip explants to form adventitious bulblets *in vitro* was tested for the first time in an experiment inoculated on 5 December 2002. *G. plicatus* bulbs were obtained, freshly lifted, from two sources, together with a well known cultivar of this species, Wendy's Gold. Tissues from *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii* were also used for comparison and to establish fresh cultures of these species. Explants from each plant type were inoculated onto half-strength MS and G media. The full experimental design was two basal media (0.5G and 0.5MS) x six snowdrop types (two samples of *G. plicatus*, *G. plicatus* Wendy's Gold, *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*), 12 treatments in all. Each treatment was replicated with ten bulbs of each snowdrop type, two chip explants from each bulb being inoculated onto each medium (240 cultures in total).

Control of hyperhydration in G. elwesii

By the end of the second culture passage a physiological disorder known as hyperhydration (vitrification) developed in multiplying bulblet cultures in both 2000 and 2001. Hyperhydration is characterised by tissues becoming very swollen, translucent in appearance, deformed and brittle. This disorder is caused by an excessive uptake of water (Debergh *et al.,* 1992).

Although no systematic scoring of hyperhydration was attempted, general observations were made. Occurrence of the disorder varied from explant to explant and even within different regions of the same explant. Whole scale leaf segments, present in the original bulb chip explant became very swollen and glassy in appearance. In the most extreme instances these scales expanded to the extent were they pressed against both sides of the culture vessels (21mm i.d.). Tissues of newly initiated bulblets also became hyperhydrated, forming swollen and distorted scales and leaves. Tissues of *G. elwesii* were much more prone to hyperhydration than *G. nivalis*. With this species hyperhydration occurred on all media tested. Hyperhydration is usually regarded as a problem because it is difficult to reverse, and hyperhydrated plantlets are difficult to acclimatise to *in vivo* conditions. It was therefore important to develop methods to restrict hyperhydration, particularly with *G. elwesii.*

A major factor controlling hyperhydration in plant tissues is the concentration and type of gelling agent used (Debergh, Harbaoui and Lemeur, 1981; Debergh, 1983). Choice of cytokinin can also influence hyperhydration, with BA, the cytokinin used in the current study, being implicated as a cause of the disorder in some circumstances (Kataeva *et al*., 1991; Debergh *et al.,* 1992). Experiments were therefore established to examine if manipulation of these factors could control hyperhydration in *G. elwesii*. Since it was also noted that hyperhydration in *G. elwesii* cultures was less frequent and severe on G medium compared with MS medium (Table 1.6 and Figure 1.2), the effects of changing the basal medium were also tested.

Agar concentration and basal medium

G. elwesii bulb chip explants were inoculated onto media solidified with either 7, 9 or 11 g/l Oxoid purified agar. Each agar concentration was repeated with both half-strength G and MS media. In this experiment the general surface sterilisation treatment with PPM was modified to improve its efficacy. Explants were treated in groups of three in 30ml aliquots of 5% PPM. The full experimental design was two basal media (0.5G and 0.5MS) x three agar concentrations, six treatments in all. Each treatment was replicated with 26 bulbs, one chip explant from each bulb being inoculated onto each medium (156 cultures in total).

Agar concentration and cytokinin

G. elwesii bulb chip explants were inoculated onto media solidified with either 7, 9 or 11 g/l Oxoid purified agar. Each agar concentration was repeated with half-strength MS media supplemented with either 1mg/l BA or an equimolar concentration of an alternative cytokinin kinetin (Kin). The full experimental design was two cytokinins (BA and Kin) x three agar concentrations, six treatments in all. Each treatment was replicated with 25 bulbs, one chip explant from each bulb being inoculated onto each medium (150 cultures in total).

Attempted initiation of shoot cultures

In the project proposal it was anticipated that adventitious shoots would be formed by bulb chip explants facilitating the rapid multiplication of tissues. In all initiation experiments examined to date, the only adventitious structures to be formed were bulblets rather than shoots or leaves, regardless of the composition of the culture medium (see previous reports). Even shoots derived from lateral bulb initials, pre-formed in the bulb chip explants, also rapidly developed into bulblets under the culture conditions used here. Further experiments were therefore set up in an attempt to initiate proliferating shoot cultures on bulb chip explants, rather than bulblets. All experiments were replicated with *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*. Experiments were designed examining the effects of reduced temperature, reduced photoperiod and enhanced blue light, and inhibition of endogenous ABA synthesis.

Reduced temperature

Since high temperature is known to be a strong stimulus to bulb formation in many species, cultures were initiated at 6ºC by incubating in a Sheerer CEL 512-37 growth chamber set to provide a 16h photoperiod and a light quality and PAR equivalent to the growth room. Cultures incubated at 18ºC in the growth room, described previously, were used as a control (two temperature treatments x three snowdrop types, six treatments in all). Each treatment was replicated with 16 chip explants (96 cultures in total).

Reduced photoperiod and enhanced blue light

Formation of many storage organs is under the control of photoperiod, the onset of long days (LD) often being an induction signal for organ formation, with short days (SD) being prohibitive. Culture initiation in LD and SD conditions was therefore investigated. Since some *Galanthus* species evolved in high altitude mountainous conditions, the effect of blue light supplementation was also tested. This was examined because light quality is known to change, with blue light increasing with altitude (van der Linde, personal communication).

Four Fisons 600G3/THTL growth cabinets were used to give the following sets of conditions:

a. 16h photoperiod (LD) provided by cool white fluorescent tubes (control)
b. 8h photoperiod (SD) provided by cool white fluorescent tubes
c. LD provided by cool white tubes supplemented with Osram L 36W/67 blue fluorescent tubes
d. SD provided by cool white tubes supplemented with Osram L 36W/67 blue fluorescent tubes

Photosynthetically active radiation (PAR) was equalised between treatments to 100 μmoles/m^2/s by use of SR9910 scanning spectroradiometer (Macam Photometrics Ltd). Spectra from these growth cabinets are shown in Figure 1.1. The full experimental design was two photoperiods x two blue light treatments x three snowdrop types, 12 treatments in all. Each treatment was replicated with 20 bulb chip explants (240 cultures in total).

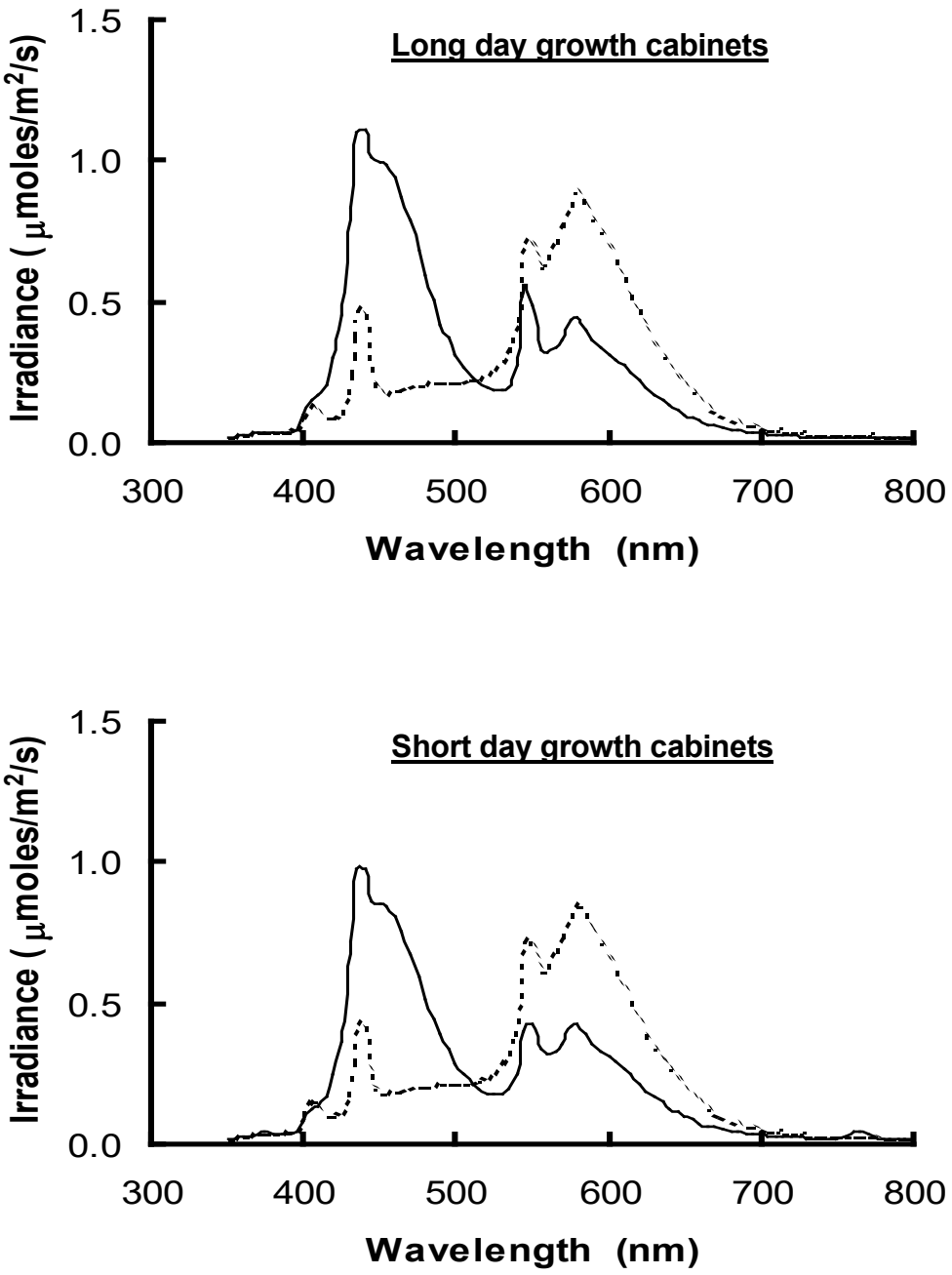


Figure 1.1. Light quality of the four growth cabinets used to assess the effects of photoperiod and blue light enhancement on shoot/bulblet formation by snowdrop bulb chip explants. Cabinets were fitted with either cool white fluorescent tubes (dashed line) or mixture of cool white fluorescent tubes and Osram L 36W/67 blue fluorescent tubes (solid line).

Inhibition of endogenous ABA synthesis
Abscisic acid (ABA) is normally synthesised by plants when they are stressed, and this plant growth regulator can also act as an inducer of storage organ formation (De Hertogh and Le Nard, 1993 and references therein). Thus bulb chip explants, when inoculated to culture conditions, may be stressed to the extent that they synthesise sufficient ABA to promote bulblet formation, to the exclusion of leaf and shoot development. Adding the herbicide fluridone, a potent inhibitor of ABA synthesis, to the culture medium could block this mechanism and may stimulate shoot formation.

Three concentrations of fluridone (0, 1 and 10μM) were tested in both continuous darkness and normal lighting conditions. The light conditions were supplied by using two identical incubators (LEEC LT3), one illuminated with two cool white tubes in the door. The full experimental design was three fluridone concentrations x two light treatments x three snowdrop types, 18 treatments in all. Each treatment was replicated with 15 bulb chip explants (270 cultures in total).

Stimulation of bulblet growth

Sucrose and activated charcoal on MS basal medium
The effects of increasing the sucrose concentration and supplementing the medium with activated charcoal (Sigma C6289) on bulblet growth were examined on MS based medium free of plant growth regulators. Two sucrose concentrations, 30 and 60 g/l, were used in all combinations with 0, 1 and 5 g/l activated charcoal. All media were set at pH 6.0 prior to autoclaving. This higher than normal pH allowed for an acidification in charcoal-containing media during autoclaving. Each treatment was repeated with bulblet clumps, with averages of 2.74, 3.96 and 2.09 bulblets for *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*, respectively. The full experimental design was two sucrose x three charcoal concentrations x three snowdrop types, 18 treatments in all. Each treatment was replicated with 15 bulblet clumps (270 cultures in total).

Sucrose and activated charcoal on half-strength G basal medium
The effects of sucrose and activated charcoal were investigated further in two separate experiments, one with *G. nivalis* and the other with *G. nivalis* Flore Pleno. Both used half-strength plant growth regulator free G medium. These experiments tested the effects of 30, 60 and 90 g/l sucrose with and without 5 g/l activated charcoal (six media treatments in all). Twenty replicates were used for each snowdrop type (240 cultures in total).

Cold treatment
Since in nature pre-formed lateral bulb units are subjected to a winter cold treatment prior to them forming flowering, terminal bulbs in the spring the effect of cold treatment on subsequent bulblet growth was investigated. Bulblet clump cultures of *G. nivalis* and *G. nivalis* Flore Pleno were subjected to 0, 4, or 6 weeks cold treatments by incubating at 5ºC in a Sheerer CEL 512-37 growth chamber. Bulblet clumps from each treatment were inoculated onto half-strength plant growth regulator-free G medium containing 30 or 60 g/l sucrose plus or minus 5 g/l activated charcoal. The experiment was designed so that all cultures of each snowdrop type were inoculated at the same time with all three cold treatments. The full experimental design was three cold treatments x two sucrose x two charcoal concentrations x

two snowdrop types, 24 treatments in all. Each treatment was replicated 15 times (360 cultures in total).

Results and discussion

Initiation of bulblets

Bulb scale leaf explants

Bulb scale explants from all three bulb positions were capable of forming bulblets with all snowdrop types (Table 1.3). Use of bulb scale explants could clearly improve bulblet production compared with whole chip explants, particularly with *G. nivalis* and *G. elwesii*. However there was a highly significant interaction between species and explant type, caused by bulb scale explants from inner bulb positions 1 and 2 of *G. nivalis* Flore Pleno showing significantly lower bulblet numbers compared with the whole chip and the outer scale explant (position 3).

Table 1.3. The effect of explant type on the formation of bulblets 2 months after inoculation onto MS medium. Single scale explants were prepared from bulb chips following the final surface sterilisation with PPM. These consisted of scales with a small section of base plate left attached. Scale one was the innermost scale formed in the centre of the mother bulb, and scale three the outermost. The data were normalised by square root transformation before analysis, but untransformed data are shown in parenthesis.

Species	Explant type				Species means
	Whole chip	Scale 1	Scale 2	Scale 3	
G. nivalis	1.87 (3.88)	1.41 (2.20)	1.43 (2.91)	1.32 (2.68)	1.51 (2.92)
G. nivalis **Flore pleno**	2.14 (6.03)	0.77 (2.45)	0.60 (2.41)	1.47 (3.36)	1.25 (3.56)
G. elwesii	2.51 (7.48)	2.33 (6.44)	3.01 (11.67)	2.69 (8.77)	2.63 (8.59)
Explant means	2.17 (5.80)	1.50 (3.70)	1.68 (5.66)	1.83 (4.94)	

Analysis of variance summary

	d.f. (m.v.)	SED	Significance
Species (SP)	2	0.126	***
Explant type (E)	3	0.146	*
SP x E	6	0.253	**
Residual	129 (47)		

Basal medium composition
In MS medium P and Cu were in under-supply, whilst K, Zn and Mn were in over-supply, relative to nitrogen, when compared to the mineral composition of bulbs (Table 1.2). From this Table it can also be seen that the newly formulated G medium rectifies these discrepancies and supplies minerals to the tissues in ratios closely related to those found in bulb tissues. However, there are several significant differences in mineral composition between the three snowdrop types (Table 1.1), and therefore it would have been ideal to calculate separate media for each snowdrop type. In particular *G. elwesii* had significantly less of the three main macronutrients (N, P, and K) and notably more Ca than the other two types.

Bulblet initiation on G medium
Overall bulblet induction was greater on G medium than on MS medium, however there were highly significant interactions between snowdrop type and both basal medium ($p < 0.01$) and medium dilution ($p < 0.01$) (Table 1.4). With both *G. nivalis* and *G. nivalis* Flore Pleno G medium was significantly superior to MS medium throughout the dilution range, whilst with *G. elwesii,* MS medium gave a greater number of bulblets. The interaction between snowdrop type and medium dilution was more complex. With *G. nivalis* dilution had little effect on bulblet production, whereas with *G. elwesii* the two highest dilutions gave significantly fewer bulblets than the half-strength medium. Medium dilution showed the most benefit with *G. nivalis* Flore Pleno, in that the two highest dilutions supported the highest bulblet production.

Bulblet multiplication on G medium
Bulblet multiplication in bulblet clump cultures decreased with increasing dilution of the basal medium ($p = 0.002$), but the other main factors, species and basal medium, were not significantly different and there were no significant interactions between any of these factors (Table 1.5). The highest medium dilution (0.12) yielded significantly lower bulblet multiplication than all of the other dilution treatments.

If the bulblet multiplication data were analysed separately for each species (analysis not shown), medium dilution down to 0.12 was found to be inhibitory with *G. elwesii* [$p = 0.006$, LSD (5%) = 1.57] but not with *G. nivalis* ($p = 0.105$). Likewise, separate analysis of the data for the two species indicated that G medium was close to being statistically better for supporting bulblet multiplication than the MS medium ($p = 0.068$) with *G. nivalis*, but not with *G. elwesii* ($p = 0.948$).

Thus the data shown on Tables 1.4 and 1.5, taken together, indicate strongly that G medium is better suited for *G. nivalis* growth than for *G. elwesii*. This suggests that further improvements may be achieved for *G. elwesii* if the basal medium were redesigned using the mineral composition of bulbs of this species alone. Use of G medium with *G. elwesii* was, however, found to have an unexpected benefit in both bulblet initiation and bulblet multiplication experiments. Tissues of this species were markedly less hyperhydrated on the G medium compared with MS medium (Table 1.6 and Figure 1.2). Therefore, the use of G medium with *G. elwesii* could offer a method of controlling hyperhydration in this species, even though the medium is sub-optimal for bulblet initiation and multiplication. Dilution of both G and MS media was also found to restrict hyperhydration with *G. elwesii*. Root initiation was also greatly enhanced by use of G medium, compared with MS (Figure 1.2).

Table 1.4. The effect of basal medium composition on the formation of bulblets by bulb chip explants 3 months after inoculation. Media used were MS and medium G devised from the mineral composition of snowdrop bulbs (see Table 1.2). Each medium was used at full-strength (FS) and at three dilutions. The data were normalised by square root transformation before analysis, but untransformed data are shown in parenthesis.

Species	Basal medium	Medium dilution FS	0.50	0.25	0.12	Species x medium	Species
G. nivalis	MS	1.93 (3.86)	2.22 (5.63)	2.49 (6.81)	2.24 (5.37)	2.22 (5.42)	2.52 (7.10)
	G	2.70 (7.92)	2.80 (8.53)	3.12 (10.87)	2.64 (7.80)	2.82 (8.78)	
G. nivalis **Flore pleno**	MS	1.00 (1.42)	1.01 (1.56)	1.78 (3.47)	1.91 (3.80)	1.42 (2.56)	1.74 (3.89)
	G	1.38 (2.46)	2.13 (5.71)	2.38 (6.37)	2.30 (6.30)	2.05 (5.21)	
G. elwesii	MS	3.26 (12.98)	3.76 (17.18)	2.41 (7.71)	2.54 (10.43)	2.99 (12.22)	2.87 (10.64)
	G	2.54 (7.52)	2.94 (9.77)	2.89 (11.09)	2.63 (7.84)	2.75 (9.05)	
Species x medium dilution							
G. nivalis		2.31 (5.89)	2.51 (7.08)	2.81 (8.84)	2.44 (6.59)		
G. nivalis **Flore pleno**		1.19 (1.94)	1.57 (3.63)	2.08 (4.92)	2.11 (5.05)		
G. elwesii		2.90 (10.25)	3.35 (13.77)	2.65 (9.40)	2.59 (9.13)		
Dilution means		2.13 (6.03)	2.48 (8.16)	2.51 (7.72)	2.38 (6.92)		
Medium means		MS = 2.21 (6.73)		G = 2.54 (7.68)			

Analysis of variance summary

	d.f. (m.v.)	SED	Significance
Species (SP)	2	0.096	***
Medium (M)	1	0.079	**
Dilution (D)	3	0.111	NS
SP x M	2	0.136	**
SP x D	6	0.193	**
M x D	3	0.157	NS
SP x M x D	6	0.272	NS
Residual	265 (57)		

Table 1.5. The effect of basal medium composition on the multiplication of bulblets (number of bulblets at the end of the culture passage / number of bulblets inoculated). Media used were MS and medium G devised from the mineral composition of snowdrop bulbs (see Table 1.2). Each medium was used at full-strength (FS) and at three dilutions.

Species	Basal medium	Medium dilution FS	0.50	0.25	0.12	Species x medium	Species means
G. nivalis	MS	3.33	4.14	3.08	2.93	3.37	
							3.82
	G	4.83	5.17	4.01	3.08	4.27	
G. elwesii	MS	6.67	4.33	4.69	2.10	4.45	
							4.48
	G	4.87	5.13	4.37	3.69	4.51	
Species x medium dilution							
G. nivalis		4.08	4.65	3.55	3.00		
G. elwesii		5.77	4.73	4.53	2.89		
Dilution means		4.92	4.69	4.04	2.95		
Medium means		MS = 3.91		G = 4.39			

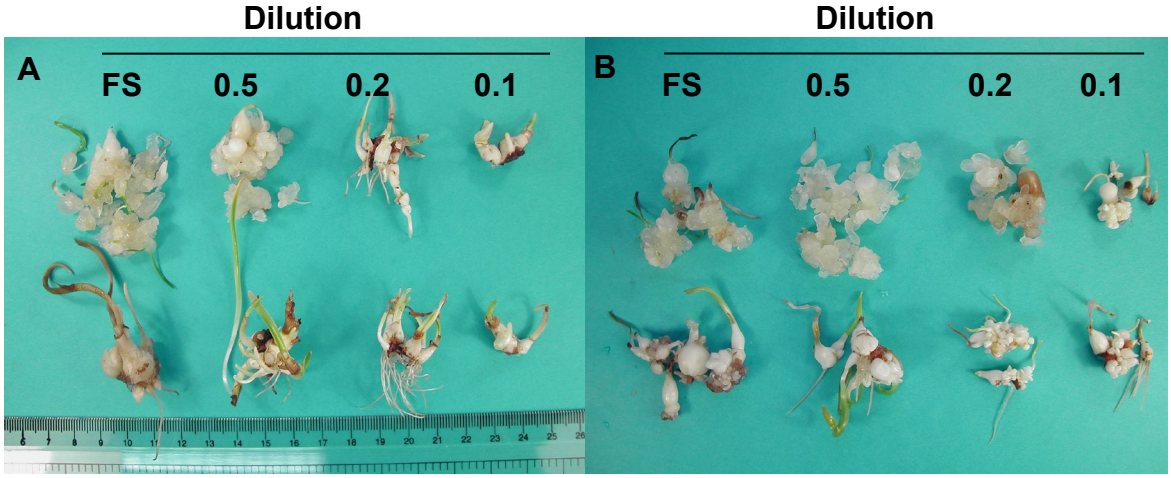
Analysis of variance summary

	d.f. (m.v.)	SED	Significance
Species (SP)	1	0.276	NS
Medium (M)	1	0.276	NS
Dilution (D)	3	0.390	**
SP x M	1	0.390	NS
SP x D	3	0.552	NS
M x D	3	0.552	NS
SP x M x D	3	0.781	NS
Residual	283 (2)		

Table 1.6. Percentage of cultures showing hyperhydration in bulblet tissues on various dilutions of MS and G basal media in the bulblet multiplication experiment at the end of the first 5 month culture passage.

Species	Basal medium	Medium dilution FS	0.50	0.25	0.12
G. nivalis	MS	5	15	0	0
	G	5	0	0	0
G. elwesii	MS	45	30	21	10
	G	32	5	5	5

Figure 1.2 Hyperhydration in multiplying *G. elwesii* cultures grown on dilutions of MS medium (top row) and G medium (bottom row) at the end of a second 7 month culture passage. A and B represent two different genotypes of *G. elwesii* used as inoculum. Glassy and swollen hyperhydrated tissues can be seen with both genotypes on the FS and 0.5 dilution of the MS medium and at the 0.25 dilution of MS medium with genotype B.



Attempted initiation of shoot cultures

Reduced temperature

No leaves or shoots were formed by bulb chip explants incubated at the reduced temperature of 6ºC. Few bulblets were formed at 6ºC but these were all derived from pre-formed lateral bulb units contained within the original chip explants and no bulblets formed *de novo*. Control chips cultured at 18ºC formed on average 4.23, 2.88, and 6.36 bulblets per explant for *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii*, respectively. Chips cultured at the lower temperature remained healthy in appearance for many months, their scale leaf segments became swollen in appearance and leaf tissues from both the terminal flowering-bulb units and lateral bulb units grew out and formed chlorophyll. Thus leaf tissues were able to grow at the lower temperature *in vitro* as would be expected from the natural growth cycle of snowdrops that develop leaves in mid-winter. Perhaps marginally higher temperatures are required for the initiation of new shoot primordia or these primordia may only differentiate after three or four bulb scale initials have been produced. Girmen and Zimmer (1988) also reported that *G. elwesii* chip explants grown at 10ºC formed bulblets, albeit at a reduced rate compared with tissues cultured at higher temperatures. Thus it can be predicted that if it is possible for bulb chip explants to produce proliferating shoot cultures *in vitro*, it will require a temperature between 6 and 10ºC.

Reduced photoperiod and enhanced blue light

Neither shortening the photoperiod nor greatly enhancing the blue light irradiance (Figure 1.1) was able to change the pattern of differentiation in the cultured bulb chip explants. The explants continued to form bulblets and no shoots or isolated leaves were differentiated in any of the four light treatments. Blue light supplementation had no effect on the rate of bulblet production (data not shown), whilst reducing the photoperiod to 8h significantly increased bulblet production rates compared with the 16h photoperiod control (Table 1.7).

Inhibition of endogenous ABA synthesis

Fluridone treatment failed to stimulate the formation of shoots or leaves on bulb chip explants, regardless of whether they were incubated in constant darkness or in a 16h photoperiod. Nevertheless explants formed adventitious bulblets, in the previously reported fashion, in all of the treatments (Table 1.8). The tissues were clearly taking up the herbicide, since carotenoid synthesis was inhibited. This was evidenced by the formation of white, non-pigmented leaves by sprouting bulblets in the light incubated tissues. Control tissues without fluridone formed normal pigmented leaves. This indicated that endogenous ABA levels were greatly depressed in the tissues grown on fluridone supplemented media and suggests that endogenous ABA levels were not a critical factor triggering bulb initiation in *Galanthus* species. Indeed it can be seen from Table 1.8 that the 10 μM fluridone treatment actually stimulated a small but significant increase in the production of bulblets compared to the other two treatments ($p < 0.05$). The behaviour of *Galanthus* tissues reported here contrasts with findings with *Lilium*, where fluridone was successfully used to promote leaf formation *in vitro* (Kim, Davelaar and DeKlerk, 1994; Shimasaki and Fukumoto, 2000).

Although no statistical comparison could be made between the dark and the 16h photoperiod treatments, owing to their lack of replication, bulb chips formed more bulblets in continuous darkness in all treatments with all three snowdrop types. This, together with the previous finding that reducing the photoperiod from 16h to 8h also stimulates bulblet production, indicates that light may be inhibitory to adventitious bulblet initiation.

Although failure to induce shoot formation in the bulb chip explants was disappointing, since this mode of organogenesis potentially offers high multiplication rates, it was not essential to the micropropagation of *Galanthus* species. In reality, direct formation of bulblets, that readily multiply *in vitro,* offers a simpler micropropagation system requiring less stages and media changes than one involving a shoot multiplication phase.

Table 1.7. The effect of photoperiod on the production of bulblets by bulb chip explants after 3 months incubation. For each photoperiod the data shown are the means of the cabinet fitted with cool white fluorescent tubes and the cabinet with blue light supplementation. Data were normalised by square root transformation before analysis, but untransformed data are shown in parenthesis.

Species	Photoperiod (h)		Species means
	8	16	
G. nivalis	1.88 (4.44)	1.40 (2.87)	1.64 (3.66)
G. nivalis **Flore pleno**	1.23 (2.24)	0.92 (1.36)	1.08 (1.80)
G. elwesii	2.10 (5.25)	2.04 (5.37)	2.07 (5.31)
Photoperiod means	1.74 (3.98)	1.45 (3.20)	

Analysis of variance summary

	d.f. (m.v.)	SED	Significance
Species (SP)	2	0.106	***
Photoperiod (P)	1	0.014	*
Light (L)	1	0.014	NS
SP x P	2	0.123	NS
SP x L	2	0.123	NS
Residual	212 (18)		

Table 1.8. The effect of fluridone on the formation of bulblets by bulb chip explants 3 months after inoculation. Since the dark and 16h photoperiod treatments were not replicated, but set up in two separate incubators, ANOVA was used on each light treatment separately and the two light treatments together, without analysing for light effects. The data were normalised by square root transformation before analysis, but untransformed data are shown in parenthesis.

Species	Fluridone (μM)			Species
	0	1	10	means
MEAN OF DARK AND 16H PHOTOPERIOD TREATMENTS				
G. nivalis	2.38	2.46	2.92	2.59
	(6.96)	(7.69)	(9.40)	(8.01)
***G. nivalis* Flore pleno**	3.10	3.11	3.48	3.23
	(11.27)	(11.57)	(14.21)	(12.35)
G. elwesii	2.91	2.84	3.04	2.93
	(9.79)	(9.25)	(10.26)	(9.77)
Fluridone means	2.80	2.80	3.15	
	(9.34)	(9.50)	(11.29)	
DARK				
G. nivalis	2.83	3.00	3.35	3.06
	(8.97)	(10.12)	(11.71)	(10.27)
***G. nivalis* Flore pleno**	4.05	3.95	4.43	4.14
	(17.39)	(16.73)	(20.97)	(18.37)
G. elwesii	3.36	3.13	3.24	3.24
	(12.26)	(11.51)	(12.07)	(11.95)
Fluridone means	3.41	3.36	3.67	
	(12.88)	(12.79)	(14.92)	
16h PHOTOPERIOD				
G. nivalis	1.92	1.92	2.50	2.11
	(4.80)	(5.07)	(7.11)	(5.66)
***G. nivalis* Flore pleno**	2.20	2.27	2.55	2.34
	(5.53)	(6.40)	(7.67)	(6.53)
G. elwesii	2.42	2.57	2.71	2.57
	(6.97)	(7.14)	(7.87)	(7.33)
Fluridone means	2.18	2.25	2.59	
	(5.77)	(6.20)	(7.55)	

Analysis of variance summary

Dark and 16h photoperiod treatments analysed together

	d.f. (m.v.)	SED	Sig
Species (SP)	2	0.112	***
Fluridone (F)	2	0.112	*
SP x F	4	0.193	NS
Residual	199 (33)		

Dark and 16h photoperiod treatments analysed separately

	DARK			16h PHOTOPERIOD		
	d.f. (m.v.)	SED	Sig	d.f. (m.v.)	SED	Sig
Species (SP)	2	0.152	***	2	0.159	NS
Fluridone (F)	2	0.152	NS	2	0.159	NS
SP x F	4	0.263	NS	4	0.276	NS
Residual	90 (22)			101 (11)		

Stimulation of bulblet growth

Sucrose and charcoal on MS basal medium

Bulblet growth was greatly stimulated by the addition of activated charcoal to the culture medium (Table 1.9 and Figure 1.3A, B and C). In contrast, doubling the amount of sucrose in the medium to 60g/l had only marginal effect on bulblet growth in the absence of charcoal with all three snowdrop types. Addition of activated charcoal and sucrose together was also found to have a synergistic effect on bulblet growth, as indicated by the significant interaction between these two factors. In fact the benefit of adding 5g/l charcoal as opposed to 1g/l charcoal could only be seen at the higher sucrose concentration with all the bulb growth parameters assessed.

Bulblets produced by addition of extra sucrose and activated charcoal were found to have a normal looking anatomy in longitudinal section and they formed up to three scale leaves per bulb (Figure 1.3D). Although many of the bulbs were sprouting and producing new leaves, and even lateral bulb units developed in larger bulbs, none formed flowers *in vitro*. Thus it would be expected that *in vitro* produced bulbs would require further treatment(s) to stimulate flower initiation or a minimum of one season in field conditions to achieve flowering size.

Activated charcoal also induced the formation of large numbers of roots in the bulblet clumps and stimulated root elongation (Table 1.10 and Figure 1.3E). As with bulblet growth, benefits of adding the higher level of charcoal (5g/l) were only seen when the sucrose concentration was also increased to 60g/l. There was also a strong interaction between snowdrop type and charcoal concentration for root formation (both for root numbers and length of the longest root). This interaction was caused by *G. nivalis* Flore Pleno reacting differently to the other two species in response to charcoal addition. Both *G. nivalis* and *G. elwesii* continued to show increased root development with increasing charcoal concentration, whilst with *G. nivalis* Flore Pleno addition of 1g/l charcoal was optimal and further addition of charcoal to 5g/l was supra-optimal.

Table 1.9. Bulblet growth characteristics in the first bulblet growth experiment examining the effects of sucrose and activated charcoal concentration. Cultures were maintained on full-strength MS medium without plant growth regulators.

Species	Sucrose (g/l)					
	30			60		
Charcoal (g/l)	0	1	5	0	1	5
Bulblet multiplication						
G. nivalis	1.44	1.39	1.51	1.71	1.66	1.89
Flore Pleno	2.28	1.95	1.96	1.97	1.87	1.53
G. elwesii	1.47	1.45	1.53	1.51	1.40	1.06
Charcoal x sucrose means	1.73	1.60	1.70	1.73	1.64	1.49
Total fresh weight of the bulblet clump (g)						
G. nivalis	0.25	0.40	0.40	0.11	0.25	1.01
Flore Pleno	0.38	1.16	0.98	0.28	1.03	1.04
G. elwesii	0.25	1.14	0.64	0.67	0.60	2.27
Charcoal x sucrose means	0.29	0.90	0.67	0.35	0.63	1.44
Fresh weight of the largest bulblet (g)						
G. nivalis	0.06	0.18	0.18	0.03	0.05	0.19
Flore Pleno	0.07	0.26	0.17	0.05	0.22	0.42
G. elwesii	0.10	0.43	0.28	0.17	0.24	1.37
Charcoal x sucrose means	0.08	0.29	0.21	0.08	0.17	0.66
Diameter of the largest bulblet (mm)						
G. nivalis	3.76	5.39	5.14	3.48	4.20	5.82
Flore Pleno	3.64	5.69	4.54	3.94	5.84	7.59
G. elwesii	3.92	6.38	5.99	4.76	5.42	9.13
Charcoal x sucrose means	3.77	5.82	5.22	3.91	5.15	7.51
Diameter of the second largest bulblet (mm)						
G. nivalis	2.15	2.90	3.54	2.52	3.37	4.56
Flore Pleno	2.81	4.60	3.46	2.67	4.33	5.17
G. elwesii	2.76	4.14	3.97	3.15	3.85	4.64
Charcoal x sucrose mean	2.57	3.88	3.66	2.78	3.85	4.79

Analysis of variance summary

	d.f (m.v.)	Multiplication		Fresh weight				Diameter			
				Total		Largest bulb		Bulblet 1		Bulblet 2	
		SED	sig	SED	sig	SED	sig	SED	sig	SED	sig
SPECIES (SP)	2	0.241	NS	0.055	***	0.033	***	0.212	***	0.171	*
SUCROSE (SU)	1	0.197	NS	0.045	**	0.027	**	0.173	*	0.140	*
CHARCOAL (C)	2	0.241	*	0.055	***	0.033	***	0.212	***	0.171	***
SP x SU	2	0.341	NS	0.078	***	0.047	***	0.300	NS	0.242	NS
SP x C	4	0.418	NS	0.096	***	0.057	***	0.367	NS	0.297	NS
SU x C	2	0.341	NS	0.078	***	0.047	***	0.300	***	0.242	*
SP x SU x C	4	0.591	**	0.135	***	0.081	***	0.519	NS	0.419	NS
Residual	229 (9)										

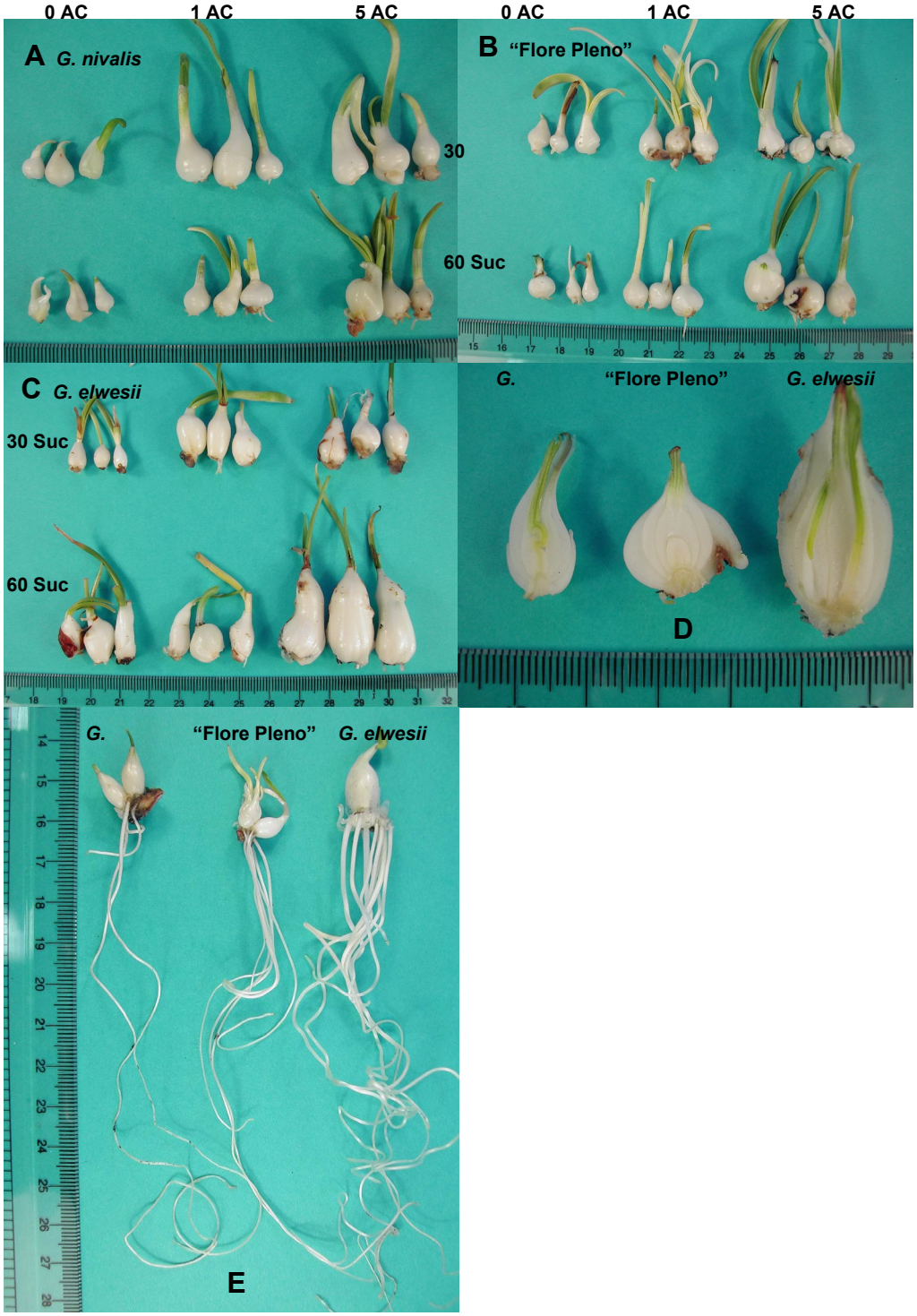
Table 1.10. Rooting in the first bulblet growth experiment examining the effects of sucrose and activated charcoal concentration. Root numbers were normalised by square root transformation before analysis but untransformed data are shown in parenthesis.

Species	Sucrose (g/l)					
	30			60		
Charcoal (g/l)	0	1	5	0	1	5
Root numbers per bulblet clump						
G. nivalis	0.02	1.37	0.99	0.00	0.88	1.84
	(0.08)	(2.40)	(1.47)	(0.00)	(1.13)	(3.73)
Flore Pleno	0.41	3.00	2.56	0.46	2.91	2.63
	(0.65)	(9.80)	(7.67)	(0.73)	(9.27)	(7.80)
G. elwesii	0.13	1.02	1.44	0.57	1.07	1.95
	(0.13)	(1.87)	(3.73)	(1.00)	(2.13)	(5.07)
Charcoal x sucrose means	0.19	1.80	1.66	0.34	1.62	2.14
	(0.29)	(4.69)	(4.29)	(0.55)	(4.18)	(5.53)
Length of longest root (mm)						
G. nivalis	1.9	37.7	19.1	0.0	17.5	65.3
Flore Pleno	6.4	186.5	139.5	0.8	145.3	135.4
G. elwesii	0.4	71.5	90.2	3.9	53.1	114.6
Charcoal x sucrose means	2.9	98.5	82.9	1.2	72.0	105.1
Charcoal x species interaction	**Root numbers**			**Longest root length (mm)**		
G. nivalis	0.00	1.12	1.41	0.00	27.6	42.2
Flore Pleno	0.43	2.95	2.60	3.6	165.9	137.4
G. elwesii	0.35	1.05	1.69	2.2	62.3	102.4

Analysis of variance summary

	d.f (m.v.)	Root number		Root length	
		SED	sig	SED	sig
SPECIES (SP)	2	0.090	***	6.15	***
SUCROSE (SU)	1	0.073	NS	5.02	NS
CHARCOAL (C)	2	0.090	***	6.15	***
SP x SU	2	0.127	NS	8.70	NS
SP x C	4	0.156	***	10.65	***
SU x C	2	0.127	*	8.70	*
SP x SU x C	4	0.220	NS	15.07	NS
Residual	229 (9)				

Figure 1.3 Stimulation of bulblet growth by activated charcoal (AC) supplied at either 1 or 5 g/l in combination with 30 or 60 g/l sucrose (SUC) with three snowdrop types (A, B and C). Bulblets of all snowdrop types were normal looking in longitudinal section, producing up to three scale leaves, except that no flowers were formed even in the largest bulbs (D). Extensive root development was also stimulated by AC (Figure E shows bulblet clumps on 5g/l AC and 60 g/l SUC treatment). All scales are in mm.



Sucrose and charcoal on half-strength G basal medium

Initial observations indicated that *G. nivalis* is forming rooted bulblets on the half-strength G based medium, and some genotypes are rapidly forming large well developed bulbs even on the control medium where neither sucrose is elevated nor charcoal added (Figure 1.4). Interestingly, *G. nivalis* Flore Pleno bulblet clumps are continuing to multiply rapidly on all of the same media and are failing to form enlarged bulbs (Figure 1.5). This contrasts with the previous experiment where *G. nivalis* Flore Pleno bulblets readily grew into enlarged bulbs on a full-strength MS based medium. Further investigation is therefore needed comparing MS with G media at varying strengths in a single experiment with both snowdrop types.

Figure 1.4. Rapid growth of *G. nivalis* bulblets on half-strength G medium with varying levels of sucrose (SUC g/l) with and without 5 g/l activated charcoal. The boiling tubes shown have a 21mm i.d. and cultures were inoculated 3 months previously with bulblets about 1mm in diameter.

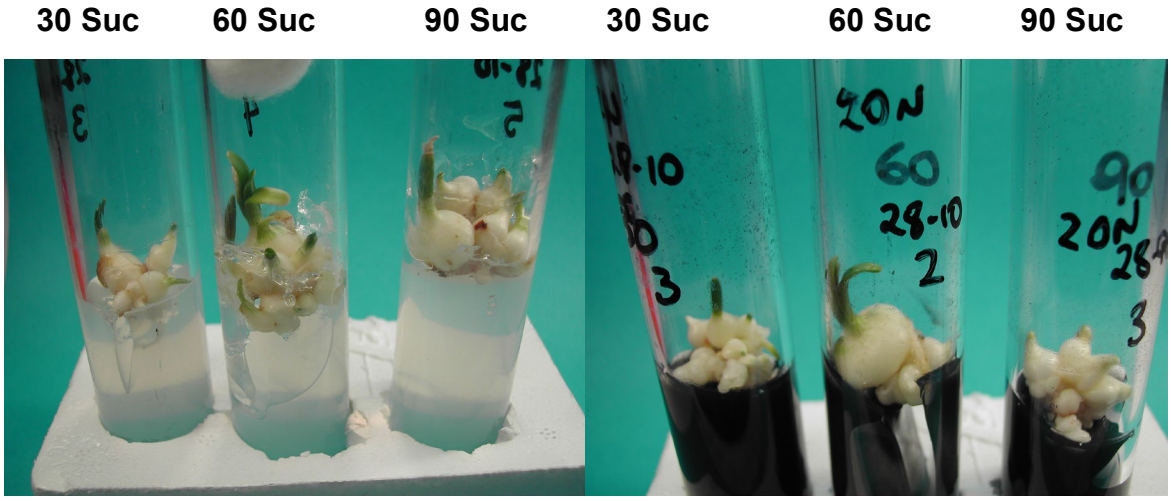
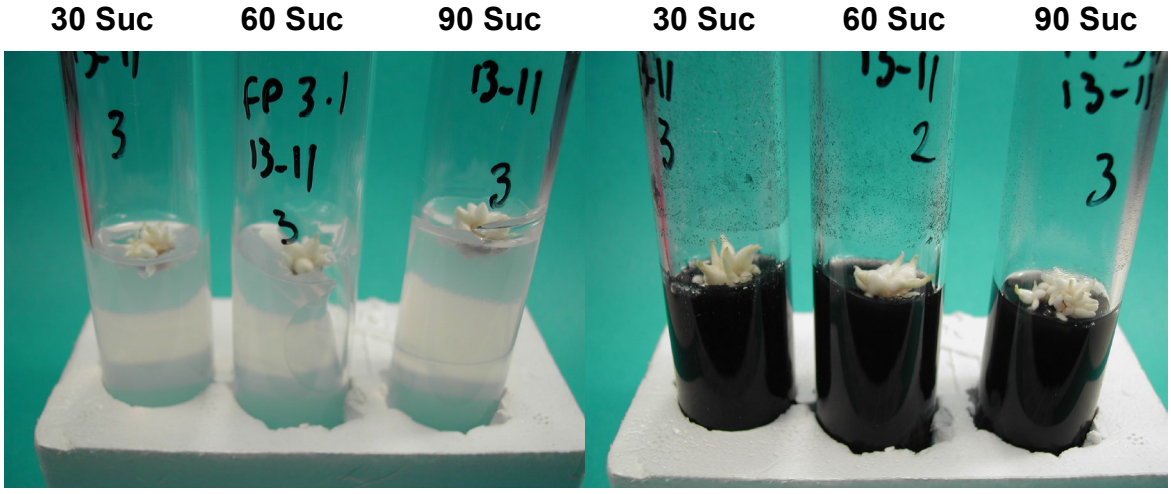


Figure 1.5. Poor growth of *G. nivalis* Flore Pleno bulblets on half-strength G medium with varying levels of sucrose (SUC g/l) with and without 5 g/l activated charcoal. The boiling tubes shown have a 21mm i.d. and cultures were inoculated 2.5 months previously with bulblets about 1mm in diameter.



Cold treatment

These results will be reported in the next report.

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Agronomy

Materials and methods

Plant material

Forty thousand bulbs of 'field-grown' *Galanthus nivalis*, grade 4–5 cm circumference, were purchased in August 2000 from a UK supplier. They were stored in net bags in a non-lit store at 15°C and about 70% relative humidity until planted. Since the bulbs supplied were somewhat variable, the largest and soundest were selected for experiment 1, the next best for experiment 2, and the remaining bulbs were planted as stock. At planting, bulb samples were taken and examined for the presence of *Botrytis galanthina* by Cheryl Brewster (then at HRI Stockbridge House). About 10% of the bulbs had *Botrytis* sclerotia. Difficulties were encountered in culturing *Botrytis* from the bulbs, because of the overwhelming presence of *Penicillium*, and it was not practical to confirm the presence of *B. galanthina*.

Further bulbs (*G. nivalis* and *G. elwesii*) were bought and planted in 2002, but were not used in experiments until 2003.

General methods for field experiments

Field trials were set up in 2000 at HRI Kirton, Lincolnshire, in an open field situation typical of the South Lincolnshire area. The soil was a coarse silty marine alluvium. The previous crop was barley (giving a MAFF N index of 0). Before use the field was ploughed and cultivated. Standard agricultural soil analysis revealed the following levels: pH 7.7, P index 4, K index 3 and Mg index 4. Conforming to MAFF fertiliser recommendations for bulbs, no additional fertilisers were applied pre-planting, but N (70kg/ha) was applied in winter. Because of the precocity of the crop, the nitrogen application was made carefully by hand along the beds on 8 January 2001.

The layout of trials was based on 1.200m-wide beds separated by 0.600m-wide pathways, allowing for tractors on 1.830m 'wheelings' working over the beds. The beds were aligned approximately north-west to south-east, at right angles to the prevailing south-westerly winds. The beds were marked in and cultivated, and the position of plots was marked with canes and labels. Along each bed, the plots were 2.475m-long and were separated by 0.825m-long unplanted 'guard' areas. 204 bulbs were planted in each plot, 5-10cm deep and in six rows along the beds, with between-bulb spacings of 7.5cm between and within the rows (making a planted area 37.5cm wide across the bed). The bulbs in each plot comprised three, 60-bulb sub-plots, each sub-plot being separated by a row of six 'guard' bulbs. The sub-plots allowed for sampling in each of three years of the experiment. Bulbs were planted by hand using trowels on 25-27 September 2000. After planting, the area was raked over to tidy and consolidate the soil. When the experimental plots were planted, further bulbs were planted in rows in the same field to provide stocks for storage experiments; these received the same routine husbandry treatments as the experimental plots except that some areas were sprayed with herbicides to determine suitable materials.

Electric fencing and bird scarers were set up to deter predators. After planting, herbicide (diquat + paraquat) was applied across the whole area. After crop emergence, herbicide was applied carefully to pathways only using a knapsack sprayer, applying cyanazine 'pre-emergence' in early-December 2000, and chlorpropham + linuron 'post-emergence' in early-January 2001. The planted areas were maintained weed-free by hand weeding as required. A regular fungicide spray programme, consisting of alternating vinclozolin, iprodione and dichlofluanid, was applied. All pesticides listed in this report were applied at standard rates. Since seed pods were to be collected and assessed each year from the appropriate sub-plots (see below), seed pods from other sub-plots were removed in June 2001 in order not to confound plant counts in subsequent years.

After the snowdrop foliage had died down in spring/summer 2001, the trials area was made tidy, irrigation and shading were checked, etc. The following treatments were applied across the whole area: methiocarb (slug pellets, 5 November 2001), thiram (as a moss killer, 6 November 2001), and diquat + paraquat (13 November 2001). The pathways were treated with cyanazine herbicide on 19 November 2001, weed control in plots being by hand. Post-emergence herbicide and fungicide applications were applied as before. Similar procedures were used in 2002-2003.

Agronomy experiment 1: The effect of shade, shelter and soil moisture treatments

Plots were set up with three treatment factors:
(a) Shading: either no shading (control) or shaded with green polyethylene mesh (Netlon Agroshade), either 40, 50 or 70% shade factor
(b) Windbreak: either no shelter, or plot sheltered on one side by black polyethylene mesh (Netlon Tensar Windbreak, 55% protection factor)
(c) Plots either (1) irrigated and mulched or (2) neither irrigated nor mulched

There were thus 16 treatment combinations (four shading levels x two windbreak levels x two irrigation/mulching levels). The layout was a lattice square design for the 16 treatments in five squares, each of four rows and four columns; the one-, two- and three-year sub-plots were allocated randomly within plots. Shading consisted of a single layer of mesh stretched horizontally over the plots, 45cm above ground level. Windbreak consisted of a single layer of mesh (35cm high) held vertically on the windward (south-west) side of the plots 15cm from the edge of the planted area. Mulching consisted of a layer of straw about 5cm deep placed over the planted area in November before emergence. Irrigation was provided from one line of drip tape (T-Tape, T-Systems International Inc, specification TSX 510-15-1000) placed centrally along the planted area. Runs of drip tape were connected across non-irrigated plots by plain pipe. Shading, windbreak, mulching and irrigation extended beyond the appropriate plot, halfway into the adjacent guard areas.

Due to consistently wet weather, irrigation was not necessary during the 2000-2001 or 2001-2002 growing seasons. In summer 2001, following complete die-down of the foliage, the remaining straw mulch was removed. It was replaced in early-November 2001. Similar procedures were followed in 2002-2003.

Agronomy experiment 2: The effect of shading and inter-cropping

Plots were set up with six treatments:
(1) Control (no shading, no inter-cropping)
(2) Shading using 40% shade factor mesh, as above
(3) Shading using 70% shade factor mesh, as above
(4) One row of narcissus bulbs (cv. Carlton, 12-14cm grade) planted 15cm deep along each side of the planted area, 15cm from the edge of the planted area and using 26 bulbs per m, after planting snowdrop bulbs
(5) One row of spring barley sown along each side of the planted area, 15cm from the edge of the planted area after planting bulbs
(6) Planting area over-sown with perennial rye-grass after planting bulbs, extending 15cm beyond the edge of the planted area

The layout was a balanced row and column design with five replicates; the one-, two- and three-year sub-plots were allocated randomly within the plots. As in Experiment 1, shading and inter-crop plantings extended beyond the appropriate plot, halfway into the adjacent guard areas. The initial barley sowing was lost due to predation, and so replaced by a further sowing and also by transplanting of module-raised seedlings.

In October 2001 any remaining barley was removed and wheat was sown in its place. In November 2001 the rye-grass was cut close to ground level using a strimmer. Narcissus bulbs were left in place. Similar procedures were followed in 2002-2003.

Agronomy experiments - crop records

Crop production was assessed on one sub-plot (60 planted bulbs) of each plot annually for three years starting in 2001. Seed pods were left *in situ* until ready for collection. The following records were made on each sub-plot:

- Number of shoots (February 2001 and January 2002; recorded only from non-mulched plots in 2002 to avoid crop damage)
- Number of flower stems (February 2001 and 2002)
- Percentage of foliage die-back (24 May 2001)
- Number of seed pods and seeds (June 2001 and 2002)
- Number and weight of bulbs <4 cm and >4 cm circumference (after lifting, cleaning and lightly surface-drying bulbs in July 2001 and 2002)

Environmental data

The following parameters were logged at 30-minute intervals during the growing season in representative plots:

- Soil temperature at bulb depth (Delta-T TM1 sensor)
- Air temperature at median leaf height (Delta-T TM1 sensor)
- Soil moisture at bulb depth (Delta-T M1M sensor ('Theta probes')
- Level of photosynthetically active radiation (PAR) at mid leaf height (Delta-T QS sensor)

Occasional obviously erroneous readings from the logger (due to equipment malfunctions) were deleted. Soil moisture at bulb depth was additionally recorded using Irrometer 'water sensors' checked at about weekly intervals.

Results and discussion

Main experiment

The results for the first year of the experiment, 2000-2001, were presented in the previous annual report. In that year, and despite initially uniform planting, fewer shoots emerged in the non-sheltered plots than in sheltered plots (suggesting that wind damage caused some loss of foliage), and fewer in mulched than in non-mulched plots (suggesting that the presence of the straw mulch impeded the emergence of weaker shoots). In the second year, 2001-2002, the main effect on shoot numbers was due to shading, with more shoots emerging in shaded plots than in non-shaded controls (Table 2.1). Presumably this reflected the overall better growth under shading seen in the first growing season.

In the first year there were no significant effects of treatments on the numbers of flower stems, which was as expected since flower initiation in snowdrops would have been determined in the previous summer. There were only minor significant effects of treatments in the second year (Table 2.1). There was a significant effect (at the 5% level of probability) of shading on the numbers of flower stems produced, with fewer stems in non-shaded than shaded plots. Minor significant interactions (at the 5 or 10% level) on stem and seed pod numbers were due to particularly weak growth in plots that had neither shade, shelter nor mulch. For example, the mean stem number was 36.4 per sub-plot (60 bulbs planted) for this treatment, compared with a grand mean across all treatments of 48.2. As in the case of shoot numbers, these results reflected the benefits to snowdrop growth of, particularly, shading in the first year.

In the first year, bulb yields (numbers and weights) were consistently higher in shaded than in non-shaded plots, although this effect did not always achieve statistical significance and there was, surprisingly, no clear 'dose-response' relationship between bulb yields and shading density, which will be further investigated. Results for the second year (Table 2.2) confirmed the beneficial effects of shading on bulb yield (at the 10% level of probability) and, especially, on the mean weight of harvested bulbs (1% level). There were more significant benefits of mulching on bulb yield, mulching producing higher yields in the larger bulb grade (5% level or better) and higher mean bulb weights (0.1% level). Statistically significant second-order interactions (i.e. between shading, shelter and mulch; 10% level or better) resulted from:

- Very poor yields in plots with neither shading, shelter nor mulch; and
- Very high yields in plots with mulch but neither shade nor shelter (Figure 2.1).

It was clear that the beneficial effects of shading and mulching, seen in the previous year, were being compounded by a further year's treatment. Without other protection, such as a mulch, natural light intensities on this exposed site were unfavourable to bulb growth. Simple shading and mulching were beneficial but, in this situation, a side windbreak appeared to provide little extra benefit.

Figure 2.2. shows bulb yields after one and two growing seasons. The fact that yields have declined from year one to year two in the absence of mulch (except where dense shading was used), may explain why attempts at growing *Galanthus nivalis* in a field situation may have

failed. In this case, the best bulb increases were obtained where a mulch had been used. Mulching could reduce reliance on herbicides. As shading appeared to work by prolonging the growing season (see first annual report), this might also be achieved through fungicide spray programmes that delay foliar senescence. Fungicide sprays will be investigated in a further trial in 2002-2003.

Table 2.1. Effects of shading, windbreak and mulch and irrigation treatments on snowdrop growth in the second year (2002). To avoid crop damage, the numbers of emerging shoots were not counted in mulched plots. Main effect means only presented.

Factors and treatments	Numbers per sub-plot (60 bulbs planted)			
	Shoots	Flower stems	Seed-pods	Seeds
Shading				
None	32.8	43.8	15.9	107
Light	45.6	50.6	18.8	166
Medium	42.2	45.8	18.1	152
Dense	46.0	52.5	14.3	105
SED (60 d.f)	-	3.12	2.67	35.9
SED (28 d.f)	4.40	-	-	-
Windbreak				
None	39.5	48.7	16.0	135
Yes	43.7	47.7	17.5	131
SED (60 d.f.)	-	2.20	1.88	25.4
SED (28 d.f.)	3.11	-	-	-
Mulch and irrigation				
None	-	49.6	15.2	113
Yes	-	46.8	18.3	152
SED (60 d.f.)	-	2.20	1.88	25.4
Analysis of variance summary [a]				
Shading (S)	*	*	NS	NS
Windbreak (W)	NS	NS	NS	NS
Mulch and irrigation (M)	-	NS	NS	NS
S x W	NS	NS	NS	NS
S x M	-	NS	(*)	NS
W x M	-	NS	NS	NS
S x W x M	-	*	NS	NS

[a] NS, not significant; (*), *, ** and ***, significant at the 10, 5, 1 and 0.1% levels of probability, respectively. These significance levels indicate that the observed differences between treatment means could have arisen by chance in 1 out of 10, 1 out of 20, 1 out of 100 and 1 out of 1000 occasions, respectively.

Table 2.2. Effects of shading, windbreak and mulch and irrigation treatments on snowdrop bulb yield in the second year (2002). Main effect means only presented.

Factors and treatments	Bulb yields per sub-plot (60 bulbs planted)						Mean bulb weight (g)
	Numbers			Weight (g)			
	< 4cm	> 4cm	Total	< 4cm	> 4cm	Total	
Shading							
None	19.5	22.6	42.1	21.0	72.2	93.2	1.94
Light	26.0	26.7	52.7	30.9	89.7	120.6	2.24
Medium	20.8	20.9	41.6	24.8	75.1	99.9	2.35
Dense	19.5	30.7	50.2	22.2	109.1	131.3	2.53
SED (60 d.f)	3.05	4.16	5.20	4.39	15.99	17.14	0.155
Windbreak							
None	22.2	25.3	47.5	25.7	88.5	114.2	2.28
Yes	20.7	25.2	45.9	23.8	84.5	108.3	2.25
SED (60 d.f.)	2.16	2.94	3.68	3.11	11.30	12.12	0.109
Mulch and irrigation							
None	20.8	21.7	42.5	23.1	68.7	91.8	2.07
Yes	22.1	28.8	50.9	26.4	104.3	130.7	2.46
SED (60 d.f.)	2.16	2.94	3.68	3.11	11.30	12.12	0.109
Analysis of variance summary							
Shading (S)	NS	(*)	(*)	NS	(*)	NS	**
Windbreak (W)	NS	NS	NS	NS	NS	NS	NS
Mulch and irrigation (M)	NS	*	*	NS	**	**	***
S x W	**	NS	NS	**	NS	NS	NS
S x M	NS	NS	NS	NS	NS	NS	NS
W x M	NS	NS	NS	NS	NS	NS	NS
S x W x M	NS	**	**	NS	**	*	*

Figure 2.1. Snowdrop bulb yields after 2 years of treatment

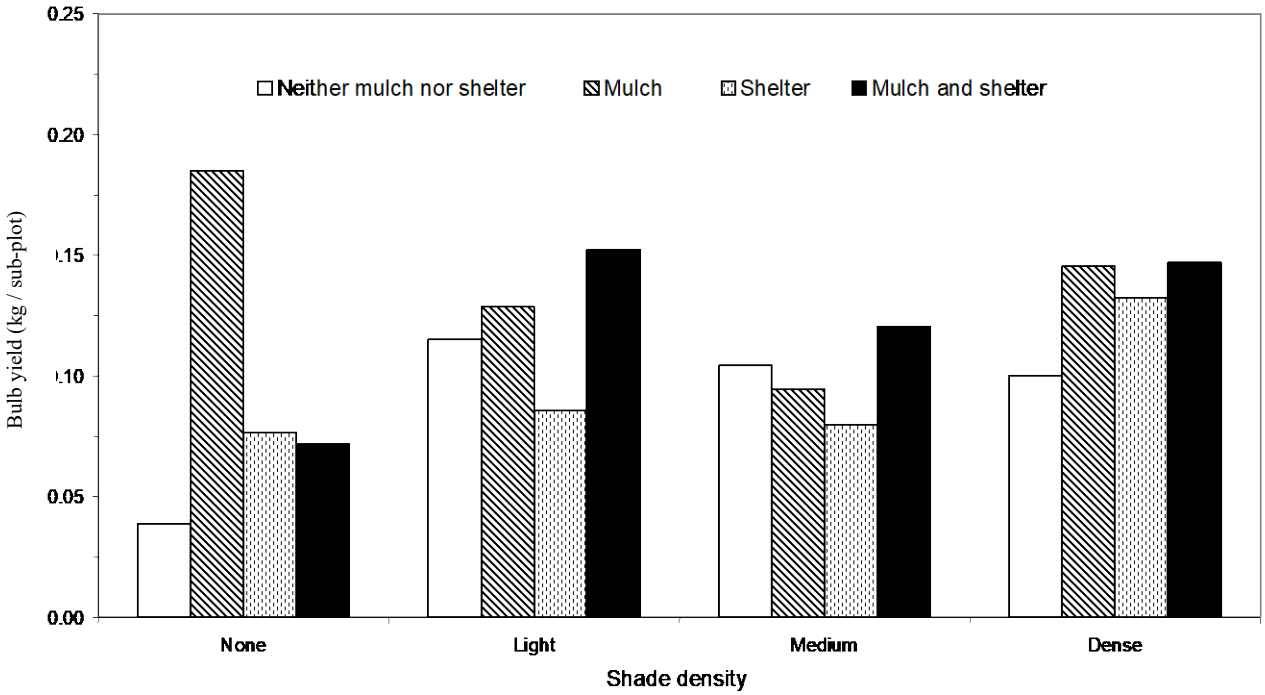
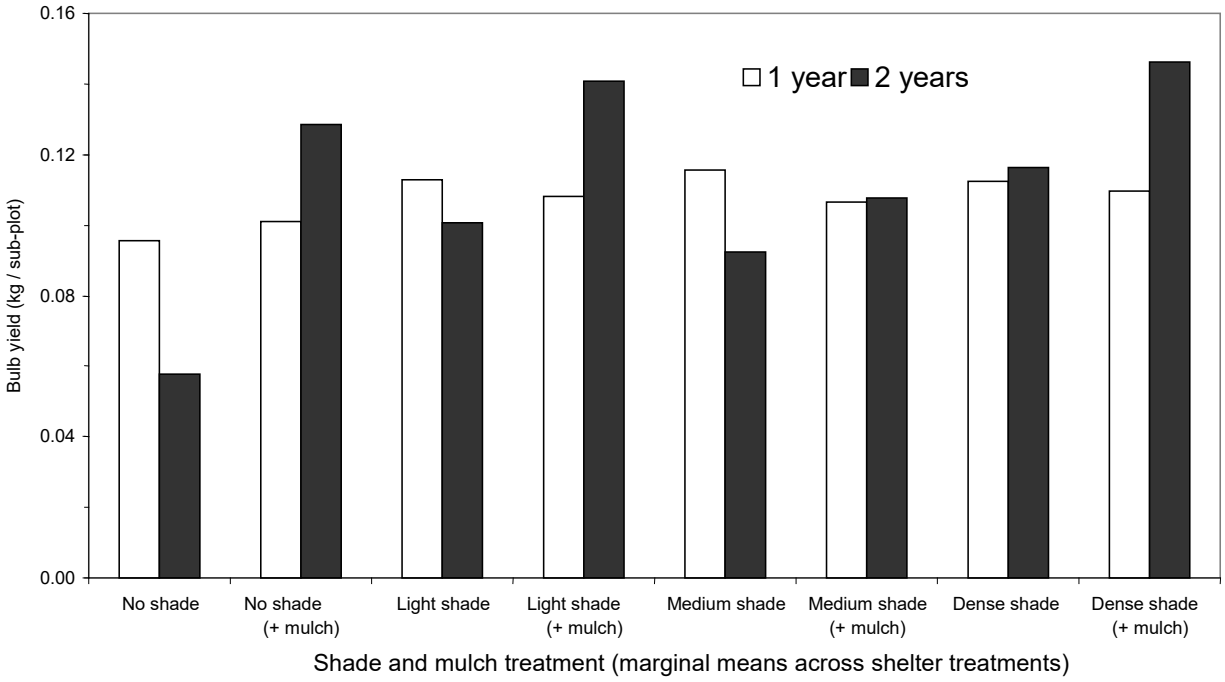


Figure 2.2. Snowdrop bulb yields after 1 and 2 years



Inter-crops experiment

In the first year of the experiment there had been no significant effects of treatment on the numbers of shoots, flower stems, seed pods or seeds obtained per sub-plot. In the second year the results showed that the adverse or beneficial effects of treatments on growth in the previous year were continuing to show clear effects on plant performance (Table 2.3). There were significant effects of treatment (at the 5% level), with best yields of stems and seeds under the medium-density shade, and much poorer yields where inter-crops, and especially over-sown rye-grass, had been used.

As in the first year, in the second there was a significant effect of treatment on total bulb yields and yield of the larger bulbs (at the 5% level or better), but not on the yield of smaller bulbs (Table 2.4). Corresponding with the effects on stem and seed production (see above), the best yields were produced from plots under the higher density shade, with poorer yields from the inter-cropped plots. However, bulb yields (and the mean weight of harvested bulbs) were greater from the rye-grass plots than from the narcissus or cereal plots, indicating a marked recovery of the snowdrops over-sown with rye-grass, which were very poor in the first year. However, early indications in the third growing season (in January 2003) confirmed an improvement in bulb growth in the rye-grass plots.

The generally weaker growth of bulbs in this experiment was due to the better bulbs being used for experiment 1 (see Materials and Methods).

In general these results confirm the beneficial effect of artificial shading on growth, seen in the main experiment. The effect of inter-cropping, which from the first year's results appeared too competitive or otherwise unsuitable, needs a further year's observation.

Table 2.3. Effects of shading and inter-cropping on snowdrop growth in the second year, 2001-2002.

Treatments	Numbers per sub-plot (60 bulbs planted)		
	Flower stems	Seed pods	Seeds
None	51.0	14.0	61.0
Light shading	49.8	11.0	50.6
Medium shading	56.6	13.6	84.8
Narcissus intercrop	46.2	9.4	41.0
Cereal intercrop	40.2	9.4	45.6
Rye-grass oversown	35.2	7.4	22.4
SED (20 d.f.)	5.75	1.85	14.96
Significance	*	*	*

Table 2.4. Effects of shading and inter-cropping on snowdrop bulb yield in the second year, 2001-2002.

Treatments	Bulb yields per sub-plot (60 bulbs planted)						Mean bulb weight (g)
	Numbers			Weight (g)			
	< 4cm	> 4cm	Total	< 4cm	> 4cm	Total	
None	24.2	20.6	44.8	25.0	62.4	87.4	1.98
Light shading	29.0	18.6	47.6	32.7	56.0	88.8	1.84
Medium shading	24.0	30.4	54.4	27.7	92.9	120.6	2.04
Narcissus inter-crop	24.0	8.4	32.4	23.0	23.3	46.3	1.40
Cereal inter-crop	15.2	8.8	24.0	16.8	23.6	40.4	1.55
Rye-grass over-sown	20.0	15.4	35.4	21.7	46.3	67.9	1.93
SED (20 d.f.)	4.62	6.30	9.58	4.96	18.36	20.84	0.247
Significance	NS	*	*	(*)	**	**	(*)

Meteorological data

Over the 2002 growing period the medium shading reduced mean PAR by about 20% and heavy shading by about 30%, compared with non-shaded controls (Table 2.5).

Table 2.5. Effect of shading on PAR over the 2002 growing season.

	PAR (mmol)		
Treatment	Mean	Mean as % of control	Maximum
Control[a]	0.089	100.0	1.750
Shade (light)	0.092	103.4	1.194
Shade (medium)	0.071	79.8	1.069
Shade (heavy)	0.064	71.9	1.129

[a]Control has no shade, windbreak or mulch

All plots remained very wet during the 2002 season, with only minor differences in mean soil moisture between the treatments.

Unlike the previous year, air temperatures differed little between the various treatments (Table 2.6).

Table 2.6. Effect of various treatments on air temperatures over the 2002 growing season.

	Air temperature (°C)		
Treatment	Minimum	Mean	Maximum
Control	-5.9	8.2	25.8
Mulch	-5.3	8.2	24.9
Shade (heavy) + windbreak	-5.4	8.3	24.8
Shade (heavy) + windbreak + mulch	-5.0	8.2	24.6

Compared with control plots and those with a windbreak only, mean soil temperature in other plots (with shading, mulch or combinations of treatments) was slightly lower (Figure 2.3). Figure 2.4 shows how the treatments affected the range of soil temperatures (i.e. the range from minimum to maximum recorded soil temperatures over the growing season). The temperature range was highest in control plots and in plots with windbreak only or cereal or narcissus inter-cropping, and lowest in plots with shading plus mulch. These temperature patterns were somewhat dissimilar to the previous year, and the implications of this will be considered when the full three years' data are available.

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Fig. 2.3. Mean soil temperature through 2002 growing season

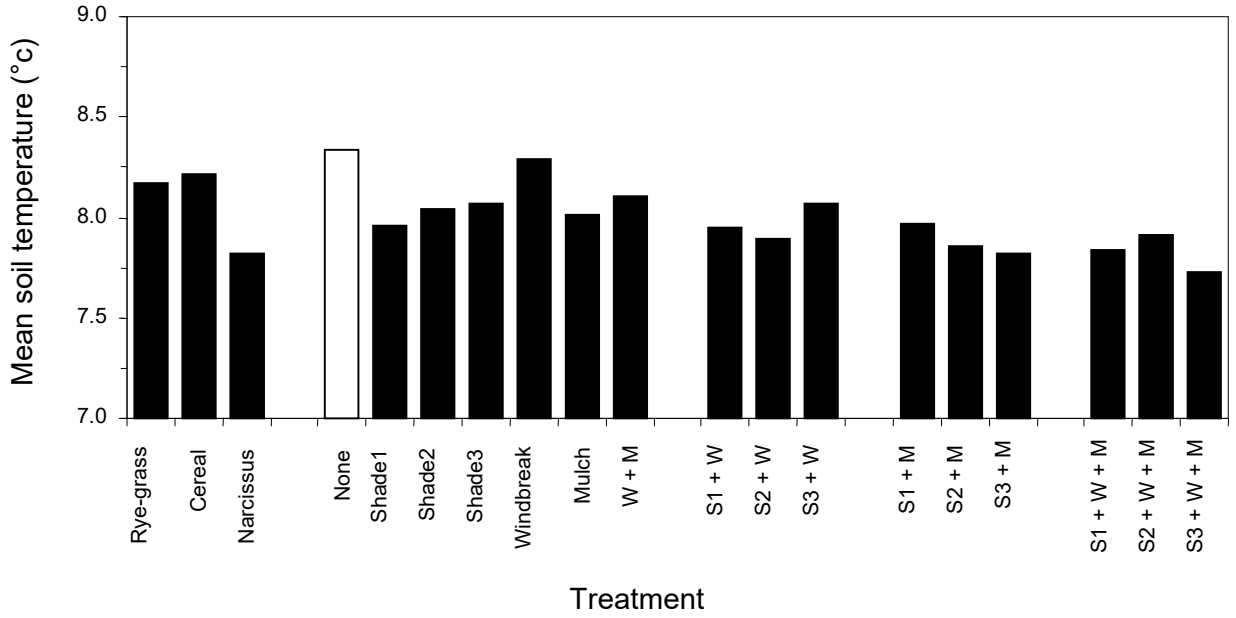
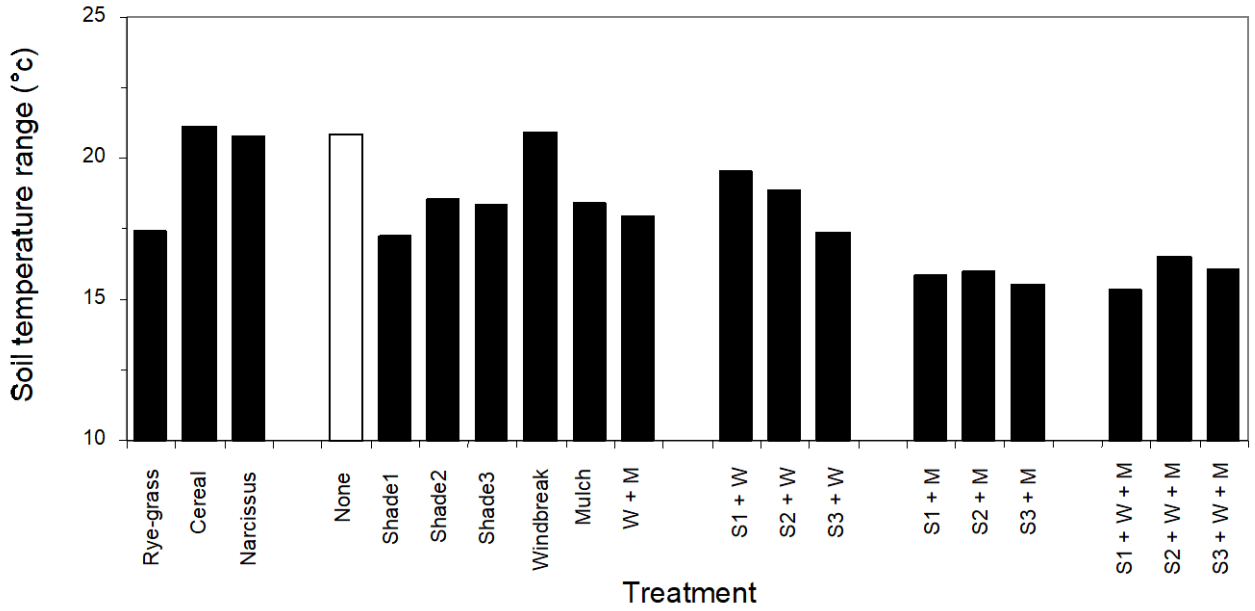


Fig. 2.4. Soil temperature range through 2002 growing season



Bulb Storage

Materials and methods

Stock bulbs of *G. nivalis*, planted in 2000, were lifted for use in a storage experiment on 27 March 2002 (foliage still fully green), 23 April 2002 (foliage in early stages of senescence) and 15 May 2002 (foliage completely died down). About 500 bulbs were lifted on each date.

On the day of lifting, bulbs were dipped in aqueous benomyl + captan (as 40g Benlate Fungicide + 100g Captan per 10 litres) for 15 minutes at ambient temperatures, and allowed to drain overnight at ambient temperatures under gentle air movement from a fan to produce surface-dry bulbs by the next morning. The remaining foliage was excised at the top of the bulb, and any damaged bulbs or bulbs falling outside the range 3-5 cm in circumference were discarded. Ninety lots of five bulbs each were allocated at random and weighed. Nine lots were allocated to and set up in each of ten treatments:

Treatment no.	Storage temperature (°C)	Storage medium	Typical humidity (% r.h.)
1	10	Silver sand in polythene bag	-
2	13	Silver sand in polythene bag	-
3	17	Silver sand in polythene bag	-
4	20	Silver sand in polythene bag	-
5	13	Open seed tray	70
6	17	Open seed tray	70
7	13	Polythene bag (loosely folded)	90
8	17	Polythene bag (loosely folded)	90
9	13	Perspex propagator with damp paper	>90
10	17	Perspex propagator with damp paper	>90

The bags, trays and propagators were placed in controlled temperature rooms at the required temperatures and 65-75 % r.h.

Three replicate lots of each treatment were removed from the stores after 4, 8 and 12 weeks, and the bulbs weighed. After weighing, the bulbs lifted after 4 or 8 weeks were placed in polythene bags of silver sand in the 13°C store, and storage was continued to a total period of 12 weeks. For statistical analysis, recovered bulb weights were adjusted by using the initial weight (i.e. weight after lifting from the field) as a co-variate.

After the 12-week period any rotted or desiccated bulbs were removed, and the remaining bulbs were counted and planted in a proprietary potting compost (Levington M2) in 14 cm-diameter flower pots and grown in a cold glasshouse (with frost protection heating set at 3°C and automatic ventilation at 10°C). The numbers of bulb shoots and flower stems was recorded for each pot in winter/spring 2002, and the weight of bulbs recovered from each pot will be recorded after complete foliage senescence.

Further bulb storage experiments are under way and will be reported later.

Results and discussion

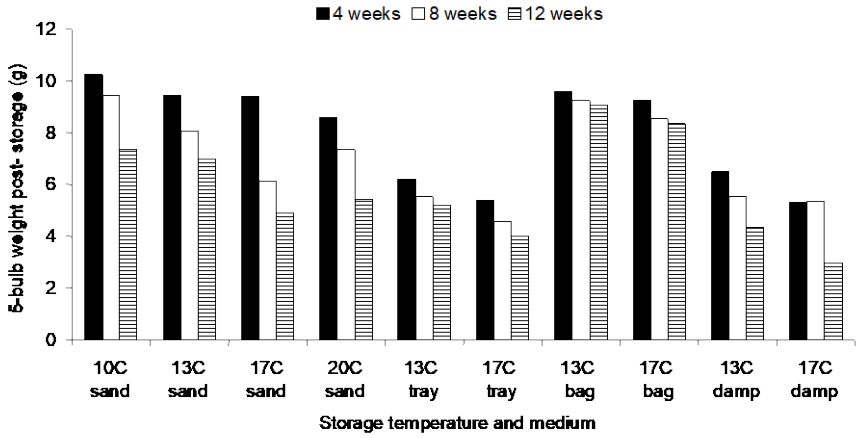
Bulb weight at the end of 4-, 8- or 12-week storage in different temperatures and media, following lifting bulbs at three dates, are shown in Figure 3.1. The effects of all three factors (harvest date, storage temperature and medium, and storage duration) were statistically significant (at the 0.1% level), and the second-order interaction (harvest date x storage temperature and medium x storage duration) was also significant (5% level; for analysis of variance summary, see Table 3.1). Of the three experimental factors, harvest date had the least impact on bulb weight: the overall means for the three dates were 6.9, 7.9 and 7.8g, respectively. Storage in 'damp' conditions was unacceptable due to fungal infection resulting in weight loss, and storage in open trays resulted in much desiccation. Standard storage in silver sand gave reasonable protection from desiccation, but storing bulbs in loosely closed polythene bags gave excellent weight retention, even over 12 weeks' storage. Within the temperature range studied, the lower the storage temperature the better the results. In silver sand and loosely closed polythene bags, storage at 13°C, the usual recommended temperature, was satisfactory.

A variable number of the stored bulbs were deemed suitable for potting and growing-on, any rotted or desiccated bulbs being discarded. For the means of the various treatment combinations, between 67 and 100% of the stored bulbs were deemed suitable for growing-on. These data, along with the subsequent performance of the bulbs, are summarised in Figure 3.2 (for analysis of variance summary see Table 3.1). Statistical analysis showed that storage temperature and medium, but not harvest date nor storage duration, significantly affected both the percentage of bulbs re-planted (at the 0.5% level) and the percentage of these bulbs sprouting in the following spring (0.1% level). The highest percentage of sprouting bulbs was obtained following storage in polythene bags (at either temperature) or in silver sand at 20°C. Figure 3.2 also shows a score that combines the effect of treatments on bulb quality (bulbs re-planted and sprouting): the same three treatments clearly give the best results when expressed in this way. However, it should be noted that the sprouting results are only interim, since, at the time of writing, further shoots are still emerging.

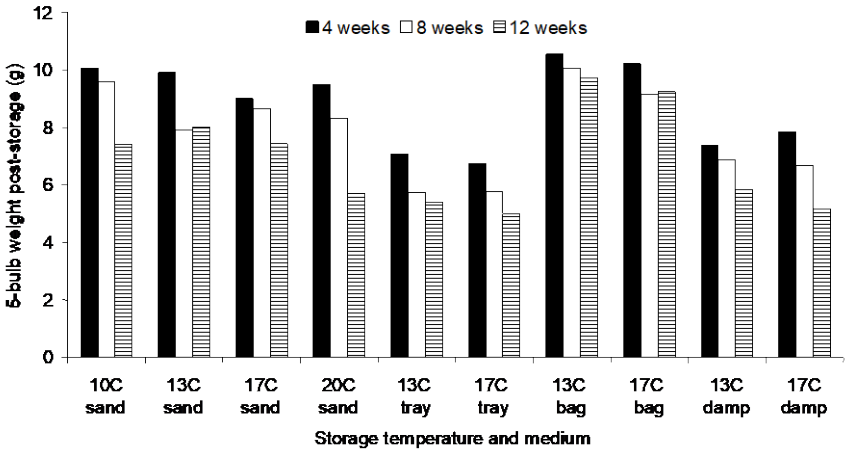
Table 3.1. Analysis of variance table for storage experiment 1.

Factor or interaction	d.f.	Probability		
		Bulb weight after storage (g)	Sound bulbs recovered after storage (%)	Bulbs sprouting (% of planted)
Harvest date (HD)	2	<0.001***	0.581 NS	0.114 NS
Storage temp. and medium (STM)	9	<0.001***	0.006**	<0.001***
Storage duration (SD)	2	<0.001***	0.945 NS	0.052(*)
HD x STM	18	0.001**	0.208 NS	<0.001***
HD x SD	4	0.315NS	0.189 NS	0.900 NS
STM x SD	18	<0.001***	0.285 NS	0.169 NS
HD x STM x SD	36	0.014*	0.902 NS	0.004**
Co-variate (initial bulb weight)	1	<0.001***	-	-

Fig. 3.1. Effect of storage conditions and duration on bulb weight.
(a) First bulb harvest date.



(b) Second bulb harvest date.



(c) Third bulb harvest date.

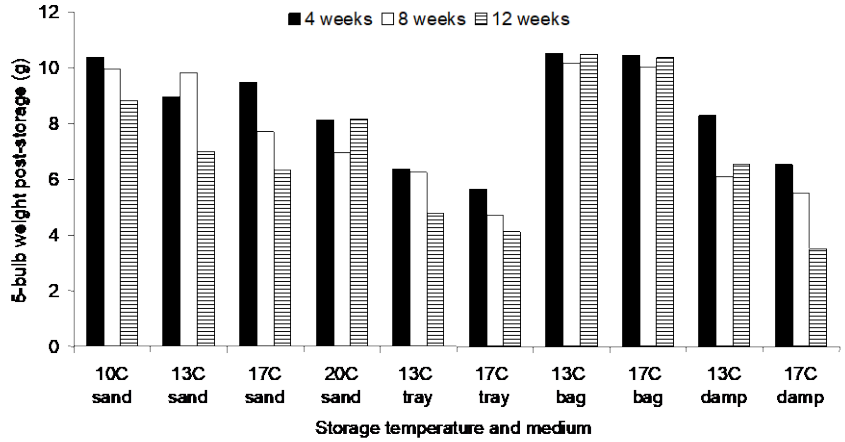
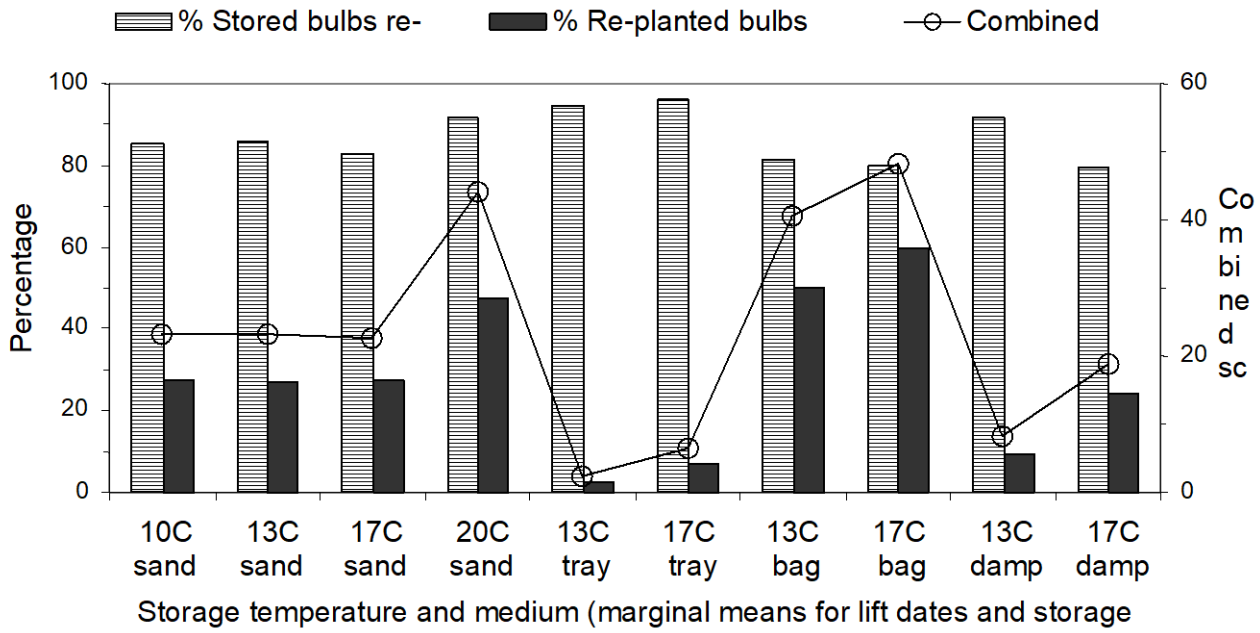


Fig. 3.2. Effect of storage conditions on bulb performance*.

*Sprouting on 27 January 2003; combined score = (% re-planted x %



Conclusions

1. The continuing demand in the Bulb Trade for snowdrop (*Galanthus*) bulbs cannot be met from present sources. Prosecutions for the illegal lifting of snowdrop bulbs are still occurring. Furthermore, existing supplies are often of poor quality, with small, desiccated and diseased bulbs. Supplies in bulk are largely limited to 'ordinary' *G. nivalis*, with the choicer and more varied species and hybrids being highly sought after by galanthophiles but largely unappreciated by the wider gardening public.
2. In setting up the current project, three problems associated with the commercial exploitation of snowdrop bulbs were identified for study: a lack of good quality bulb stocks, the apparent unsuitability of snowdrop bulbs for normal commercial cultivation in the field, and the poor storage characteristics of the bulbs. These problems have been addressed through studies of micropropagation, agronomy and storage methods.
3. An intensive study of the micropropagation of snowdrops is being conducted, involving *G. nivalis*, *G. nivalis* cv Flore Pleno, *G. elwesii*, *G. plicatus* and *G. plicatus* cv Wendy's Gold. The main conclusions so far are:
   3.1. Tissues from all species and cultivars could be successfully sterilised by an ethanol rinse followed by treatments with hypochlorite and 'Plant Preservative Mixture'.
   3.2. Bulblets initiated on outer surfaces of bulb scales and division and transfer to fresh culture medium could readily multiply these, thus forming the basis for rapid propagation of selected bulbs. Bulblet numbers can be increased with *G. nivalis* and *G. elwesii* by individual culture of scale leaf explants as opposed to bulb chips.
   3.3. A mineral analysis of snowdrop bulbs indicated that basal MS medium, often used with bulbous species, was probably not supplying minerals in the optimum ratios for snowdrops. This could be rectified by use of a redesigned *Galanthus* medium (G).
   3.4. *G. elwesii* was much more prone to the physiological disorder, hyperhydration, than other snowdrop types. This could be corrected to some extent by changing to the basal medium G or dilution of the MS or G media.
   3.5. Removal of plant growth regulators from the medium and addition of activated charcoal could stimulate bulblet growth and the initiation of roots. Improved growth of bulblets was achieved by increasing the sucrose supply in combination with addition of charcoal. Bulblets greater than 1cm in diameter, that had up to three scale leaves but no flowers, could be produced by this method.
4. In further micropropagation experiments, factors controlling hyperhydration in *G. elwesii*, bulblet growth and acclimatisation in a range of species, are being investigated.
5. Field trials have been conducted in which simple mulching, shading and shelter techniques have been applied to plots of *G. nivalis*, to simulate conditions that might be more appropriate to the genus. The main conclusions are:
   5.1. Simple shading, using horticultural netting, enhances snowdrop growth, giving higher bulb yields through more vigorous growth and delayed foliar senescence.
   5.2. A straw mulch was also effective in enhancing growth and bulb yields.
   5.3. Inter-cropping snowdrops with cereals or narcissus bulbs, or over-sowing snowdrop plantings with perennial rye-grass, appeared from initial results to be unsuitable because these other species were too competitive. However, the effects of growing under rye-grass are still being investigated.
6. Field trials on the effects of fungicide spray programmes are being conducted in 2003. Fungicide applications are needed to control *Botrytis galanthi* and to increase bulb growth

through delaying natural leaf senescence.

7. Field trials will also be conducted in 2003-2004 to compare the field growth of 'ordinary' bulbs, 'chipped' bulbs, seedlings and *ex vitro* plantlets or bulblets.
8. Experiments have confirmed the value of storing *G. nivalis* bulbs in silver sand at 13 or 17°C. Storage in loosely closed polythene bags at these temperatures was also successful in preventing desiccation and ensuring acceptable subsequent plant growth. Further storage experiments are being carried out in 2003.
9. The results obtained so far suggest that commercial snowdrop production could be enhanced through the availability of high quality nuclear planting stocks of a variety of species and cultivars (a) derived from micropropagation and (b) grown-on in the field under shading and (or) using a mulch.
10. While the machinery and handling aspects of snowdrop bulb growing is not a part of this project, this will need to be addressed. However, it is suggested that the Dutch technique of planting and lifting bulbs in nets would be a practical possibility.
11. In order to enhance the prospects for successful snowdrop bulb production and sales in the UK, an exploitation plan should be developed in 2003-2004 involving researchers, micropropagation managers and specialist bulb growers.