Project title:	**Snowdrops: Developing cost-effective production methods through studies of micropropagation, agronomy and bulb storage**
Report:	**Year 2 Annual Report (December 2001)**
Previous reports:	**Year 1 Annual Report (December 2000)**
HDC Project number:	**BOF 48**

Project leaders:

Gordon R Hanks
Crop and Weed Science Department
Horticulture Research International (HRI)
Kirton
Boston
Lincs PE20 1NN

Dr Chris Selby
Department of Applied Plant Sciences
The Queen's University of Belfast (QUB)
Newforge Lane
Belfast BT9 5PX

Location: **HRI Kirton and QUB Belfast**

Project Co-ordinators:

Dr Gordon J Flint
Winchester Growers Ltd
Lacey's Nursery
Herdgate Lane
Pinchbeck
Spalding
Lincs

Brian Taylor
O A Taylor & Sons Ltd
Washway House Farm
Holbeach
Spalding
Lincs PE12 7PP

Date commenced: **April 2000**

Date completion due: **December 2003**

Keywords: ***Galanthus*, snowdrop, bulb, micropropagation, agronomy, storage, shade, irrigation, mulches, bulblets, *Galanthus nivalis*, *Galanthus nivalis flore pleno*, *Galanthus elwesii***

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The contents of this publication are strictly private to HDC members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission of the Horticultural Development Council.

© 2001 Horticultural Development Council

PROJECT LEADERS

C Selby BSc, PhD
G R Hanks BSc, MPhil, MHort, MBPR(Hort), CBiol, MIBiol

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

....	....
(signature)	(signature)
G R Hanks	Dr Chris Selby
Crop and Weed Science Department	Department of Applied Plant Sciences
Horticulture Research International	The Queen's University of Belfast (QUB)
Kirton	Newforge Lane
Boston	Belfast BT9 5PX
Lincs PE20 1NN	
Date........................	Date........................

Report authorised by:

....
(signature)

Dr D Gray
Head, Crop and Weed Science Department
Horticulture Research International
Wellesbourne
Warwicks CV35 9EF

Date........................

© 2001 Horticultural Development Council

CONTENTS

Page no.

PRACTICAL SECTION 1
Objectives and background 1
Summary of results 1
Action points for growers 2
Practical and financial benefits from study 2

EXPERIMENTAL SECTION 3
Introduction 3
Micropropagation 4
Materials and methods 4
Plant material 4
Bulb preparation for micropropagation 4
Media preparation and culture conditions 5
Sterilisation methods 5
Experiment 1 5
Experiment 2 6
Experiment 3 6
Experiment 4 7
Initiation of shoot cultures 7
Effects of plant growth regulators 7
Bulblet initiation on bulb scale leaf explants 8
Additional initiation experiments 8
Multiplication of bulblet cultures 9
Results and discussion 9
Sterilisation methods 9
Initiation of shoot cultures 10
Effects of plant growth regulators 18
Bulblet initiation on bulb scale leaf explants 18
Multiplication of bulblet cultures 18
Hyperhydration 19
Root initiation 20

Agronomy 25
Materials and methods 25
Plant material 25
General methods for field experiments 25
Agronomy experiment 1: The effect of shade, shelter and soil moisture treatments 26
Agronomy experiment 2: The effect of shading and inter-cropping 26
Agronomy experiments – crop records 27
Environmental data 27
Storage experiments 27
Results and discussion 28
Main experiment 28
Inter-crops experiment 31
Meteorological data 32
Acknowledgements 36
References 36

© 2001 Horticultural Development Council

PRACTICAL SECTION FOR GROWERS

OBJECTIVES AND BACKGROUND

There is a demand for snowdrop 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 increase snowdrop bulb sales, both of dry bulbs sold in bulk, and of choice species sold in growth.

SUMMARY OF RESULTS

Micropropagation

Work focused on the initiation and multiplication phases of micropropagation using bulb chip explants of *Galanthus nivalis* and *G. elwesii*. The main conclusions so far are:

- 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. The main differences were:
  - 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 and included:
  - 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

© 2001 Horticultural Development Council

effective with *G. nivalis*.
- Root initiation of bulblets was spontaneous, particularly on PGR-free medium.

Agronomy

Experiments were set up with *G. nivalis* to study the effects of shading, shelter, irrigation and mulching on plant, seed and bulb production. The mulching treatment included drip irrigation via T-Tape irrigation lines. Only the first year's data are available, and the main conclusions so far are:

- 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 ryegrass, were too competitive to snowdrops to be used as alternatives to artificial shading.

ACTION POINTS FOR GROWERS

- The results from micropropagation experiments so far are encouraging, so growers interested in taking up snowdrop growing in due course might find it worthwhile to consider sources of good stocks or superior species or cultivars of snowdrops at this early stage.
- The findings to date suggest that the erection of simple shading materials over snowdrop crops would provide some protection and would delay leaf senescence, allowing greater bulking of bulbs (and greater seed production). Foliar fungicides that delay leaf senescence may be useful for the same reason.
- Mulching and the provision of windbreaks have smaller effects on snowdrop development, but mulching might be worthwhile as it would also have the effect of controlling weeds.
- The project is in its early stages and there are no further action points at present.

PRACTICAL AND FINANCIAL BENEFITS FROM STUDY

Again, an assessment of these benefits must await the completion of further work. The success of micropropagation, however, suggests there is every prospect of establishing good, sustainable stocks that would stimulate demand. 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, considerably more for double-flowered or choice types.

Brian Taylor, HDC Co-ordinator for the project, comments: "With the huge demand for snowdrops from the gardening public, this is just the kind of diverse R&D that the bulb industry needs."

© 2001 Horticultural Development Council

EXPERIMENTAL SECTION

INTRODUCTION

There is a demand for snowdrop bulbs that has in the past 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 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.

This project was set-up in 2000, and a literature review and results for the first year of the project were included in the previous Annual Report. In that first part of the project, the initial work on *micropropagation* was concentrated on finding appropriate methods for achieving sterile cultures of bulb explants, using *Galanthus nivalis*, *G. nivalis* Flore Pleno and *G. elwesii*. High concentrations of hypochlorite were found to be necessary to achieve the surface sterilisation of explants. Hot-water treatment, prior to surface sterilisation, increased (rather then decreased, as expected) subsequent contamination. Treatment with a commercial product, 'Plant Preservation Mixture' (PPM), after surface sterilisation with lower concentrations of hypochlorite, gave better control of contamination, although high concentrations of PPM initially inhibited the growth of explants. Following successful surface sterilisation, there were found to be additional benefits of incorporating fungicides in the culture medium, a mixture of carbendazim and imazilil completely eradicating residual infection. Steeping explants in imazilil before inoculation was highly effective in controlling infection, particularly when combined with incorporating imazilil in the culture medium. There were early indications that *in vitro* bulbil formation was occurring in most treatments where microbial infection had been controlled.

Agronomy experiments were set up with *G. nivalis* to study the effects of shading, shelter, irrigation and mulching on plant, seed and bulb production. As well as using artificial shading materials, narcissus and cereal inter-crops and over-sowing with ryegrass were evaluated. Bulb *storage* experiments will be carried out in 2002.

This second Annual Report describes further progress on the micropropagation of *Galanthus* species, and updates results of the agronomy experiments.

© 2001 Horticultural Development Council

MICROPROPAGATION

<u>Materials and methods</u>

Plant material
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 2000 and 2001, and were stored at room temperature. Additional *G. nivalis* bulbs were sampled from the same batch as used in the Kirton field trials. These were used in later micropropagation experiments to provide planting stocks, of the same genetic origin as those used in the shading and bulb storage trials, for comparative field trialing at Kirton. Approximately 180 bulbs of each species were planted in 13cm (*G. nivalis*) or 17cm (*G. nivalis* Flore Pleno and *G. elwesii*) pots using a steam-sterilised loam of pH 5.8. The drainage in the compost for *G. elwesii* was improved by mixing the loam with coarse grit and sharp sand (3:1:1 loam:grit:sand). The bulbs were planted to a depth three times their height. For *G. nivalis* and smaller bulbs of *G. nivalis* Flore Pleno eight bulbs were planted per pot. Six and five bulbs per pot were planted for the larger *G. nivalis* Flore Pleno bulbs and *G. elwesii*, respectively. Initially these plants were grown in a well-ventilated unheated glasshouse, but were then transferred to a shaded cold frame and the pots embedded in sand to keep the root systems cool.

***N. B.** Bulbs purchased in October 2000 as *G. nivalis* Flore Pleno produced single flowers instead of the expected doubles. The true identity of these bulbs is still to be confirmed but was thought to be *G. nivalis* by Jacques Amand International. Results produced with these *G. nivalis* Flore Pleno bulbs are therefore termed '*G. nivalis* 2' to distinguish them from the authentic bulbs of this species.

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. For *G. nivalis* in all but experiment 1, trimming of basal tissues was restricted to the removal of roots and loose dead tissues, because the base plate tissues of these bulbs were too thin to allow trimming both before and after sterilisation. Apical bud 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 surface sterilised in pairs in 100ml Erlenmeyer flasks (*G. nivalis* and *G. nivalis* Flore Pleno) or singly in wide form 100ml beakers (*G. elwesii*). Sterilant was then added to completely cover the bulbs, the vessel capped in aluminium foil and shaken at 130 r.p.m. on a reciprocating shaker.

After sterilisation the bulbs were cut aseptically into explants. Bulbs were first trimmed top and bottom to remove tissues damaged by the sterilant. Basal trimming was such as to allow a minimum of 1mm of base plate to remain. Apical trimming then produced bulb sections approximately 8mm in height for *G. nivalis* and *G. nivalis* Flore Pleno or 10mm in height for *G. elwesii*. Longitudinal cuts were then made through the centre of the bulbs to produce four (*G. nivalis* and *G. nivalis* Flore Pleno) or six (*G. elwesii*) chip explants per bulb.

© 2001 Horticultural Development Council

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) 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). This was supplemented with 30g/l sucrose and plant growth regulators as described in individual experiments. 6-benzyladenine (BA), naththaleneacetic acid (NAA) and potassium salt of naphthaleneacetic acid (KNA) were all cell culture quality tested (Sigma Aldrich Co. Ltd.). 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 Fisons 600G3/THTL growth cabinets at a constant temperature of 18ºC. A photoperiod of 16h was provided by cool white fluorescent tubes supplemented at the red end of the spectrum with 40W incandescent bulbs giving a PAR of 100µmol/m^2/s at bench height.

Sterilisation methods

A series of four experiments was completed examining the effects of various surface sterilisation methods. Hypochlorite treatments were used either alone, or in combination with an initial hot-water treatment (HWT) of whole bulbs, or in combination with steeping treatments in Plant Preservative Mixture (PPM) or various fungicides applied to bulb chip explants. The effects of incorporating PPM or fungicides in the culture medium were also investigated. PPM, whose main active constituents are methylisothiazolinone and methylchloroisothiazolinone, is a specially formulated product used in plant tissue culture systems (Plant Cell Technology Inc.). PPM and the fungicides benomyl (Benlate 50% w/w a.i., Du Pont UK Ltd.), carbendazim (Derosal 80% w/w a.i., Hoechst UK Ltd.) and imazalil (100% imazalil sulphate, Janssen Pharmaceuticals Ltd.) were added to culture media prior to adjusting the media pH and autoclaving.

The binary infection data (infection, not infected) from sterilisation experiments were statistically analysed using logistic analysis of the treatment factors and their interactions.

Experiment 1

In this experiment relatively mild hypochlorite sterilisation methods using dilutions of Domestos (Lever Brothers Ltd.) were tested alone and combined either with HWT or PPM treatments. Domestos is manufactured to have a hypochorite concentration of 4.6%, but this can fall to 4.2% with time.

The six sterilisation treatments tested are described below:

1 Bulbs were shaken in 70% ethanol for 30sec then in 10% Domestos (0.42 - 0.46% hypochlorite) for 20min followed by six rinses in sterile distilled water.
2 The same as treatment 1 except the Domestos concentration was increased to 30% (1.26 - 1.38% hypochlorite).
3 Cleaned bulbs were HWT at 43ºC for 45min then sterilised as in treatment 1.
4 Cleaned bulbs were HWT at 43ºC for 45min then sterilised as in treatment 2.

© 2001 Horticultural Development Council

5 Bulbs were sterilised as in treatment 1 and bulb chip explants prepared as described above. Explants were then steeped in 2% PPM for 9h. Chip explants were treated in groups of five, in 20ml aliquots of PPM solution prepared in 50mg/l $MgSO_4$, in 100ml Erlenmeyer flasks that were shaken at 100 r.p.m. Explants were inoculated directly to the culture media without rinsing.
6 The same as treatment 5 except the PPM concentration was increased to 4%.

Following sterilisation, explants were inoculated onto four culture media described below:
1 Basal medium lacking plant growth regulators (see media preparation above).
2 The same as medium 1 supplemented with 0.1mg/l NAA and 1mg/l BA.
3 The same as medium 2 supplemented with 20mg/l (a.i.) benomyl.
4 The same as medium 2 supplemented with 1ml/l PPM.

The full experimental design was 6 sterilisation treatments x 4 culture media x 3 snowdrop types, 72 treatments in total. Each treatment was replicated with six bulb chip explants (432 cultures in total).

Experiment 2
In this experiment surface sterilisation with higher hypochlorite and PPM concentrations was examined in combination with incorporating fungicides in the culture medium. The three sterilisation treatments used were:

1 Cleaned bulbs were shaken in 70% ethanol for 1min then in 50% Chloros (5% hypochlorite) for 20min followed by six rinses in sterile distilled water. Ten drops per litre of Tween 20 were added to the Chloros solution to act as a wetting agent.
2 The same as treatment 1 except the Chloros concentration was increased to 100% (10% hypochlorite).
3 Cleaned bulbs were shaken in 70% ethanol for 1min then in 50% Domestos (2.1 – 2.3% hypochlorite) for 20min followed by six rinses in sterile distilled water. Bulb chip explants were then prepared and steeped in 5% PPM for 9h as described previously (experiment 1 treatment 5).

Following sterilisation, explants were inoculated onto four culture media described below:

1 Same plant growth regulator supplemented medium number 2 as in experiment 1 (control).
2 The same as medium 1 supplemented with 20mg/l (a.i.) carbendazim.
3 The same as medium 1 supplemented with 20mg/l imazalil.
4 The same as medium 1 supplemented with 20mg/l (a.i.) carbendazim and 20mg/l imazalil.

The full experimental design was 3 sterilisation treatments x 4 culture media x 3 snowdrop types, 36 treatments in total. Each treatment was replicated with six bulb chip explants (216 cultures in total).

Experiment 3
Cleaned bulbs were shaken in 70% ethanol for 1min then in 50% Domestos (2.1 – 2.3% hypochlorite) for 20min followed by six rinses in sterile distilled water. Bulb chip explants

© 2001 Horticultural Development Council

were prepared and steeped in fungicide solutions for 1h (as described for PPM in treatment 5, experiment 1) then transferred without washing to the same four media used in experiment 2, as described below:

Steeping treatment	Media inoculated
1 Water control	All four media.
2 20mg/1 carbendazim	Control medium and 20mg/l carbendazim medium.
3 40mg/1 carbendazim	Control medium and 20mg/l carbendazim medium.
4 20mg/l imazalil	Control medium and 20mg/l imazalil medium.
5 40mg/l imazalil	Control medium and 20mg/l imazalil medium.
6 20mg/l carbendazim & 20mg/l imazalil	Inoculated onto control medium and the medium with a mixture of 20mg/l carbendazim & 20mg/l imazalil.
7 40mg/l carbendazim & 40mg/l imazalil	Inoculated onto control medium and the medium with a mixture of 20mg/l carbendazim & 20mg/l imazalil.

The full experimental design was 6 fungicide treatments x 2 media x 3 snowdrop types + water control (1treatment x 4 media x 3 snowdrop types), 48 treatments in all. Each treatment was replicated with six bulb chip explants (288 cultures in total).

Experiment 4
Bulb chip explants were prepared from surface sterilised bulbs as described in experiment 3 then subjected to the treatments described below:
1 Inoculated directly to culture media.
2 Steeped in water for 1h.
3 Steeped in 40mg/l imazalil for 1h.

Explants were then inoculated onto a fungicide-free medium or a medium with 20mg/l imazalil. The full experimental design was 3 post-explant chipping treatments x 2 media x 2 snowdrop types (*G. nivalis* and *G. elwesii*), 12 treatments. Each treatment was replicated with 20 bulb chip explants with *G. nivalis* and 22 bulb chip explants with *G. elwesii* (252 cultures in total).

Initiation of shoot cultures
In the project proposal it was anticipated that adventitious shoots would be formed by bulb chip explants. In all culture initiation experiments examined to date the only adventitious structures to be formed have been bulblets rather than shoots regardless of the composition of the culture medium. The only shoots formed have derived from lateral bulb initials already present in the bulb chip explants. Under the culture conditions used here these shoots also rapidly developed into bulblets. Counts were therefore made of the numbers of bulblets formed by explanted tissues approximately 10 – 12 weeks after inoculation of cultures. Bulblet counts were made for sterilisation experiments 2-4, because there was too much infection in most of the treatments in experiment 1 to make collection of data worthwhile.

Effects of plant growth regulators
Twenty-four replicate bulb chip explants of each of *G. nivalis, G. nivalis* Flore Pleno and *G. elwesii* were inoculated on to each of the six media detailed below to test for growth regulator effects on bulblet initiation:
1 Control = no growth regulators

© 2001 Horticultural Development Council

2 NAA (0.083 mg/l)
3 KNA (0.1 mg/l)
4 BA (1mg/l)
5 BA (1mg/l) & NAA (0.083 mg/l)
6 BA (1mg/l) & KNA (0.1 mg/l)

The sterilisation method used involved shaking cleaned bulbs in 70% ethanol for 1min then in 30% Domestos for 20min followed by six rinses in sterile distilled water. Chip explants were then prepared and treated for 9h with 4% PPM solution as described previously.

Bulblet initiation on bulb scale leaf explants
Since bulblets were observed to form in the outer surface of the scale leaves of the bulb chip explants (see results) some scale leaves were isolated, for separate culture, to examine their organ forming abilities. Scale leaves, that had not formed bulblets at the end of the initiation culture passage in sterilisation experiment 1, were dissected away from the bulb chip explants and sub-cultured on the appropriate culture medium. For each of the three species 10 scale leaves were prepared from tissues cultured on each of the four culture media used and transferred onto media of the same compositions. Bulblet formation was assessed periodically.

A second experiment was established in October 2001 examining if scale leaf position within the bulb influences bulblet production. Cleaned bulbs were sterilised by shaking in 70% ethanol for 1 min, 50% Domestos for 20 min and were then rinsed seven times in sterile water. Bulb chip explants were then prepared and treated with 4% PPM for 9h. Four chip explants were prepared from each of 18 bulbs of *G nivalis, G. nivalis* Flore Pleno and *G elwesii*. After PPM treatment two explants from each bulb were inoculated directly to the culture medium. The remaining two explants were cut into single scales each with a section of bulb basal plate still attached and these were numbered from one to three for the most inner scale to the outer scale respectively. Scales were individually inoculated base plates down onto the culture medium supplemented with 0.1mg/l NAA and 1mg/l BA.

Additional initiation experiments
Further experiments were set up in an attempt to initiate proliferating shoot cultures on bulb chip explants rather than bulblets. In each of these experiments the sterilisation method and culture medium was the same as in the bulb scale experiment described previously and each was replicated with all three species of snowdrop. Experiments were designed examining the effects of:

1 Reducing the incubation temperature from 18ºC to 7ºC since high temperature is known to be a strong stimulus to bulb formation in many bulbous species. Cultures incubated at 18ºC were used as a control.
2 Incorporating the herbicide fluridone in the culture medium to inhibit the synthesis of the stress/bulb dormancy related growth regulator compound abscisic acid (ABA). Three concentrations of fluridone (0, 1 and 10µM) were tested in both continuous darkness and normal lighting conditions.
3 Reduced photoperiod and enhanced blue light. Four Fisons 600G3/THTL growth cabinets were used to give the following sets of conditions:
   a. A photoperiod of 16h (LD) provided by cool white fluorescent tubes. (Control)

© 2001 Horticultural Development Council

b. A photoperiod of 8h (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.

PAR was equalised between treatments by use of a Macam scanning spectroradiometer.

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. A snowdrop medium was devised using these methods that had a set nitrogen composition of 40mM NO_3 and 20mM NH_4 (the same as in MS medium). Media dilutions of 0, 0.5, 0.25 and 0.125 were tested with both MS (controls) and the new snowdrop medium for their ability to support bulblet initiation in bulb chip explants.

Multiplication of bulblet cultures

The standard subculture method used involved the cutting away of any necrotic tissues and the shortening of any leaves that had developed down to about 1cm from the bulb tissues. The remaining tissues consisted mainly of bulblets at various stages of development (see Figure 1.1B) and swollen white/grey tissues that had derived from the scale leaves in the original explant. These tissues were divided using a sharp scalpel and subcultured onto fresh medium. Culture passages of 12 weeks were used.

The effects of longitudinally splitting *in vitro* produced bulblets at subculture on bulblet multiplication rates was established with *G. nivalis* and *G. elwesii*. In August 2001 bulblets that were formed on the imazalil-free medium in experiment 4 were subjected to the following physical treatments:

1 Clumps of bulblets with an average of 4.3 and 1.9 with *G. nivalis* and *G. elwesii* per culture respectively.
2 Single isolated bulblets with most of the supporting scale leaf tissue cut away.
3 Half bulblets prepared by cutting vertically through the centre of bulbs.
4 Quarter bulblets prepared by cutting vertically through the centre of bulbs.

For each species treatments were replicated with bulbs derived from the same initial culture, and initial cultures being used for *G. nivalis* and *G. elwesii* respectively. Owing to the small size of the *G. nivalis* bulbs treatment 4 was omitted.

Results and discussion

Sterilisation methods

Last year we showed that high hypochlorite concentrations or PPM treatment were important in reducing microbial contamination in bulb chip explants. These results were confirmed by a statistical analysis of the data from experiments 1 and 2. For instance, in experiment 1 increasing the Domestos concentration from 10% to 30% gave significantly improved control of infection ($p < 0.05$). In experiment 1 both the 2% and 4% PPM treatments gave very highly significantly reduced levels of infection compared to the 10% Domestos control (p <

© 2001 Horticultural Development Council

0.001). Similarly, in experiment 2, use of 5% PPM gave significantly better control of contamination than the Chloros treatments ($p < 0.05$). In both of these experiments no two or three way interactions were found between sterilisation treatment, snowdrop species or culture medium composition. However inclusion of a mixture of carbendazim and imazalil in the culture medium was effective at reducing residual contamination following surface sterilisation with Chloros in experiment 2 ($p < 0.01$). Steeping bulb explants in an imazalil solution was also found to significantly reduce contamination compared to the water steeped control in experiment 4 ($p < 0.05$).

Initiation of shoot cultures
When bulb chip explants are inoculated onto the culture medium their scale leaf sections increase in length and thickness within a few days. However, the production of any adventitious organs was slow, taking up to 8 weeks before primordia were observed. Bulblets rather than leaves and shoots were formed in all cultures, regardless of sterilisation method or culture medium used. In almost all instances bulblets developed from the abaxial side of the swollen scale leaves. With *G. nivalis,* bulblets usually formed in a row on this outer scale leaf surface 1-2mm above the point of attachment to the bulb base plate (Figure 1.1A). In contrast, the positions of bulblet formation were much more variable with *G. elwesii,* although bulblets were still confined to the outer surface of the scale leaves with this species. With *G. elwesii* many bulblets still formed close to the base plate, but others formed on other parts of the outer scale leaf surface. These were sometimes arranged in horizontal rows perpendicular to the root - shoot axis of the bulb well away from the base plate, including the cut surface of the explant most distal to the base plate (Figure 1.1B).

All three snowdrop populations were capable of producing bulblets under the culture conditions used. Bulblet numbers per explant were similar between the authentic *G. nivalis* and *G. elwesii* samples in sterilisation experiments 2 (Table 1.1), 3 (Table 1.2) and 4 (Table 1.3) and the experiment comparing the effects of plant growth regulators (Table 1.4). However, the *G. nivalis* 2 sample produced significantly more bulblets than either of these two species in all experiments in which it was included (all $p < 0.001$), except for experiment 2 where there were complex two way interactions between species and sterilisation method ($p < 0.05$) and media ($p < 0.05$) which may have modified overall species differences (Table 1.1). This indicates that the *G. nivalis* samples were either genetically distinct or of a different nutritional or physiological condition. *G. nivalis* 2 bulbs were larger and had thicker scale leaves than the authentic *G. nivalis* and therefore produced larger explants that may have been more resistant to damage during surface sterilisation. This suggests that there might be some advantages in selecting large bulbs for micropropagation with this species.

In sterilisation experiment 2, PPM treatment significantly ($p < 0.001$) reduced the numbers of bulblets formed by the explants compared to both chloros treatments (Table 1.1). There was also a significant interaction between sterilisation method and culture medium for bulblet production ($p < 0.05$). This interaction was due to the inhibitory effect of PPM only occurring when explants were transferred to media to which fungicides were added, but was not significant in PPM treated explants transferred to the fungicide-free control medium, indicating that combining PPM treatment with fungicides in the medium may be harmful to the cultured tissues. There was also a more complex interaction between sterilisation treatment and snowdrop species ($p < 0.05$). Treatment with 100% chloros was either stimulatory or had little effect on bulblet production by the two *G. nivalis* populations, whilst

© 2001 Horticultural Development Council

use of the high chloros concentration significantly inhibited production by *G. elwesii* compared to the 50% chloros treatment. PPM was inhibitory with all three plant types.

Bulblet initiation rates were unaffected by steeping in solutions of the fungicides carbendazim and imazalil when these compounds were used alone in sterilisation experiment 3. Likewise, there were no interactions between fungicide steep, snowdrop species or addition of fungicide to the culture medium for bulblet production when these fungicides were used singly (Tables 1.2a & b). In contrast, steeping in a mixture of 20mg/l of each of these fungicides resulted in significantly ($p < 0.001$) more bulblets being formed compared with steeping in water or the 40mg/l fungicide mixture (Table 1.2c).

Unlike in experiment 3, sole use of a 40 mg/l imazalil steep in experiment 4 significantly ($p < 0.05$) reduced bulblet initiation compared to both the directly transferred explants and the water steep control (Table 1.3). This discrepancy between experiments may be explained by the higher concentration of imazalil used in the culture media in experiment 4 (40mg/l) compared with 20mg/l used in experiment 3 causing a combined inhibitory steep plus medium concentration of imazalil to build up in the tissues in experiment 4.

Including fungicides in the culture medium was found to significantly affect bulblet production in sterilisation experiments 2, 3 and 4 and there were also interactions of medium with both species and sterilisation treatments. Most notably *G. elwesii* bulblet initiation was significantly reduced by the inclusion of fungicides in the medium, whilst *G. nivalis* was relatively insensitive to fungicides supplied in this way. This difference between *G. elwesii* and *G. nivalis* resulted in several significant interactions between snowdrop species and fungicide in the medium being found for bulblet initiation (see Table 1.1 with a CI mixture in experiment 2 ($p < 0.05$), Table 1.2c with a CI mixture in experiment 3 ($p < 0.001$) and Table 1.3 with imazalil in experiment 4 ($p = 0.051$; with effectively normal untransformed data $p = 0.02$)).

© 2001 Horticultural Development Council

Table 1.1 Bulblet production by snowdrop bulb chip explants in sterilisation experiment 2, recorded 2 months after inoculation. The data were normalised by square root transformation before analysis, but untransformed data are shown in parenthesis.

Species	Media[1]	Sterilisation method			Species x Media means
		Chloros (50%)	Chloros (100%)	PPM (5%)	
G. nivalis	Control	1.06 (1.11)	1.87 (3.68)	1.57 (2.75)	1.50 (2.51)
	C	1.50 (2.70)	1.49 (2.42)	0.32 (0.46)	1.10 (1.86)
	I	3.26 (9.73)	2.81 (7.16)	0.71 (1.50)	2.26 (6.13)
	CI	1.64 (3.33)	1.89 (4.33)	1.30 (2.83)	1.61 (3.50)
Species x Sterilisation means		1.86 (4.22)	2.01 (4.40)	0.97 (1.89)	1.62 (3.50)
***G. nivalis* 2**[2]	Control	1.84 (4.65)	1.20 (4.14)	2.01 (4.01)	1.91 (4.27)
	C	1.65 (3.35)	2.59 (7.28)	1.36 (2.83)	1.87 (4.49)
	I	1.92 (4.83)	3.06 (9.67)	0.76 (1.17)	1.92 (5.22)
	CI	2.33 (5.41)	2.52 (7.00)	0.64 (0.83)	1.83 (4.41)
Species x Sterilisation means		1.93 (4.56)	2.55 (7.02)	1.16 (2.21)	1.88 (4.60)
G. elwesii	Control	2.85 (12.47)	1.82 (2.68)	1.82 (3.50)	2.16 (6.22)
	C	3.28 (12.76)	2.07 (5.24)	1.34 (2.50)	2.25 (6.80)
	I	2.60 (8.33)	1.57 (3.41)	0.77 (1.08)	1.65 (4.27)
	CI	1.97 (8.17)	1.67 (4.00)	0.93 (1.83)	1.52 (4.67)
Species x Sterilisation means		2.68 (10.41)	1.78 (3.83)	1.23 (2.23)	1.90 (5.49)
Media x Sterilisation means					Media means
	Control	1.92 (6.08)	1.90 (3.50)	1.76 (3.42)	1.86 (4.33)
	C	2.14 (6.24)	2.05 (4.98)	1.02 (1.93)	1.74 (4.38)
	I	2.59 (7.63)	2.48 (6.74)	0.75 (1.25)	1.94 (5.21)
	CI	1.98 (5.64)	2.03 (5.11)	0.96 (1.83)	1.65 (4.19)
Sterilisation method means		2.16 (6.40)	2.11 (5.08)	1.12(2.11)	

[1]Explants were grown on either a culture medium supplemented with 0.1 mg/l NAA and 1mg/l BA (Control) or the same plant growth regulator supplemented media to which either 20mg/l carbendazim (C), 20mg/l imazalil (I) or a mixture of 20mg/l of both carbendazim and imazalil (CI) was added.
[2]See note in the Experimental Section (plant materials).

© 2001 Horticultural Development Council

Analysis of variance summary for Table 1.1[a]

	d.f. (m. v.)	SED	Significance
Species (SP)	2	0.176	NS
Sterilisation method (ST)	2	0.176	***
SP x ST	4	0.304	*
Residual	40		
Media (M)	2	0.203	NS
M x SP	6	0.352[b]	*
		0.353[c]	
M x ST	6	0.352[d]	*
		0.353[e]	
M x SP x ST	12	0.610[f]	NS
		0.611[g]	
Residual	99 (36)		

[a] NS, not significant; *, ** and ***, significant at the 5, 1 and 0.1% levels of probability, respectively.
[b]SED for comparing media means with different species.
[c]SED for comparing media means within the same species.
[d]SED for comparing media means with different sterilisation methods.
[e]SED for comparing media means within the same sterilisation method.
[f]SED for comparing media means with a different species or sterilisation method.
[g]SED for comparing media means with the same species and sterilisation method.

© 2001 Horticultural Development Council

Table 1.2 Bulblet production by snowdrop bulb chip explants in sterilisation experiment 3, recorded 2 months after inoculation[1]. The data were normalised by square root transformation before analysis but untransformed data are shown in parenthesis.

Table 1.2a

Species	Media[2]	Carbendazim steeping treatment (mg/l)			Media means
		Water	20	40	
G. nivalis	Control	1.39 (2.77)	1.49 (2.35)	1.40 (2.50)	1.43 (2.54)
	C	1.62 (2.83)	1.98 (4.19)	1.89 (3.69)	1.83 (3.57)
	Mean	1.51 (2.80)	1.74 (3.27)	1.64 (3.10)	1.63 (3.06)
***G. nivalis* 2[3]**	Control	2.37 (5.83)	1.97 (4.03)	1.81 (3.69)	2.05 (4.52)
	C	2.24 (5.15)	2.25 (5.17)	1.62 (3.27)	2.04 (4.53)
	Mean	2.31 (5.49)	2.11 (4.60)	1.72 (3.48)	2.04 (4.52)
G. elwesii	Control	2.11 (4.85)	1.57 (3.69)	2.46 (6.77)	2.05 (5.10)
	C	1.58 (2.80)	1.61 (3.92)	1.62 (3.56)	1.60 (3.43)
	Mean	1.85 (3.82)	1.59 (3.81)	2.04 (5.16)	1.82 (4.26)
Steeping x Media means	Control	1.96 (4.48)	1.68 (3.36)	1.89 (4.32)	1.84 (4.05)
	C	1.82 (3.59)	1.95 (4.43)	1.71 (3.51)	1.82 (3.84)
Carbendazim steeping means		1.89 (4.04)	1.81 (3.89)	1.80 (3.91)	1.83 (3.95)
Species means over all treatments					
	G. nivalis		1.76 (3.42)		
	G. nivalis 2		2.10 (4.82)		
	G. elwesii		1.63 (3.83)		

[1]See the footnote at the end of the Tables for details of how the analysis of variance was performed. All bulbs were first surface sterilised by dipping in 70% ethanol for 1min then 50% Domestos for 20min followed by 6 rinses in sterile water before being cut into chip explants. After 1h steeping in the appropriate fungicide solution explants were transferred, without washing, to culture media.

[2]Media were supplemented with 0.1 mg/l NAA and 1mg/l BA (Control) or the same plant growth regulator media to which either 20mg/l carbendazim [(C, (Table 1.2a)], 20mg/l imazalil [I, (Table 1.2b)] or a mixture of 20mg/l of both carbendazim and imazalil [CI, (Table 1.2c)] was added.

[3]See note in the Experimental Section (plant materials).

© 2001 Horticultural Development Council

Table 1.2b

Species	Media[2]	Imazalil steeping treatment (mg/l)			Media means	
		Water	20	40		
G. nivalis	Control	1.39 (2.77)	1.64 (2.95)	1.74 (3.33)	1.59	(3.02)
	I	2.01 (4.50)	1.70 (2.95)	1.77 (3.27)	1.83	(3.57)
	Mean	1.70 (3.64)	1.67 (2.95)	1.76 (3.30)	1.71	(3.29)
***G. nivalis* 2[3]**	Control	2.37 (5.83)	2.13 (4.95)	2.14 (4.83)	2.22	(5.20)
	I	1.86 (3.67)	2.38 (5.83)	2.32 (5.83)	2.19	(5.11)
	Mean	2.12 (4.75)	2.25 (5.39)	2.23 (5.33)	2.20	(5.16)
G. elwesii	Control	2.11 (4.85)	1.80 (3.55)	1.27 (2.19)	1.73	(3.53)
	I	1.35 (2.83)	1.49 (3.87)	0.95 (1.15)	1.26	(2.62)
	Mean	1.73 (3.84)	1.64 (3.71)	1.11 (1.67)	1.49	(3.07)
Steeping x	Control	1.96 (4.48)	1.86 (3.81)	1.72 (3.45)	1.84	(3.92)
Media means	I	1.74 (3.67)	1.85 (4.22)	1.68 (3.42)	1.76	(3.77)
Imazalil steeping means		1.85 (4.07)	1.86 (4.01)	1.70 (3.43)	1.80	(3.84)

Table 1.2c

Species	Media[2]	CI steeping treatment (mg/l)			Media means	
		Water	20	40		
G. nivalis	Control	1.39 (2.77)	2.08 (4.68)	1.64 (3.09)	1.70	(3.51)
	CI	1.91 (3.69)	2.27 (5.61)	1.55 (2.39)	1.91	(3.90)
	Mean	1.65 (3.23)	2.17 (5.15)	1.60 (2.74)	1.81	(3.71)
***G. nivalis* 2[3]**	Control	2.37 (5.83)	2.23 (5.33)	1.59 (3.00)	2.06	(4.72)
	CI	1.56 (3.15)	2.80 (8.00)	2.26 (5.33)	2.20	(5.49)
	Mean	1.96 (4.49)	2.51 (6.67)	1.92 (4.17)	2.13	(5.11)
G. elwesii	Control	2.11 (4.85)	3.39 (13.17)	1.12 (1.88)	2.21	(6.63)
	CI	0.98 (2.17)	1.01 (1.83)	1.70 (3.09)	1.23	(2.36)
	Mean	1.55 (3.51)	2.20 (7.50)	1.41 (2.48)	1.72	(4.50)
Steeping x	Control	1.96 (4.48)	2.57 (7.73)	1.45 (2.66)	1.99	(4.96)
Media means	CI	1.48 (3.00)	2.03 (5.15)	1.84 (3.61)	1.78	(3.92)
CI steeping means		1.72 (3.74)	2.30 (6.44)	1.64 (3.13)	1.89	(4.44)

© 2001 Horticultural Development Council

Analysis of variance summary for Tables 1.2a, b & c[1]

			Fungicide(s)		
			Carbendazim	Imazalil	Carbendazim + Imazalil
Sub-set analysis on Tables			1.2a	1.2b	1.2c
	d.f. (m.v.)	SED			
Species (S)	2	0.156	***	***	***
Fungicide Steep (F)	2	0.181	NS	NS	***
Media (M)	1	0.148	NS	NS	NS
S x F	4	0.313	NS	NS	NS
S x M	2	0.256	NS(* p=0.068)	NS	***
F x M	2	0.256	NS	NS	*
S x F x M	4	0.443	NS	NS	***
Residual	187 (48)				

[a] NS, not significant, (*); *, ** and ***, significant at the 10, 5, 1 and 0.1% levels of probability, respectively.

[1]The statistical analysis was not straightforward because of the treatment combinations used. The 16 (fungicide steeping x media) treatments were viewed as comprising a sub-set with a factorial combination of fungicide steeps and medium composition plus 'other treatments'. Three different overlapping sub-sets can be identified relating to steeping with carbendazim (Table 1.2a), imazalil (Table 1.2b), and a mixture of carbendazim and imazalil (Table 1.c) (the only sub-sets overlap being the water steep control cultured on the control medium). The initial analysis (not reported) was for 48 treatments [16 (steep x media combinations) x 3 species] in 6 blocks. The data was further analysed three times, each time the factorial elements of one of the sub-sets were investigated, as were their interactions with species. In each of the analyses the effects of the 'other treatments' and the difference between these and the sub-set were analysed but are not reported. The consequence of this is that the three analyses are not independent and therefore conclusions drawn from across the analyses need to be viewed with caution.

© 2001 Horticultural Development Council

Table 1.3 Bulblet production by snowdrop bulb chip explants in sterilisation experiment 4, recorded 2 months after inoculation. The data were normalised by square root transformation before analysis but untransformed data was shown in parenthesis. All bulbs were first surface sterilised by dipping in 70% ethanol for 1min then 50% Domestos for 20min followed by 6 rinses in sterile water before being cut into chip explants. Explants were either transferred directly to the culture media or given a 1h steep in water or 40mg/l Imazalil before inoculation.

Species	Media[1]	Steeping treatment			Species x Media means
		None	Water	Imazalil	
G. nivalis	Control	1.62 (3.01)	1.62 (3.24)	1.40 (2.64)	1.55 (2.96)
	I	1.76 (3.87)	1.63 (3.20)	1.09 (1.75)	1.50 (2.94)
G. elwesii	Control	1.96 (4.60)	1.54 (3.01)	1.37 (2.38)	1.62 (3.33)
	I	1.37 (2.37)	1.25 (1.95)	0.87 (1.45)	1.16 (1.92)
Steeping treatment means		1.68 (3.46)	1.51 (2.85)	1.18 (2.06)	

[1]Explants were grown on either a culture medium supplemented with 0.1 mg/l NAA and 1mg/l BA (Control) or the same plant growth regulator media to which 20mg/l imazalil (I) was added.

Analysis of variance summary[a]

	d.f. (m. v.)	SED	Significance
Species (S)	1	0.128	NS
Fungicide Steep (F)	2	0.157	*
S x F	2	0.222	NS
Residual	45		
Media (M)	1	0.103	*
M x S	1	0.165[b]	(*) p = 0.051
		0.246[c]	
M x F	2	0.202[d]	NS
		0.179[e]	
M x S x F	2	0.253[f]	NS
		0.285[g]	
Residual	120 (54)		

[a] NS, not significant, (*); *, ** and ***, significant at the 10, 5, 1 and 0.1% levels of probability, respectively.
[b]SED for comparing species x media means across different species.
[c]SED for comparing species x media means within the same species.
[d]SED for comparing steep x media means across different steeps.
[e]SED for comparing steep x media means within the same steep.
[f]SED for comparing species x steep x media means within the same species x steep combination.
[g]SED for all other species x steep x media means.

© 2001 Horticultural Development Council

Effects of plant growth regulators
Surprisingly the use of BA, NAA or KNA failed to stimulate the production of bulblets above that found with the plant growth regulator free medium in any of the snowdrop species (Table 1.4). However, use of BA alone significantly reduced the number of bulblets formed compared with the hormone-free medium. In addition, there was a difficult to explain interaction between the auxins and addition of BA, caused by differences in bulbing responses to NAA and KNA when added alone as compared to their being used in combination with BA ($p < 0.01$). In the absence of BA there was no significant difference between the auxins, whereas when BA was used, NAA significantly stimulated more bulblets than KNA. Use of BA and NAA in combination gave significantly more bulblets than either compound used alone.

Bulblet initiation on bulb scale leaf explants
When individual bulb scale sections were cultured alone they were, like bulb chip explants, capable of forming bulblets even in the absence of plant growth regulators (Table 1.5), whilst adding hormones in this bulblet induction passage gave no improvement in bulblet numbers. Addition of benomyl or PPM to the culture medium did not interfere with bulblet formation by isolated scales. Further work is needed to assess media effects on bulblet production rates in this system owing to the low replication used here.

Bulblet initiation was on the abaxial surface of the scale explants and was mainly basal with *G. nivalis* compared to a more random scatter with *G. elwesii*. This distribution of bulblets on the scales was in good agreement with what was found with intact chip explants. If the scales were given prolonged incubation (5-6 months) bulblets formed all over the outer surface of the scales with both species.

Multiplication of bulblet cultures
Using a standard method of sub-culturing the healthy tissues every 12 weeks, bulblet multiplication was obtained for several culture passages without the need to cut away or split larger bulblets to break their apical dominance. In fact, many new bulblets often formed on fragments of scale leaf left attached to larger bulbs when these were cultured in isolation. New bulblets were formed mainly on tissues derived from the expanding scale leaf of the initial explant (Figure 1.1E) or in some instances on the outer scales of bulblets initiated in previous culture passages (Figure 1.1D). Bulblets were also formed on scale leaves that became detached from bulblets during sub-culturing process.

Table 1.6 shows the range of multiplication rates that were achieved when tissues from sterilisation experiments 1, 2 and 3 and the plant growth regulator experiment were sub-cultured for three 12 week culture passages. During the third passage bulblet multiplication was relatively low with on average only 0.49 and 0.45 new bulblets being initiated per bulblet inoculated for *G. nivalis* and *G. elwesii* respectively. Accumulative bulblet production over the three passages averaged at 26.4 and 40.8 per initial mother bulb for *G. nivalis* and *G. elwesii* respectively. This difference between species can be mostly explained by the higher numbers of bulb chip explants prepared from the larger *G. elwesii* bulbs (6) compared to *G. nivalis* (4).

The multiplication rates shown in Table 1.6 are conservative estimates since only clearly defined bulblets were counted. Small primordia that were probably going to give rise to

© 2001 Horticultural Development Council

bulblets in the next passage were not included. Likewise, all healthy looking tissues were sub-cultured regardless of whether they were forming bulblets or not. Thus no selections of highly organogenic genotypes or of individual explants forming large numbers of bulblets were made. No doubt much higher multiplication rates can be achieved by concentrating on explants initiating many bulblets and discarding tissues that do not form bulblets at an early stage. In most media treatments such highly organogenic explants can be identified like that shown in Figure 1.1E. Focusing sub-culture on such tissues, that have probably become well adapted to the culture conditions, could be expected to dramatically increase multiplication rates. Indeed, higher multiplication rates were obtained when cultures were selected for use in the experiment investigating bulblet-splitting treatments (Table 1.7). Here, inoculum cultures were selected that provided two well-developed bulblets and a small clump of bulblets initiated on a section of leaf scale. In this experiment bulblet clumps formed 1.5 or 1.8 new bulblets per inoculated bulblet with *G. nivalis* and *G. elwesii* respectively.

In contrast, when single bulblets were cultured alone very few new bulblets were formed (Table 1.7). In the case of *G. elwesii* the higher multiplication achieved with single bulblets was mainly due to one culture that formed 34 new bulblets. If this unusually prolific culture was excluded from the data the numbers of new bulblets formed per culture was reduced from 2.3 to 1.1. Basal tissues being removed from single bulbs before inoculation may explain reduced bulblet production by singly cultured bulblets. Since these basal tissues, not part of the inoculated bulblet, was usually where new bulblets were formed. Alternatively, the larger single bulblets could have exerted apical dominance that reduced new bulblet initiation.

Singly cultured bulblets of both species also showed poor growth with many dying after becoming necrotic. This suggests that the tissues cut away from the bulblets base was needed to support bulblet growth as well as being the main site of new bulblet initiation. The process of cutting basal tissues away may also have injured or caused a wounding reaction that arrested bulb growth in the culture conditions used here.

With *G. elwesii* an increase in bulblet multiplication could be achieved by splitting bulblets into halves or quarters (Table 1.7). These splitting treatments not only increased the numbers of bulblets formed per culture but also doubled the percentage of cultures forming new bulblets. The check in growth reported for singly cultured bulblets (see above) was also relieved by the splitting treatments with this species. Tissues of the split bulblets showed rapid growth and, unlike the singly cultured bulblets, did not become necrotic. This contrast between single bulbs and split bulbs reinforces the idea that a wounding response by the basal tissues of single bulbs interferes with their growth since it can be circumvented by splitting.

Little benefit was gained by splitting *G. nivalis* bulblets (Table 1.7). Although some improvement in the percentage of cultures initiating new bulblets was achieved, the new bulblets formed were very small and lacked vigour. Split *G. nivalis* bulblets also had a greater tendancy to become necrotic than those of *G. elwesii*.

Hyperhydration

By the end of the second culture passage a physiological disorder known as hyperhydration (vitrification) had developed in some cultures. This disorder was rare during the first culture passage. Hyperhydration is characterised by tissues becoming very swollen, translucent in appearance, curled and brittle and is caused by an excessive uptake of water (Debergh *et al.,*

© 2001 Horticultural Development Council

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 (Figure 1.1C). Tissues of *G. elwesii* were much more prone to hyperhydration than in the two *G. nivalis* populations. With this species hyperhydration occurred in all media tested. In addition, use of fungicides, particularly imazalil, enhanced hyperhydration especially with *G. elwesii*. Hyperhydration of *G. elwesii* in response to fungicides may explain why fungicide steeping treatments or fungicide in the culture medium reduced bulblet initiation much more with this species than with *G. nivalis* (as discussed previously and shown on Tables 1.1, 1.2c & 1.3).

Hyperhydration is usually regarded as a problem because it is difficult to reverse and hyperhydrated plantlets are difficult to acclimatise to *in vivo* conditions. In some circumstances, however, hyperhydration can be controlled to attain very high multiplication rates and can be useful particularly with recalcitrant subjects (John and Pearson, 1986). In this respect it is interesting to note that hyperhydrated snowdrop tissues were capable of initiating new bulblets.

Root initiation
Although no specific rooting trials were established some spontaneous root initiation occurred in most experiments. Tissues cultured on hormone-free medium consistently produced the highest proportion of rooted bulblets (Figure 1.1F).

© 2001 Horticultural Development Council

Table 1.4 Effects of the plant growth regulator composition of the induction medium on the numbers of bulblets formed by snowdrop bulb chip explants 2 months after inoculation. The data needed to be normalised by square root transformation before analysis (untransformed data shown in parenthesis) and 24 replicate cultures were inoculated per PGR treatment per species.

Species	Growth regulator composition of induction medium*						Species means
	Control	NAA	KNA	BA	BA + NAA	BA+ KNA	
G. nivalis	1.35 (3.04)	1.29 (2.08)	1.29 (2.23)	1.13 (1.61)	1.55 (2.78)	1.18 (2.20)	1.30 (2.32)
***G. nivalis* 2**[1]	1.93 (4.32)	1.65 (3.36)	1.95 (4.52)	1.58 (2.92)	1.92 (4.16)	1.67 (3.46)	1.78 (3.79)
G. elwesii	1.53 (3.71)	1.04 (1.78)	1.37 (2.61)	1.23 (2.33)	1.50 (4.47)	1.22 (2.32)	1.32 (2.87)
Media means	1.60 (3.69)	1.33 (2.41)	1.54 (3.12)	1.31 (2.28)	1.66 (3.81)	1.36 (2.66)	

*Control = no growth regulators, BA = 1mg/l, KNA = 0.1 mg/l, NAA = 0.083 mg/l.
[1]See note in the Experimental Section (plant materials).

Analysis of variance summary[a]

	d.f. (m. v.)	SED	Significance
Species (S)	2	0.107	***
BA (B)	1	0.087	NS
Auxin (A)	2	0.107	NS
S x B	2	0.151	NS
S x A	4	0.185	NS
B x A	2	0.151	**
S x B x A	4	0.261	NS
Residual	312 (79)		

[a] NS, not significant; (*), *, ** and ***, significant at the 10, 5, 1 and 0.1% levels of probability, respectively.

© 2001 Horticultural Development Council

Table 1.5 Numbers of bulblets formed after 12 weeks by individually cultured scale leaves, isolated from bulb chip explants at the end of the initiation culture passage of sterilisation experiment 1. None of the scale leaves were forming bulblets at the time of transfer and each was left with a short section of basal plate attached. Media used were basal medium without plant growth regulators (control), medium supplemented with 1mg/l BA and 0.1mg/l NAA (PGR), and the PGR medium supplemented with either 20mg/l benomyl (B) or 1% PPM. The percentages of scale leaf explants forming new bulblets are shown in parenthesis.

Species	Medium			
	Control	PGR	B	PPM
G. nivalis	5.0 (50)	6.5 (50)	5.7 (50)	0.8 (20)
G. nivalis 2	4.2 (70)	2.3 (30)	7.3 (70)	10.7 (60)
G. elwesii	7.6 (60)	5.0 (40)	10.7 (80)	9.0 (70)

© 2001 Horticultural Development Council

Table 1.6 Bulblet multiplication rate (final bulblet numbers / inoculated bulblet numbers) during the third culture passage and accumulative multiplication from an initial bulb in the first three culture passages assuming four and six bulb chip explants were prepared from *G. nivalis* and *G. elwesii* respectively. Fungicides used were benomyl (B), carbendazim (C) and imazalil (I).

Expt..	Media composition (mg/l)						*G. nivalis*		*G. elwesii*	
	BA 1.0	NAA 0.1	PPM 1%	B 20	C 20	I 20	In passage three	per bulb	In passage three	per bulb
1	-	-	-	-	-	-	1.05	6.7	1.57	38.1
	+	+	-	-	-	-	3.34	65.9	1.73	32.1
	+	+	+	-	-	-	1.18	37.3	1.11	39.5
	+	+	-	+	-	-	1.57	22.5	1.28	36.6
2 & 3	+	+	-	-	-	-	1.35	25.1	1.41	39.5
	+	+	-	-	+	-	1.46	21.0	1.50	54.4
	+	+	-	-	-	+	1.21	27.7	1.19	28.9
	+	+	-	-	+	+	1.19	22.7	1.29	32.2
PGR	-	-	-	-	-	-	1.18	21.8	1.28	57.5
EXPT	+	-	-	-	-	-	1.44	20.6	1.32	27.7
	-	+*	-	-	-	-	1.35	15.5	2.28	49.5
	+	+*	-	-	-	-	1.56	30.2	1.45	54.0
Mean							1.49	26.4	1.45	40.8

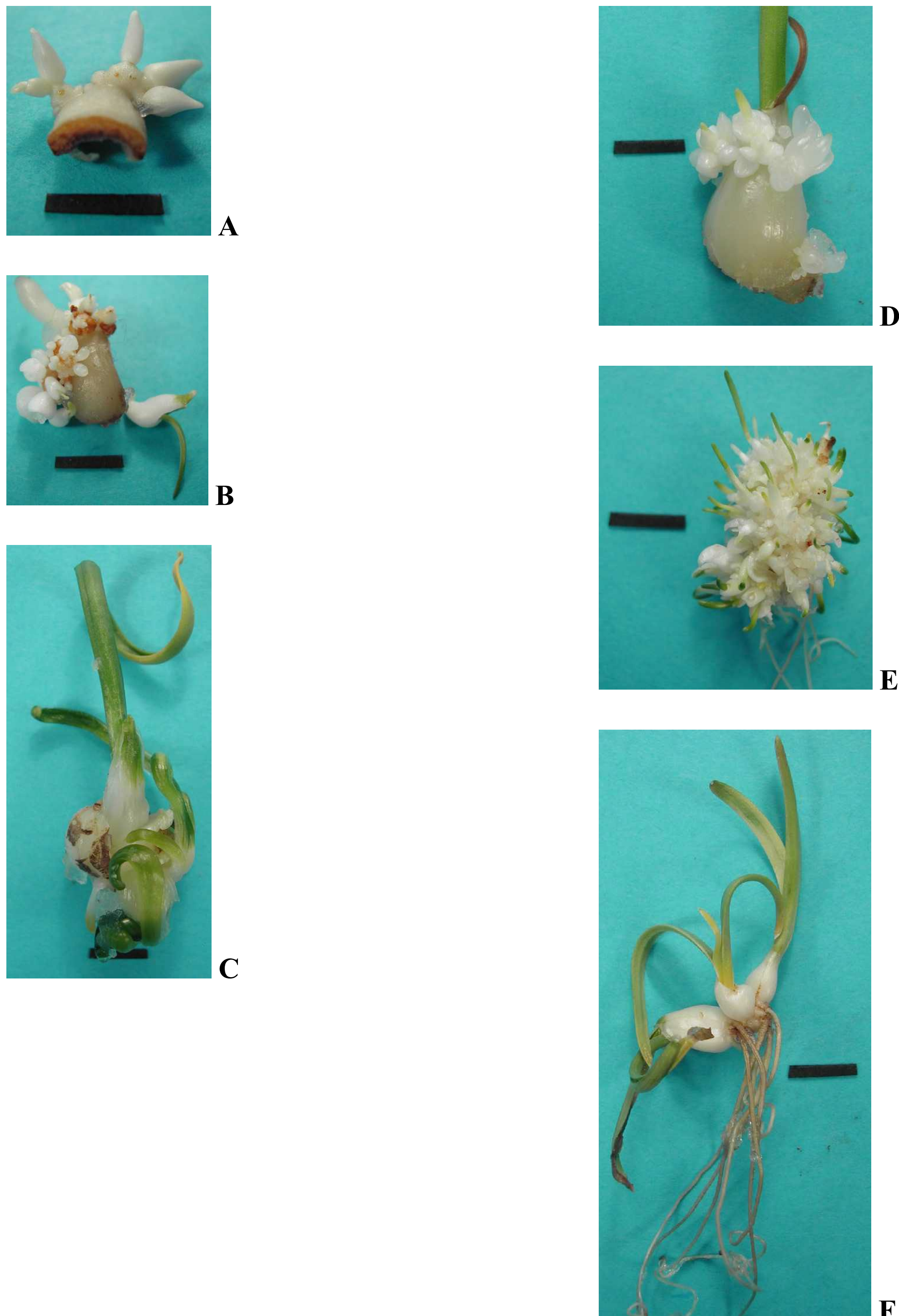
* In the PGR experiment NAA and KNA treatments were combined and transferred onto NAA medium for the third culture passage.

Table 1.7 Effects of different methods of sub-culture on bulblet multiplication. Bulblets were either transferred as small clumps, single bulbs with most basal scale leaf tissue, which the bulblet had initiated on, cut away or as half or quarter bulblet sections prepared by longitudinal cutting. The percentages of explants forming new bulblets are shown in parenthesis.

Species	Bulblet numbers per clump		Bulblet multiplication per clump	Numbers of new bulblets formed per:		
	initial	final		single bulblet	half bulblet	quarter bulblet
G. nivalis	4.3	10.9	2.5	0.5 (21)	1.0 (53)	- -
G. elwesii	1.9	5.3	2.8	2.3 (41)	3.1 (82)	2.3 (89)

© 2001 Horticultural Development Council

Figure 1.1. Basal bulblet formation on the abaxial surface of a *G. nivalis* bulb scale (A) compared to *G. elwesii* where bulblets formed more uniformly over the outer scale surface (B). Hyperhydrated *G. elwesii* bulblet showing deformation of bulblet and leaf tissue (C) that could sometimes result in new bulblets initiating on the outer surface of hyperhydrated bulblets (D). High bulblet multiplication in *G. elwesii* on a section of bulb scale from the PGR experiment (E) and rooted *G. nivalis* bulblets from the same experiment cultured on hormone-free medium (F). Bars = 1cm.



© 2001 Horticultural Development Council

AGRONOMY

Materials and methods

Plant material

40 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.

At planting, bulb samples were taken and examined for the presence of *Botrytis galanthina* by Cheryl Brewster (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*.

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

© 2001 Horticultural Development Council

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 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 will be applied as in 2000-2001.

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, half-way into the adjacent guard areas.

Due to consistently wet weather irrigation was not necessary during the 2000-2001 growing season.

In summer 2001, following complete die-down of the foliage, the remaining straw mulch was removed. It was replaced in early-November 2001.

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

© 2001 Horticultural Development Council

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 ryegrass 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 ryegrass was cut close to ground level using a strimmer. Narcissus bulbs were left in place.

Agronomy experiments - crop records

Crop production was assessed on one sub-plot 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 and flower stems (February 2001)
- Percentage of foliage die-back (24 May 2001)
- Number of seed pods and seeds (June 2001)
- Number and weight of bulbs <4 cm and >4 cm circumference (after lifting, cleaning and lightly surface-drying bulbs in July 2001)

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’) and Irrometer ‘water sensors’)
- Levels of photosynthetically active radiation (PAR) at median leaf height (Delta-T QS sensor)

Storage experiments

The bulb stocks planted alongside the agronomic experiments were allocated for use in storage experiments. Storage experiments were deferred until 2002 because of the generally small size of bulbs in these stocks.

© 2001 Horticultural Development Council

Results and discussion

Main experiment

As a check on bulb survival and the uniformity of planting, the number of emergent shoots per plot was recorded on 14 February 2001 (Table 2.1). It should be emphasised that this was a spot-check, since shoot emergence occurred over a prolonged period, so the numbers recorded at any one time were considerably fewer than the number of bulbs planted. In future years, shoot emergence will be monitored at intervals. Whilst the figures confirm the overall uniformity of planting and crop emergence, there appeared to be significant effects of both windbreak and mulching on the numbers of shoots counted. There were fewer shoots in non-sheltered plots, suggesting wind-damage and loss of some foliage, and fewer in mulched plots, suggesting that the presence of the straw mulch impeded the emergence of weaker shoots.

There were no significant effects of treatments on the numbers of flower stems, developing seed-pods and seeds per plot (Table 2.1). This was as expected, since flower initiation in snowdrops will have occurred early the previous summer, but it does confirm that flower survival and development was reasonably uniform throughout the stock and unaffected by the treatments applied. Note, however, that there was a just-significant difference between seed numbers in non-shaded plots (54 per plot) and in shaded plots (74-79 per plot).

There were strong effects of both shading and mulch (but not of windbreak) on the rate of foliage senescence (Table 2.1). By mid-May, 91% of foliage had died-back in non-shaded plots, compared with 65-68% with shaded plots, and 75% in mulched plots compared with 69% in non-mulched plots. Along with the effects of shading on seed production, this suggests that natural light intensities in an exposed site are unfavourable to post-flowering development.

Bulb yields after the first year's growth are shown in Table 2.2. Bulb yields (numbers and weights) were consistently higher in shaded than in non-shaded plots, although this effect did not always achieve statistical significance. Also, there was no clear 'dose-response' relationship between bulb yields and the density of shading used. The shading effect applied to yields of both small and large bulbs, and did not appear to operate by changing the ratio of smaller to larger grades. Mulching appeared to increase the yield of small bulbs, presumably by protection from desiccation.

Although based on only a single year's observations, these findings suggest that the erection of simple shading material over snowdrop crops would provide some protection and, particularly, would favour delayed leaf senescence thereby allowing greater bulking of bulbs (and greater seed production). Foliar fungicides that delay leaf senescence should also be evaluated for the same reason. Mulching and the provision of side-windbreaks have smaller effects on snowdrop development, but mulching might be worthwhile as it would also have the effect of controlling weeds (little information on safe herbicides for snowdrops is available).

© 2001 Horticultural Development Council

Table 2.1 Effects of shading, windbreak and mulch and irrigation treatments on snowdrop growth in the first year. Main effect means only presented.

Factors and treatments	Numbers per plot of: Shoots	Flower stems	Seed-pods	Seeds	% foliage die-back (24 May)
Shading					
None	31.4	17.3	10.1	54.4	91
Light	34.9	15.3	11.7	78.5	66
Medium	33.8	15.0	11.1	73.6	68
Dense	32.4	16.8	10.9	79.3	65
SED (60 d.f)	1.67	1.37	1.15	10.3	2.7
Windbreak					
None	31.9	16.0	10.5	69.2	72
Yes	34.4	16.2	11.3	73.7	72
SED (60 d.f.)	1.18	0.97	0.82	7.28	1.9
Mulch and irrigation					
None	35.4	16.6	11.5	70.7	69
Yes	30.8	15.6	10.4	72.2	75
SED (60 d.f.)	1.18	0.97	0.82	7.28	1.9
Analysis of variance summary [a]					
Shading (S)	NS	NS	NS	(*)	***
Windbreak (W)	*	NS	NS	NS	NS
Mulch and irrigation (M)	***	NS	NS	NS	**
S x W	NS	NS	NS	NS	NS
S x M	NS	NS	NS	NS	NS
W x M	NS	NS	NS	NS	(*)
S x W x M	NS	NS	NS	NS	NS

[a] NS, not significant; (*), *, ** and ***, significant at the 10, 5, 1 and 0.1% levels of probability, respectively.

© 2001 Horticultural Development Council

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

Factors and treatments	Bulb yields per plot					
	Numbers			Weight (g)		
	< 4cm	> 4cm	Total	< 4cm	> 4cm	Total
Shading						
None	14.7	37.4	52.0	9.2	89.0	98.2
Light	19.0	41.3	60.3	13.4	97.2	110.6
Medium	17.6	43.1	60.6	12.3	98.7	110.9
Dense	17.5	40.2	57.7	12.8	98.4	111.2
SED (60 d.f)	1.58	2.08	2.63	1.07	6.79	6.92
Windbreak						
None	17.2	41.6	58.8	11.9	97.3	109.2
Yes	17.1	39.4	56.5	11.9	94.3	106.2
SED (60 d.f.)	1.12	1.47	1.86	0.76	4.80	4.89
Mulch and irrigation						
None	16.3	41.0	57.3	11.1	98.0	109.1
Yes	18.0	39.9	57.9	12.7	93.6	106.3
SED (60 d.f.)	1.12	1.47	1.86	0.76	4.80	4.89
Analysis of variance summary [a]						
Shading (S)	(*)	(*)	**	***	NS	NS
Windbreak (W)	NS	NS	NS	NS	NS	NS
Mulch and irrigation (M)	NS	NS	NS	*	NS	NS
S x W	NS	NS	NS	NS	NS	NS
S x M	NS	NS	NS	NS	NS	NS
W x M	*	NS	NS	*	NS	NS
S x W x M	NS	NS	NS	NS	NS	NS

© 2001 Horticultural Development Council

Inter-crops experiment

There were no significant effects of treatments on the numbers of shoots, flower stems, seed pods or seeds obtained per plot (Table 2.3).

There was a significant effect of treatment on the rate of foliage dieback (Table 2.3). When assessed, only 83-84% dieback had occurred under shading, compared with 93% in the untreated plot and 98+% where inter-crops had been used.

While there were no significant effects of treatments on the relatively small yields of small bulbs, the effects on the yields of larger bulbs (>4cm circumference), and on the total yield, were significant (Table 2.4). Yields were greater in untreated and shaded plots (totals of 102-114g per plot) than in inter-cropped plots (69-88g per plot), being particularly low (69g per plot) where ryegrass had been over-sown.

Confirming the results from the main experiment, the provision of artificial shading appears to be a useful way of increasing yield in snowdrops. The inter-crops – and particularly ryegrass – appeared to be too competitive for practical use.

Table 2.3 Effects of shading and intercropping on snowdrop growth in the first year.

Treatments	Numbers per plot of:				% foliage dieback (24 May)
	Shoots	Flower stems	Seed-pods	Seeds	
None	35.4	16.4	9.4	45.0	93
Light shading	36.4	14.2	7.4	38.6	84
Medium shading	34.0	15.4	5.0	21.6	85
Narcissus intercrop	34.2	16.8	9.2	45.0	100
Cereal intercrop	29.8	16.2	6.6	38.2	98
Rye-grass oversown	30.6	14.2	4.6	21.8	100
SED (20 d.f.)	3.06	2.61	2.11	14.33	4.0
Significance	NS	NS	NS	NS	***

© 2001 Horticultural Development Council

Table 2.4 Effects of shading and inter-cropping on snowdrop bulb yield in the first year.

Treatments	Bulb yields per plot					
	Numbers			Weight (g)		
	< 4cm	> 4cm	Total	< 4cm	> 4cm	Total
None	16.6	47.4	64.0	11.2	103.2	114.4
Light shading	20.4	44.2	64.6	14.0	93.6	107.6
Medium shading	18.0	42.6	60.6	13.2	89.2	102.4
Narcissus inter-crop	17.8	36.6	54.4	12.4	76.0	88.4
Cereal inter-crop	15.8	37.0	52.8	10.4	71.8	82.2
Ryegrass over-sown	16.2	30.8	47.0	11.6	57.2	68.8
SED (20 d.f.)	3.51	4.28	5.73	2.47	10.57	11.11
Significance	NS	*	*	NS	**	**

Meteorological data

Light and medium shading reduced mean PAR by nearly 40%, and heavy shading by 53%, compared with non-shaded controls (Table 2.5). Interestingly, there was little difference in maximum PAR values between the three types of shading. Mulching did not increase light levels (a possibility due to increased reflection from the soil surface). The small differences in PAR transmission through the different densities of shading explain the lack of a clear effect of different shading materials on growth and yield (see above).

Table 2.5 Effect of shading on PAR over the growing season

Treatment	PAR (mmol)		
	Mean	Mean as % of control	Maximum
Control[a]	0.220	100.0	1.715
Shade (light)	0.139	63.0	1.041
Shade (medium)	0.137	62.1	1.088
Shade (heavy)	0.094	42.7	1.052

[a]Control has no shade, windbreak or mulch

There was little effect of treatment on mean soil moisture levels, except for a reduction of over 10% where ryegrass was used (Table 2.6). However, the maximum soil moisture levels recorded were increased by mulching and with ryegrass. The changing effect of ryegrass may have been due to reduced evaporation in the earlier part of the year, followed by greater utilisation as temperatures increased. This goes some way to explaining the poor bulb yields where snowdrops were over-sown with ryegrass.

Table 2.6 Effect of various treatments on soil moisture over the growing season

	Soil moisture (m^3/m^3)		
Treatment	Mean	Mean as % of control	Maximum
Control	0.322	100.0	0.390
Mulch	0.330	102.5	1.000
Shade (heavy) + windbreak	0.313	97.1	0.500
Shade (heavy) + windbreak + mulch	0.329	102.2	1.020
Rye-grass	0.286	88.8	1.020
Cereal	0.329	102.0	0.430
Narcissus	0.349	108.4	0.480

Mulching had relatively little overall effect on air temperature, although it did raise the minimum temperature slightly compared with the control, perhaps by trapping warm air (Table 2.7). In plots with shading and windbreak there was a considerable raising of air temperature overall, and particularly in the minimum temperature. Some of the effect of shading on snowdrop growth may be due raised leaf temperatures.

Table 2.7 Effect of various treatments on air temperatures over the growing season

	Air temperature (°C)		
Treatment	Minimum	Mean	Maximum
Control	-2.5	9.4	24.5
Mulch	-2.1	9.4	24.5
Shade (heavy) + windbreak	-0.4	10.4	25.1
Shade (heavy) + windbreak + mulch	-0.6	10.5	26.1

© 2001 Horticultural Development Council

Compared with control plots, mean soil temperature (at bulb depth) was decreased where shading was used and with increasing density of shading (Figure 2.1). Mulching had a smaller effect in decreasing mean temperature, while using a windbreak (and inter-crops, to a lesser extent), increased it. Heavy shading and, particularly, using ryegrass, greatly reduced the range of temperatures experienced by bulbs (Figure 2.1).

Figure 2.1 Mean soil temperature through 2000 growing season

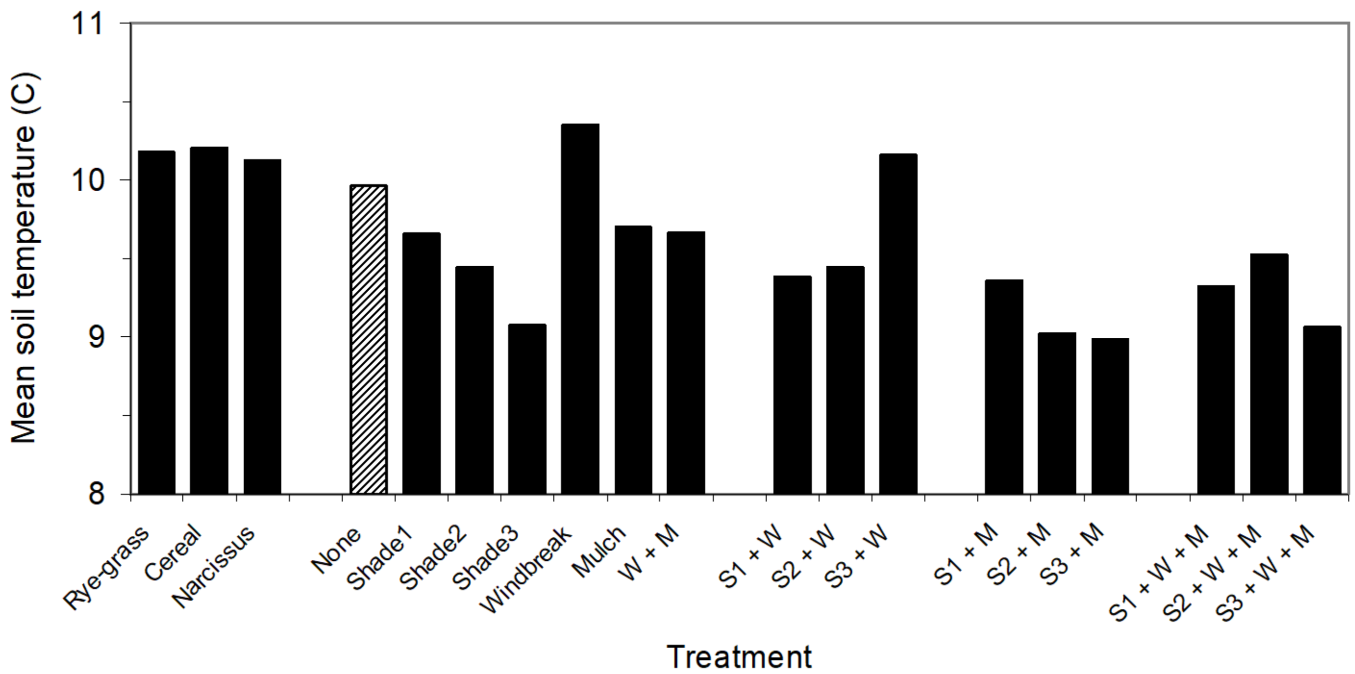
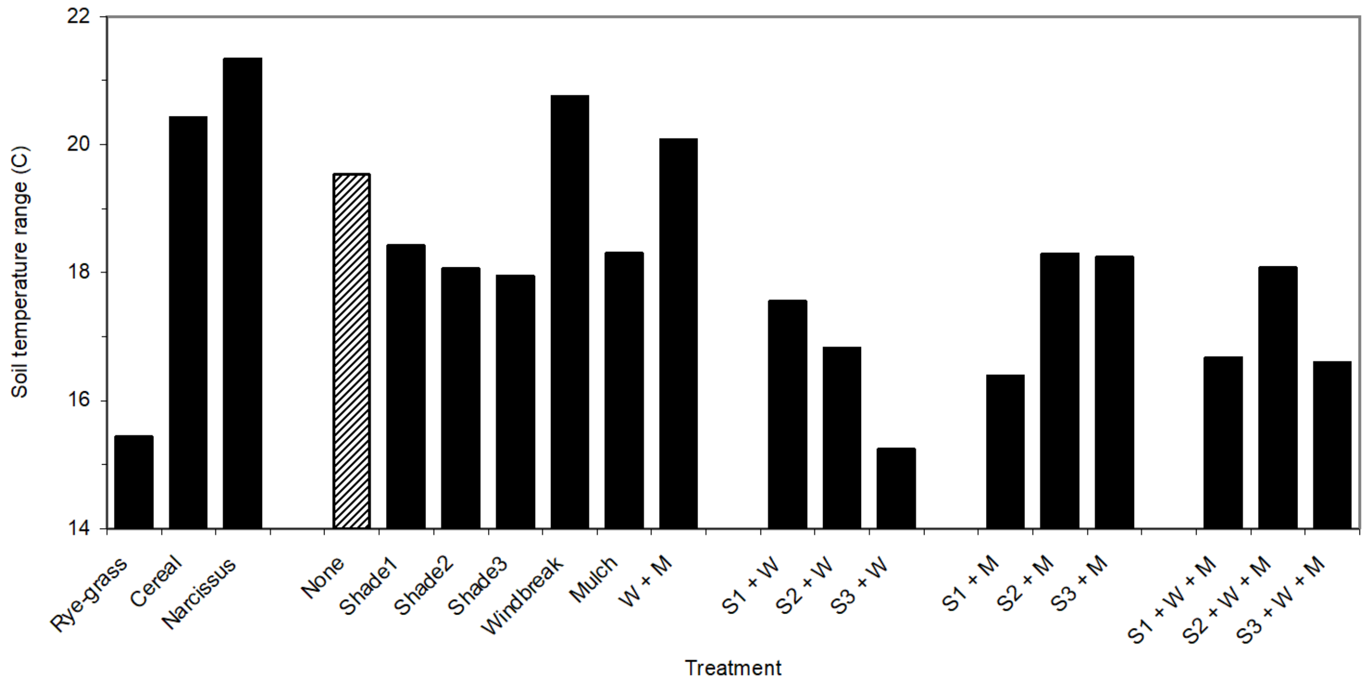


Figure 2.2 Soil temperature range through 2000 growing season



© 2001 Horticultural Development Council

ACKNOWLEDGEMENTS

The authors thank Pippa Hughes and Rod Asher for carrying out this work at HRI Kirton, Dr Sally Watson (DARD) for statistical analysis of the micropropagation, and Cheryl Brewster (HRI, Stockbridge House) for examining bulbs for *Botrytis*.

REFERENCES

BOUMAN, H., MORRIS, B. and TIEKSTRA, A. (2001) Development of new tissue culture media, using the relation between mineral composition of plant and medium. *Acta Horticulturae,* **560**, 373-376

BOUMAN, H. and TIEKSTRA, A. (2001) Mineral nutrition in tissue culture: influence on propagation and quality of the plantlets. In: *Plant nutrition - food security and sustainability of agro-ecosystems* (ed. W.J. Horst *et al.*), Kluwer Academic Publishers, Dordrecht, pp. 316-317

DEBERGH, P., AITKEN-CHRISTIE, J., COHEN, D., GROUT, B. VON, ARNOLD, S., ZIMMERMAN, R. and ZIV, M. (1992) Reconsideration of the term 'vitrification' as used in micropropagation. *Plant Cell, Tissue and Organ Culture*, **30**, 135-140

FULCHERI, C., MORARD, P. and HENRY, M. (1998) Stimulation of the growth and the triterpenoid saponin accumulation of *Saponaria officianalis* cell and *Gypsophila paniculata* root suspension cultures by improvement of the mineral composition of the media. *Journal of Agricultural and Food Chemistry*, **46**, 2055-2061

JOHN, A. and PEARSON, D.J. (1986) The induction of vitrification in *Picea sitchensis* cultures. *New Zealand Journal of Forest Research,* **16**, 328-342

MORARD, P. and HENRY, M. (1998) Optimization of the mineral composition of *in vitro* culture media. *Journal of Plant Nutrition*, **21** (8), 1565-1576

© 2001 Horticultural Development Council