Project title:	Biology, epidemiology and control of liverwort infestation of nursery plant containers
Project number:	HNS 126
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Previous reports	None
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Key words:	Liverwort, *Marchantia polymorpha*, Hardy Nursery Stock

The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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Introduction

Liverworts growing on the surface of container plant compost are a major problem to the horticultural industry, affecting both protected and hardy nursery stock, and are most prevalent in the warm, moist conditions provided in propagation systems, where plants are at their most vulnerable.

According to Horticultural Development Council (HDC) study HNS 93, the removal of liverwort, moss and weeds from pots is estimated to cost the horticultural industry 4% of total production costs, equating to £13 million annually (mainly labour costs). These figures, combined with zero tolerance for liverworts in accreditation schemes and government withdrawal of chemical approvals is putting huge pressure on the industry. Chemicals currently in use have a short-lived effect and multiple applications are necessary. (Scott and Hutchinson, 2001)

The overall aim of this project is to provide information on liverwort biology and epidemiology of infestation currently lacking, enabling future research to be targeted towards areas of weakness in the liverwort life cycle and biology. The identity of the liverwort species infesting nurseries will be established; it is commonly considered to be *Marchantia polymorpha.* There will be an investigation of non-chemical means of controlling liverwort: fungal pathogens, glucosinolate hydrolysis products obtained from seed material and cultural methods. Glasshouse trials based on the results of these investigations will consider the use of individual and integrated methods of control.

Experiment 2 investigates one aspect of vegetative reproduction used by *Marchantia polymorpha*. Gemma cups are circular structures found on the upper surface of the liverwort thallus that produce gemmae, vegetative propagules that are released when water splashes into the cup, transporting the gemmae away from the parent plant. The gemma cups of *Marchantia polymorpha* form the most efficient splash-cup mechanism, having sides at an angle of 60-70^{o} to the horizontal and lentil-shaped gemmae (Brodie, 1951). He noted dispersal distances of up to two feet by small

raindrops. Water droplets falling into the gemma cup displaces water, thrusting gemmae upwards along the cup sides.

Research has been conducted into the dispersal of spores of fungal pathogens, and can be broadly compared with liverwort gemmae dispersal. An experiment (Geagea et al., 1999) where droplets of four different uniform sizes were released from a generator positioned at three different heights above leaves covered with sporulating lesions of brown (*Puccinia recondita* f.s.*p. tritici*) and yellow (*Puccinia striiformis)* rust showed that dispersal rates and distances travelled by spores increased with increased droplet size and nozzle height.

Materials and Methods

Identification of liverwort species infesting nurseries

HDC members and ADAS consultants have been invited, via an article in the HDC News, and individual letters to ADAS consultants, to contact us with details of samples of liverworts that appear to be different to the commonly reported *Marchantia polymorpha*. No instances have been reported as yet, and no samples received. There are no current plans to visit other nurseries for liverwort collection.

Liverwort culture

Liverwort cultures have been established (separate male and female plants):

- In M51C media (Ono *et al*., 1979), substituting phytagel for agar. These cultures are maintained in growth rooms at 21^0C, and with an 8 hour day.
- In compost media, maintained under glass in short day conditions.

Both these cultures will be propagated as necessary for use during growth cabinet and glasshouse experiments.

Fungal cultures

Fungal species identified as potential parasites on liverworts have been sourced from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands: *Bryoscyphus atromarginata* and six strains of *Phaeodothis winteri*.

Further examples of fungi have been isolated from dying liverworts provided by John Atwood (ADAS). Four fungal species have been isolated from these liverworts, established on PDA and identified by CABI Bioscience, Egham as *Fusarium equiseti, Penicillium velutinum* and *Trichoderma harzianum* (2 samples).

Specimens of each fungal species have been established in culture on either Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA) media. They have been established on slopes as a longer-term maintenance procedure and in petri dishes for current experimental use. Preliminary pathogenicity testing has begun.

Experiment 1: The effect of light level and temperature on the growth and development of male and female *Marchantia polymorpha*

An experiment using four Fitotron growth cabinets compared the effect of two different temperatures (25ºC and 15ºC) and two light levels (800 µ mol m^{-2} s^{-1} and 400 µ mol m^{-2} s^{-1}) on the growth (radial expansion), dry weight accumulation and development of male and female liverworts over 6 weeks.

The two different temperatures used were replicated at cabinet level, i.e. two cabinets were set at 25ºC and two cabinets at 15ºC. Within each cabinet the shelf heights were adjusted to give the two light levels (upper shelf: 800 µ mol m^{-2} s^{-1}, lower shelf: 400 µ mol m^{-2} s^{-1}). This arrangement did not allow for random allocation of light treatments. For each treatment, equal numbers of male and female gemmae were placed in separate blocks. Humidity was set at 65% and a photoperiod of 12 hours.

In total, 288 *Marchantia polymorpha* gemmae (72 per cabinet) were placed individually onto M51C Media, in lidded 10cm diameter Bellaplast pots (one gemma per pot). The cabinets, pot positions and separate male and female areas were all randomly allocated, an example of which is shown in Figure 1.

Three pots were removed from each treatment on 6 occasions, at 7-day intervals (48 pots; 3 male and 3 female gemmae from each light treatment). The order in which samples were removed for data collection was randomly designated prior to running

High Light Treatment	M	M	M	M	M	M		F	F	F	F	F	F
	M	M	M	M	M	M		F	F	F	F	F	F
	M	M	M	M	M	M		F	F	F	F	F	F
Low Light Treatment	F	F	F	F	F	F		M	M	M	M	M	M
	F	F	F	F	F	F		M	M	M	M	M	M
	F	F	F	F	F	F		M	M	M	M	M	M

Figure 1. Example of the layout within each Fitotron cabinet.

Shelves containing high light treatment (800 µ mol m^{-2} s^{-1}) were positioned higher (nearer to the fluorescent tubes) than those containing the low light treatment levels (400 µ mol m^{-2} s^{-1}). M and F refer to Male and Female liverwort gemmae respectively.

the experiment. On each occasion each liverwort was placed on a light box and photographed using a Nikon Coolpix 995 digital camera and the area of each liverwort was calculated using ImageJ software. (Web location: http://rsb.info.nih.gov/ij/).

The number of gemma cups present on each gemma was also counted and once three had developed the number of gemmae they contained was estimated. The method developed involved transferring three gemma cups containing gemmae into an epindorf containing 500 µL water and a drop of Tween 20 to reduce surface tension. The gemma cup material was then removed and 200 µL water with gemmae were transferred to a piece of filter paper and the gemmae counted. The fresh and dry weight of the final set of replicates was also recorded.

Experiment 2: The effect of nozzle size, water pressure and nozzle height on gemmae dispersal using an overhead sprinkler system

This experiment investigates the dispersal distance of liverwort gemmae by a glasshouse overhead sprinkler system, using three different sprinkler nozzle sizes, four different water pressures (1.5, 2, 2.5 and 3 bar) and two different nozzle heights (1 and 2 metres). The nozzles used were Agridor 700 Dynamic Sprayers manufactured by Ein Dor (Ein Dor Ltd. 3 Ha'ard St, P.O.B 13129 Tel-Aviv, Israel. www.eindor.com.), colour coded by the manufacturer to denote the water flow rate they produce (Brown = 160 lh^{-1}, Blue = 105 lh^{-1} and Grey = 60 lh^{-1}).

A system was set up in the glasshouse with a hose attached to a water tap and an adjustable in-line water pressure adapter fitted (Flamco pressure reducing valve type

‘¾” PR’. Flamco UK Ltd, www.flamco.co.uk). The hose was fitted around the glasshouse cubicle to a central position along one wall with an anti-drip device and sprinkler nozzle attached to the end and held in position by a length of steel projecting towards the centre of the area, such that the nozzle could be operated at 1 and 2 metre heights (See Figure 2a and Plate 1). An Ein Dor 530 Non-Drip Valve was inserted above the nozzle to prevent the heavy water drops normally experienced as the water was turned off. A half tray (of similar height as the collection pots) with approximately 1/3 of the centre covered with mature liverwort thallus bearing gemma cups (Plate 2) was placed on the ground beneath the nozzle.

Collection pots were arranged so they were touching, as shown in Figure 2b, with three lines of 16 placed at right angles to each other. The number of collection pots required was determined by test trials. For each treatment, water was applied to the liverwort via the sprinkler system for 15 minutes, and then the number of gemmae that had fallen into each collection pot was counted. This arrangement was designed to reflect how liverwort could be dispersed within a nursery situation by water passing through an overhead sprinkler system above seed trays or pots containing liverworts.

Each nozzle is characterised by the flow rate and range of droplet sizes produced at each water pressure. Measuring the amount of water collected in a measuring cylinder in one minute and converting the result to litres per hour established the flow rate. A measure of relative droplet size for each nozzle still needs to be ascertained, using water sensitive paper.

A sequence of tests using the blue nozzle and a red dye in place of plant material, with water pressures of 2, 3 and 4 bar, indicated how gemmae are distributed. If the dye droplets travelled similar distances as the gemmae, this would imply the gemmae are splashed within the splash droplets after they hit the gemma cups. If the gemmae travelled further than the dye the implication would be that they are propelled into the air by the incident water droplets.

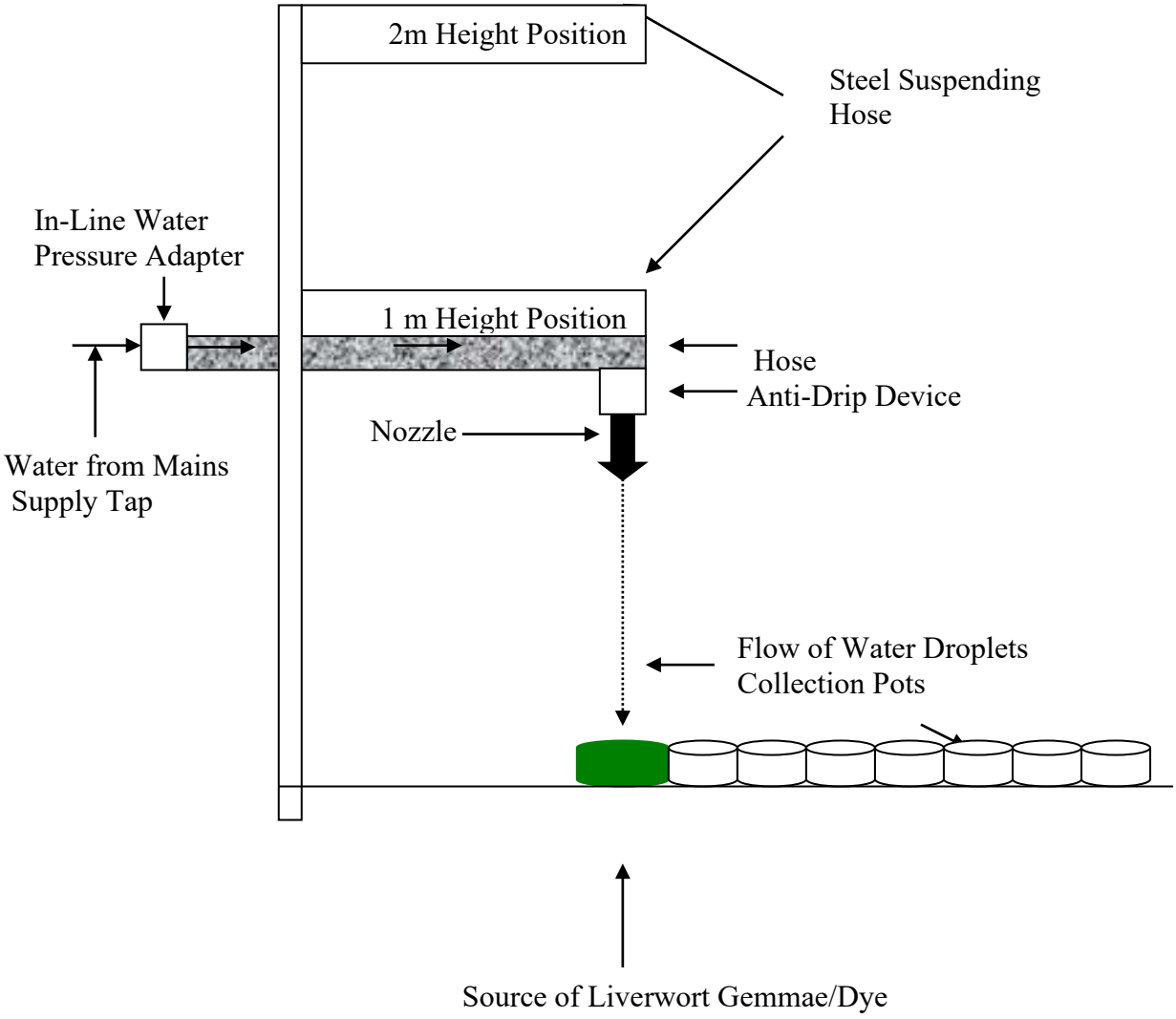


Figure 2a. Experiment Layout

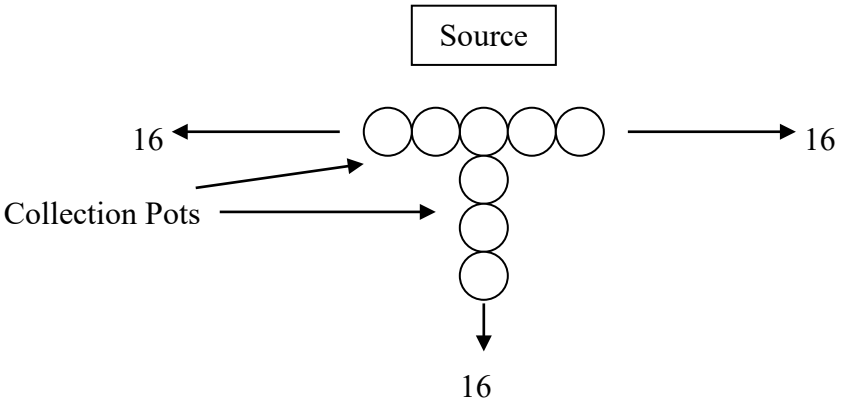


Figure 2b. Arrangement of collection pots

Plate 1. Equipment layout for experiment 2.

Plate 2. Example of liverwort used as gemmae source.

Experimental Results

Experiment 1: The effect of light level and temperature on the growth and development of male and female *Marchantia polymorpha*

Statistical analysis has not yet been applied to the results of this experiment, as the systematic arrangement of light treatments within the cabinets requires non-standard statistical software. However, the results do indicate how growth of gemmae and final fresh and dry weights were affected, shown in Figures 3a, 3b and 3c.

The results show that for the two temperature treatments there was an effect on gemma growth (radial expansion) due to light level. At 25ºC gemma growth, and fresh and dry weights after six weeks were greater when grown in low light levels compared to high light, and this trend was also seen at the lower temperature (Figures 3a, 3b and 3c). Overall, dry weights and gemma growth were greater for liverworts grown at 25ºC. When grown at 25ºC gemmalings tended to have a high relative growth rate during week 1, when compared to other treatments; this difference was markedly reduced in subsequent weeks, and in the high light, high temperature treatment relative growth rate was less in week 2 than in week 3.

Liverwort development was also monitored, with the presence and number of gemma cups and gemmae used as measurements. Gemmae development was faster in high light, high temperature treatments, with gemma cups appearing during week 2 (Figure 3d). In subsequent weeks the number of gemma cups present were variable, showing no distinct trends. Gemmae were found to clump together, making it difficult to estimate numbers. This aspect requires further investigation.

A number of observations were made concerning the morphology and colour of the gemmae throughout the experiment. By week 5 those grown in high light and high temperature conditions were a dull green colour; by week 6, 8 of the 12 replicates were developing dark brown colouration and reduced relative growth rate. The thallus had a dome-shaped appearance, rather than growing flat over the surface of the media.

Radial expansion of the gemmae was used as the measure of growth as the majority of growth is two dimensional. Fresh and dry weights were measured in the final week to confirm the validity of using gemma area as a measurement and found to broadly correspond to radial expansion measurements, although fresh weight gave the closest match.

Gemmae grown in lower temperature and light conditions were flatter and a lighter green colour. Although relative growth rates were variable they were generally sustained and, on occasions, increased to produce liverworts with more normal morphology and fresh weights similar to the high temperature, low light treatment after six weeks. These replicates may have had the potential to reach similar levels of growth as those in the high temperature, low light treatments, given a longer growth period.

Experiment 2: The effect of nozzle size, water pressure and nozzle height on gemmae dispersal using an overhead sprinkler system

The experiment was initially to be carried out at 1, 2, 3 and 4 bar. However, the nozzles did not operate below 1.5 bar, and at 4 bar very few gemmae were dispersed, thus 1.5, 2, 2.5 and 3 bar water pressures were used. The brown nozzle (160 $l h^{-1}$) did not operate at 1.5 bar, therefore no results were obtained.

A summary of the results of the preliminary experiment using red dye is shown in Table 1. Red splash droplets travelled a maximum of 45 cm, with the nozzle 2 metres high. However, when using liverworts as a source, gemmae were recorded at a maximum distance of 160 cm, with the nozzle at 1 metre high. This would indicate that whilst some gemmae may be transported within splash droplets, they were also propelled out of the gemma cups.

Table 1. Average distance travelled (cm) by splash droplets containing red dye using the blue nozzle at two different heights.

Water Pressure	1m	2m
4 Bar	33	38
3 Bar	43	37
2 Bar	28	45

Table 2 shows Nozzle Flow rates obtained. Flow rates quoted by the manufacture refer to tests carried out under laboratory conditions; differences are expected when nozzles are used in field or glasshouse situations. (Anon, Undated)

Table 2. Nozzle flow rates ($l h^{-1}$) at different water pressures

Water Pressure	Grey Nozzle	Blue Nozzle	Brown Nozzle
1.5 Bar	43.20	60.30	0.00
2 Bar	51.60	75.30	90.80
2.5 Bar	56.04	88.60	102.20
3 Bar	68.60	97.74	110.60

Results of the experiments using gemmae are shown as dispersal gradients (Figures 4a, b, c and d) and summarised in Table 3. Steeper dispersal gradients were obtained at 2 and 2.5 bar than 3 and 1.5 bar; the exception was the 3 bar, 2m height treatment using the grey nozzle. Using the grey nozzle, the distance travelled by gemmae clearly increased with increased nozzle height. However, for the blue and brown nozzles a number of gemmae travelled further when the nozzle was at the lower position; although the bulk of the gemmae did travel further when nozzles were higher.

Fewer gemmae were dispersed at the two extreme water pressures (1.5 and 3 bar) when the nozzle was set at the lower height; at the 2m nozzle height the two exceptions to this were for the brown nozzle, 3 bar treatment and the grey nozzle, 3 bar treatment. The brown nozzle (160 $l h^{-1}$) did not operate at 1.5 bar, therefore no

results were obtained. The brown nozzle (160 $l h^{-1}$) was generally the least effective at dispersing gemmae; the main exception occurring at 2 bar with the nozzle at the 2 metre height. The total number of gemmae dispersed was greater at the 2m nozzle height at all water pressures when using the brown nozzle; this was not the case in all treatments using the blue and grey nozzles.

Discussion

Without full statistical analysis of the results of Experiment 1 it is only appropriate to consider the trends observed.

Gemmae grown in high light, high temperature conditions initially appeared to benefit from this regime; in the longer term, however, these conditions proved detrimental as evidenced by the abnormal morphology and death of some replicates. When grown at the lower temperature and lower light, although relative growth rates were variable they were generally sustained and, on occasions, increased to produce liverworts with more normal morphology and fresh weights similar to the high temperature, low light treatment after six weeks. These replicates may have had the potential to reach similar levels of growth as those in the high temperature, low light treatments, given a longer growth period.

The results of this experiment do not appear to offer any indication that, by altering light or temperature within the parameters used, development of gemma cups could be reduced – in fact it was hastened by high light, high temperature treatments.

In Experiment 2 there was an interaction between water pressure, nozzle size and droplet size, and the results will be clearer once the spectrum of droplet sizes produced by each nozzle at each water pressure is obtained. The dispersal gradients (Figure 4a, b, c and d) vary considerably between treatments.

The preliminary experiment using dye appears to indicate that whilst gemmae may be dispersed within splash droplets, they are also propelled by the incident water drop. were also variable. Using the grey nozzle, the distance travelled by gemmae clearly

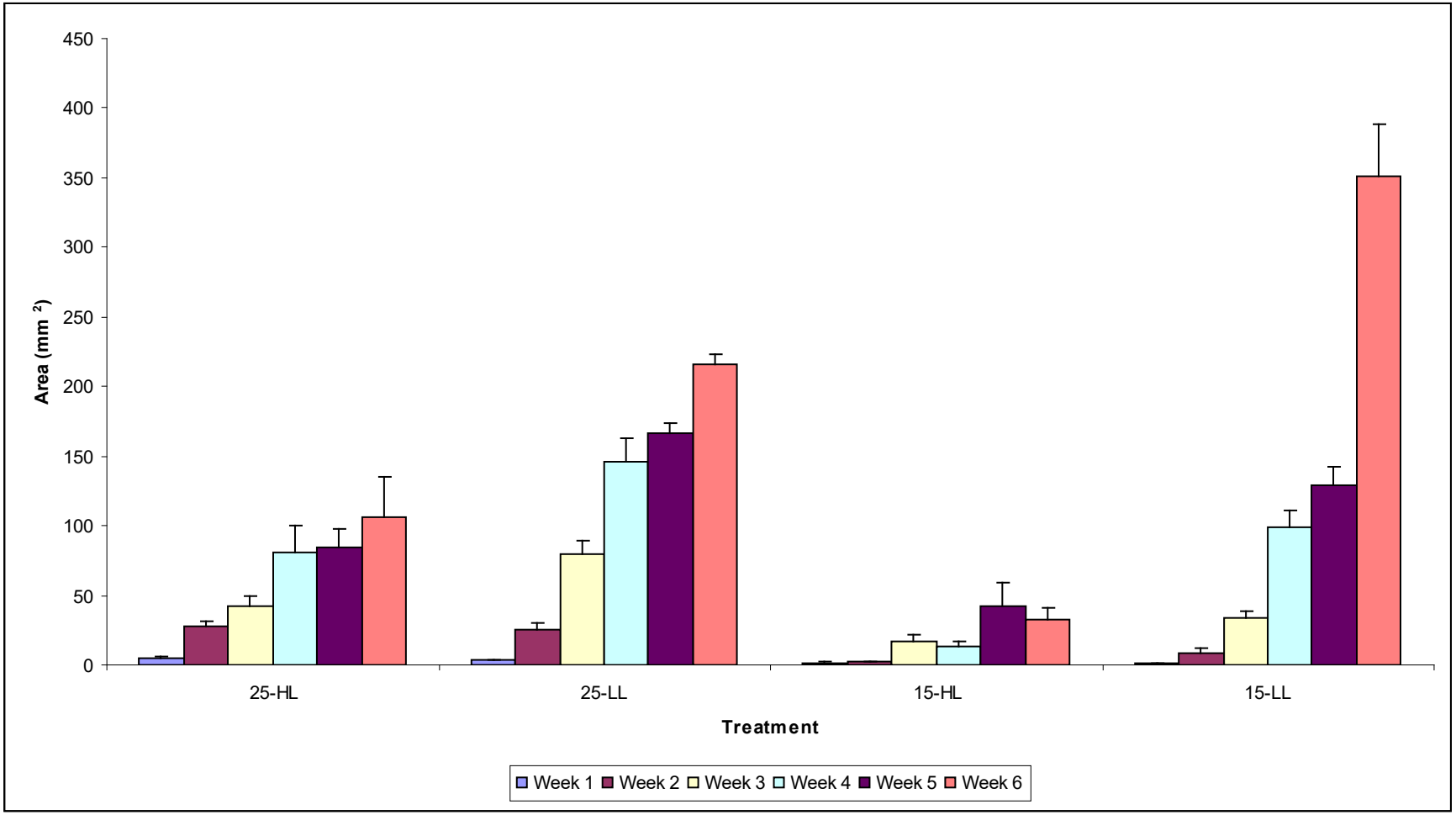


Figure 3a. Actual gemma growth under 2 light and temperature combinations.

HL=high light (800 µ mol m^{-2} s^{-1}), LL = low light (400 µ mol m^{-2} s^{-1}). Temperatures = 25ºC & 15ºC. M= male, F=female.

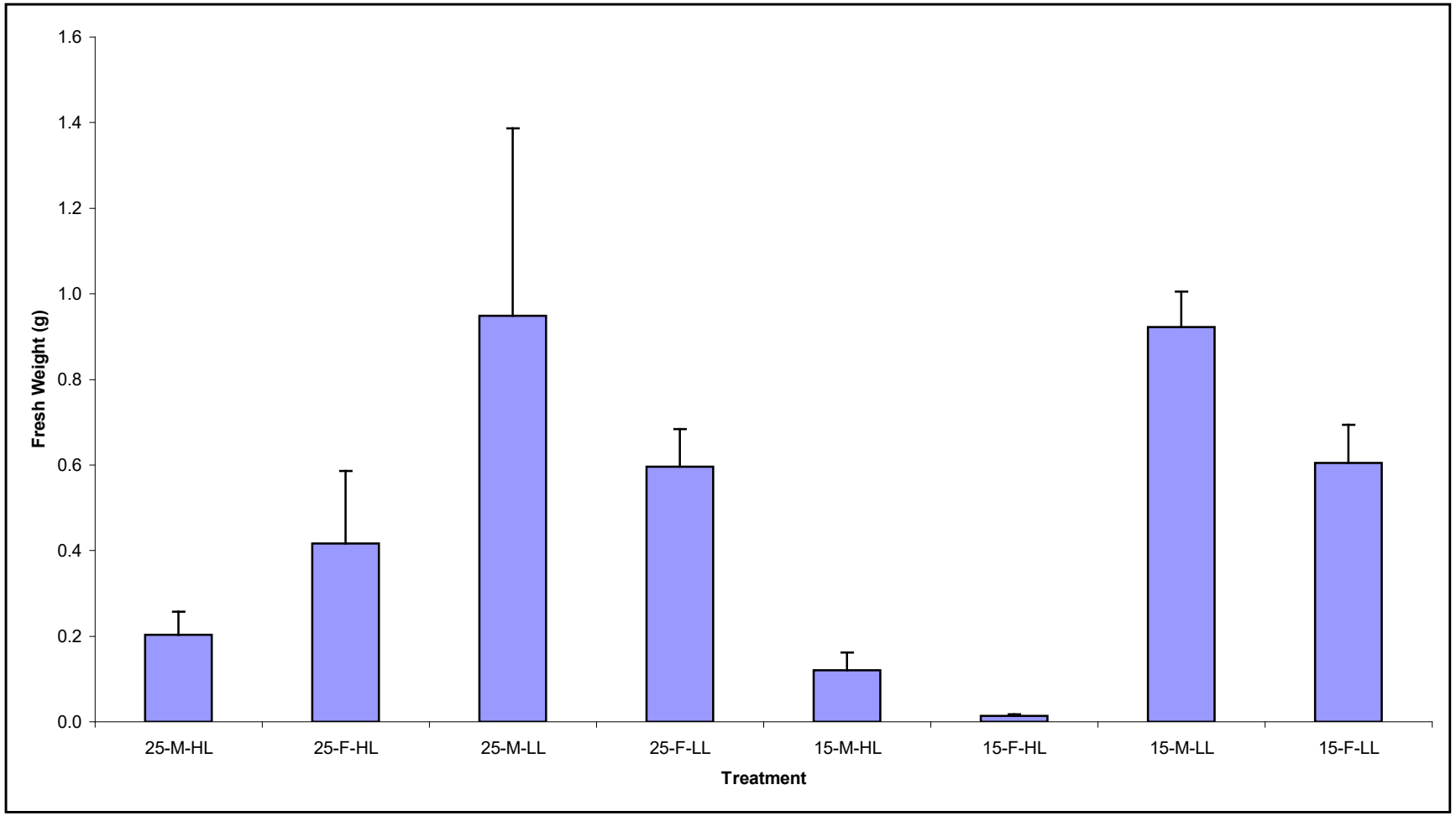


Figure 3b. Average fresh weight of gemmalings at 6 weeks.
HL=high light (800 µ mol m^{-2} s^{-1}), LL = low light (400 µ mol m^{-2} s^{-1}). Temperatures = 25ºC & 15ºC. M= male, F=female.

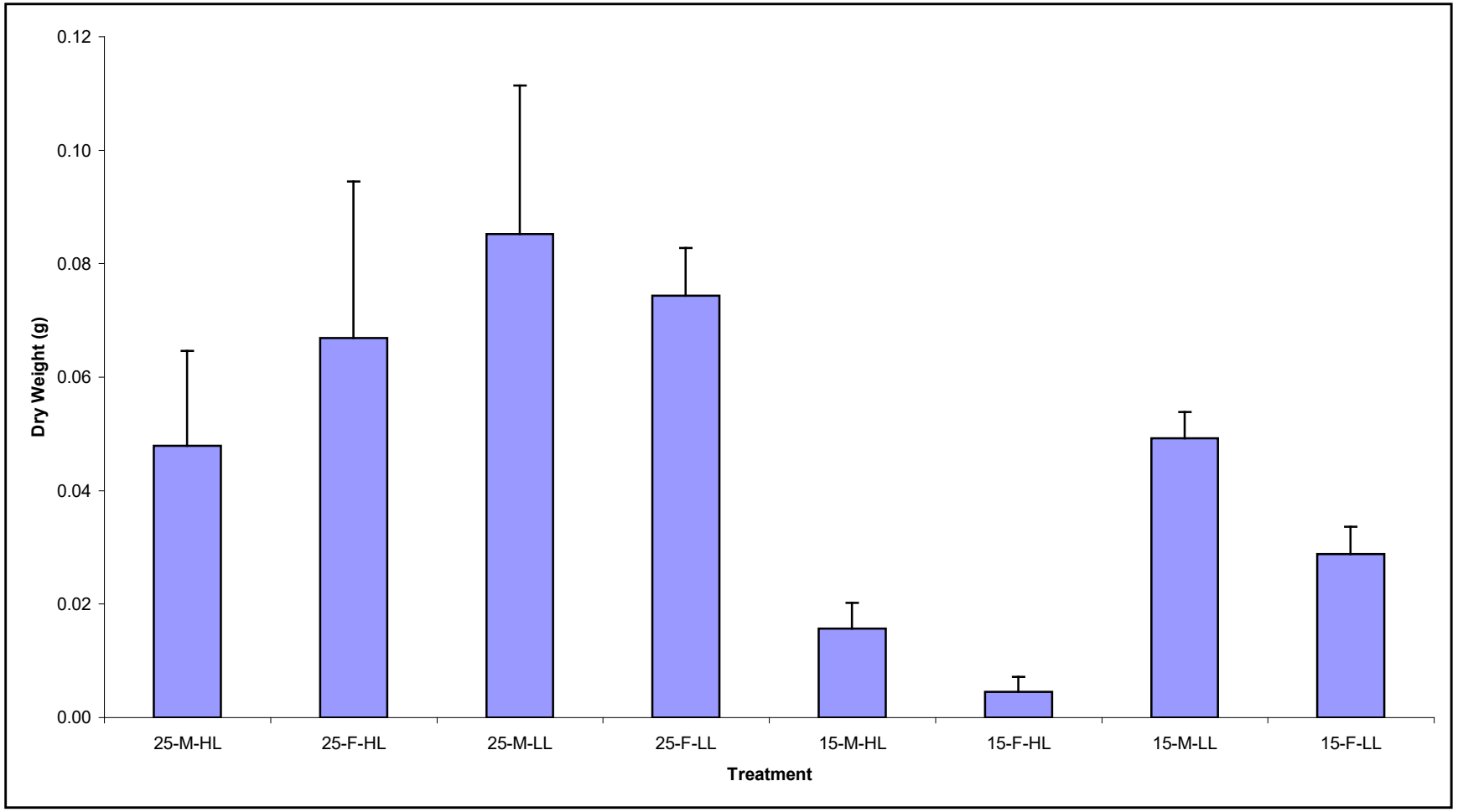


Figure 3c. Average dry weight of liverwort gemmalings at 6 weeks.

HL=high light (800 µ mol m^{-2} s^{-1}), LL = low light (400 µ mol m^{-2} s^{-1}). Temperatures = 25ºC & 15ºC. M= male, F=female.

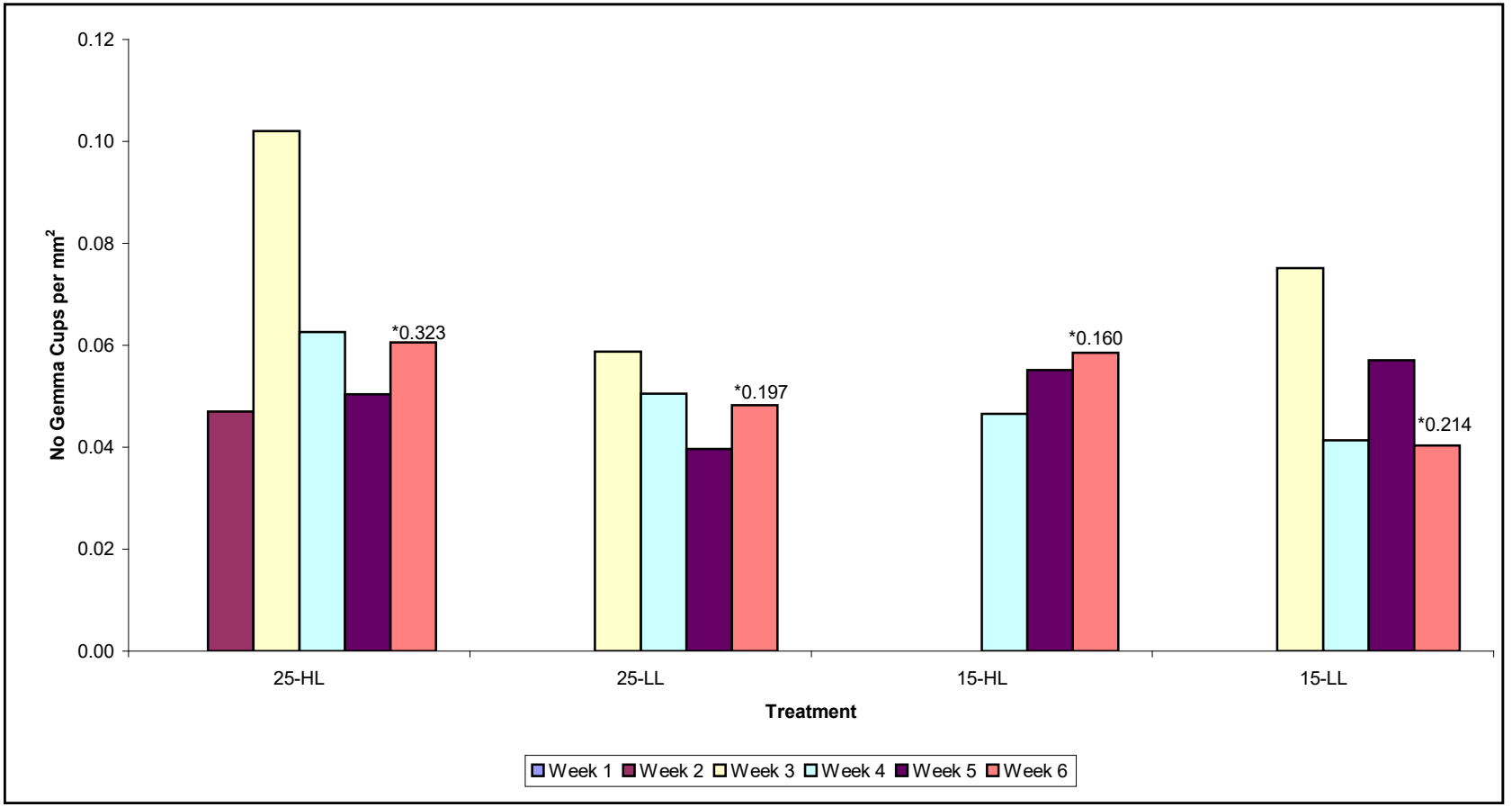
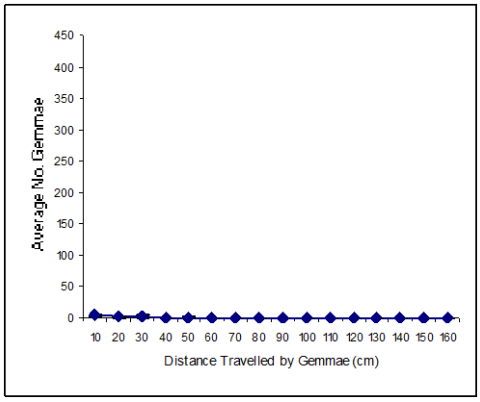


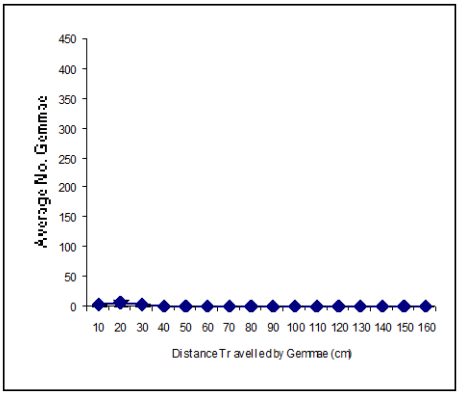
Figure 3d. No. gemma cups per mm^2.

HL=high light (800 µ mol m^{-2} s^{-1}), LL = low light (400 µ mol m^{-2} s^{-1}). Temperatures = 25ºC & 15ºC. M= male, F=female.
* = Cummulative no. of gemma cups per mm^2.

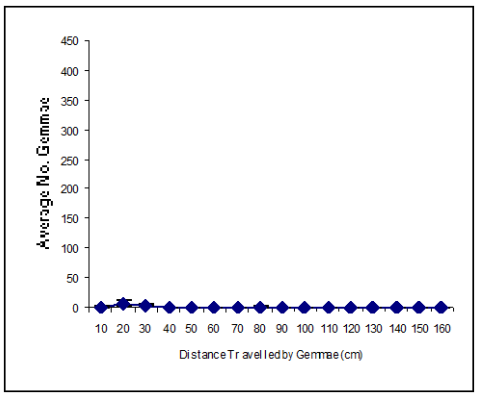
Nozzle Height → **1 Metre** **2 Metres**

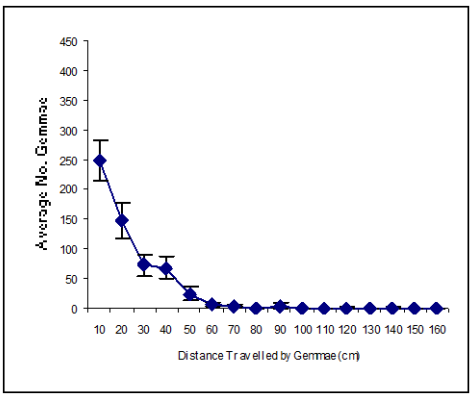
Blue Nozzle



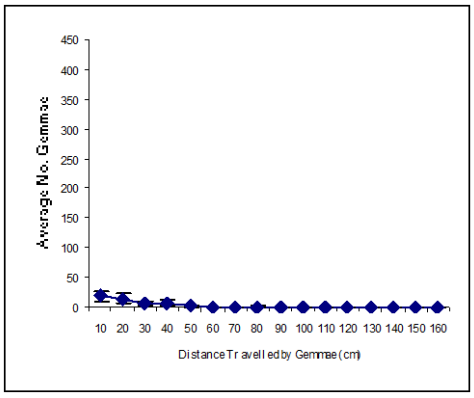


Grey Nozzle





Brown Nozzle



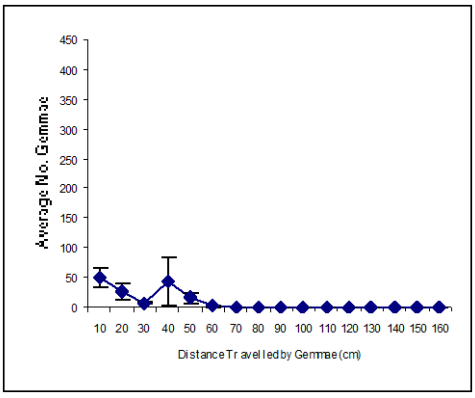
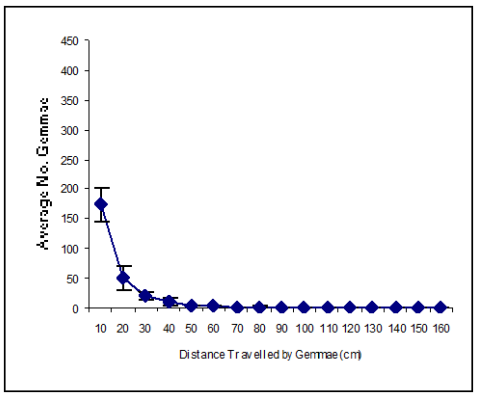
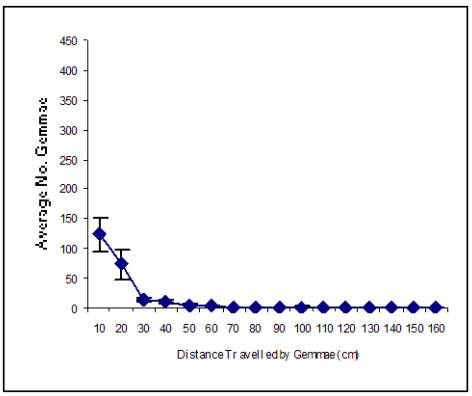


Figure 4a. Dispersal gradients obtained operating nozzles at 3 bar.

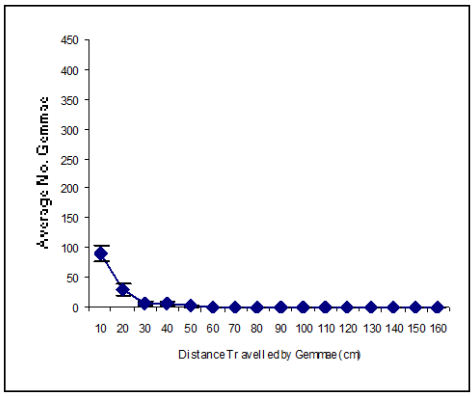
Nozzle Height → **1 Metre** **2 Metres**

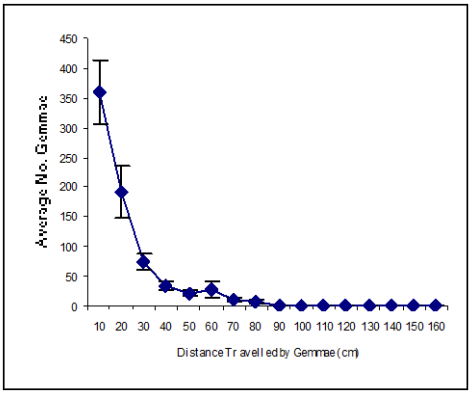
Blue Nozzle



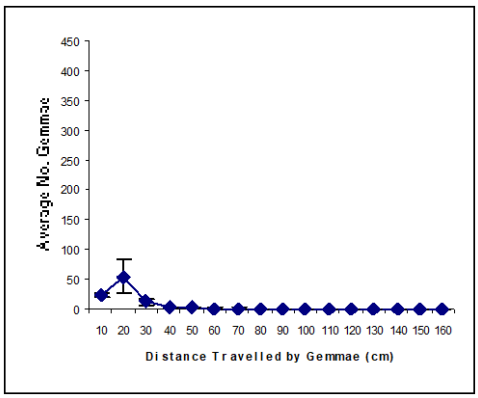


Grey Nozzle





Brown Nozzle



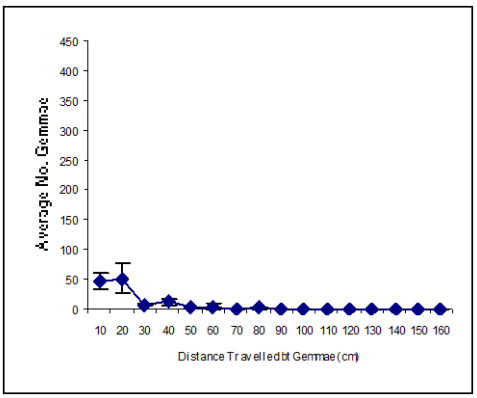
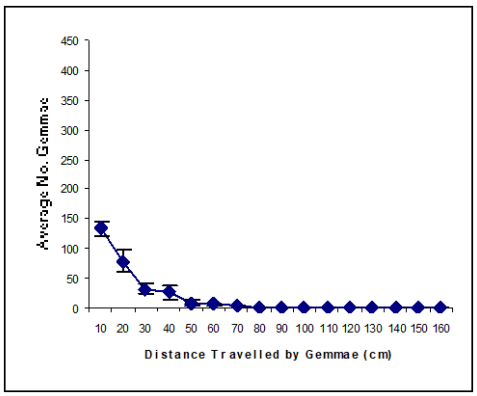
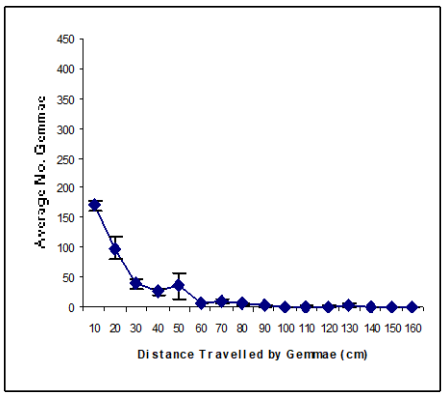


Figure 4b. Dispersal gradients obtained operating nozzles at 2.5 bar.

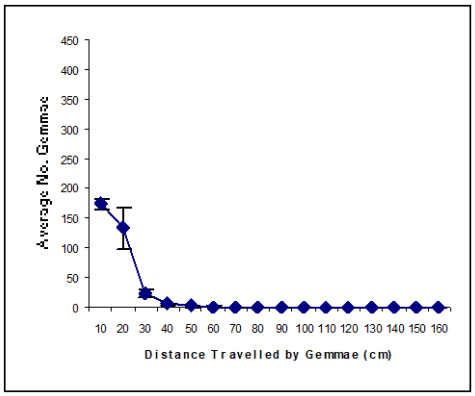
Nozzle Height → **1 Metre** **2 Metres**

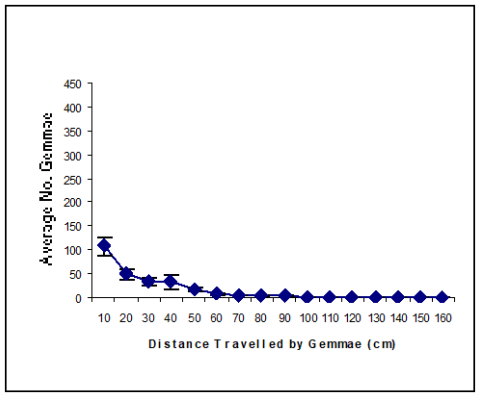
Blue Nozzle



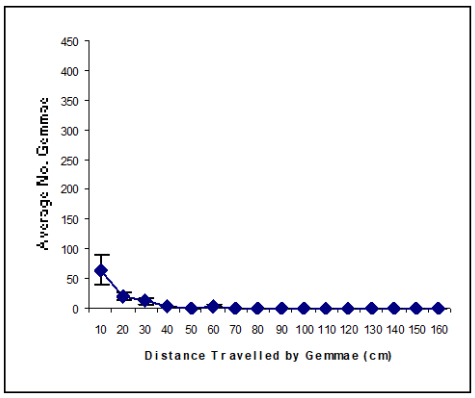


Grey Nozzle





Brown Nozzle



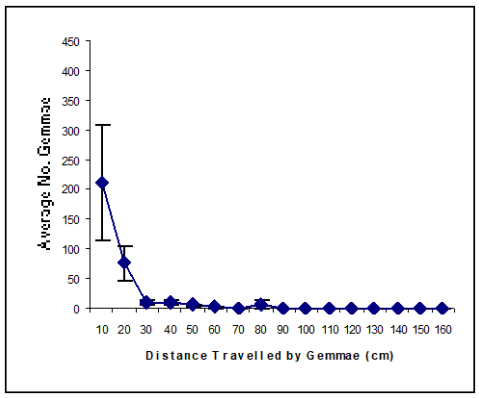
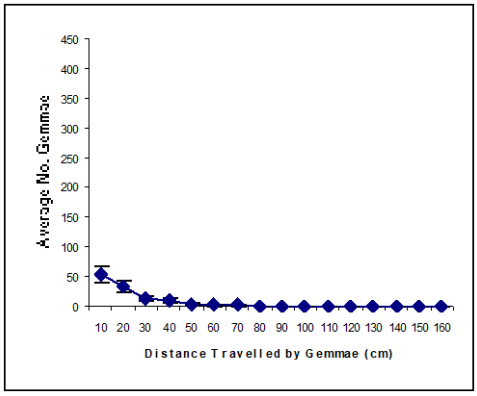
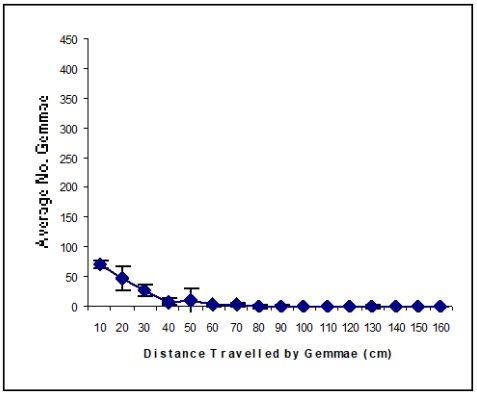


Figure 4c. Dispersal gradients obtained operating nozzles at 2 bar.

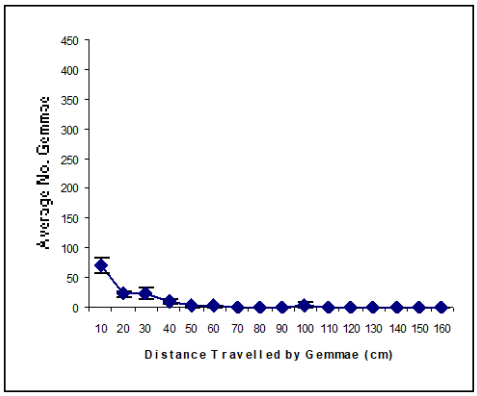
Nozzle Height → **1 Metre** **2 Metres**

Blue Nozzle





Grey Nozzle



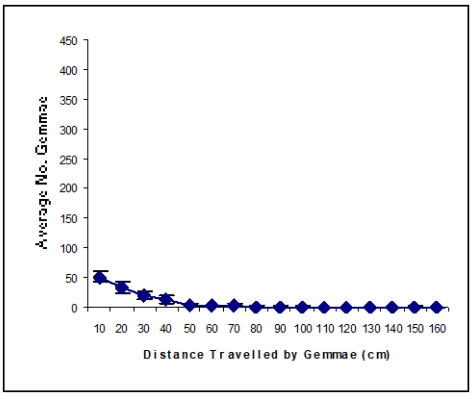


Figure 4d. Dispersal gradients obtained operating nozzles at 1.5 bar.

Table 3. Average no. of liverwort gemmae dispersed

	Grey Nozzle				Blue Nozzle				Brown Nozzle			
					1 Metre Nozzle Height							
Distance Travelled by Gemmae (cm)	**1.5 Bar**	**2 Bar**	**2.5 Bar**	**3 Bar**	**1.5 Bar**	**2 Bar**	**2.5 Bar**	**3 Bar**	**1.5 Bar**	**2 Bar**	**2.5 Bar**	**3 Bar**
10	70.00	173.33	89.33	1.67	53.00	133.00	173.33	4.67	0	65.00	24.00	20.00
20	22.22	133.44	30.78	8.22	33.56	78.11	50.22	2.11	0	19.78	55.33	15.00
30	22.56	24.67	7.22	4.22	12.33	31.33	20.67	3.22	0	12.11	12.00	6.56
40	10.00	6.56	5.56	0.22	9.89	25.56	10.11	0.11	0	3.44	2.78	8.33
50	1.78	3.11	2.89	0.44	4.78	8.00	2.78	0.78	0	0.67	2.78	2.33
60	2.56	1.33	0.56	0.78	2.11	5.22	2.33	0.00	0	4.89	1.67	0.00
70	0.78	0.11	0.11	0.44	2.00	1.89	0.33	0.56	0	0.67	1.22	0.00
80	0.67	0.00	0.11	1.56	1.00	1.00	1.00	0.44	0	0.00	0.33	1.00
90	0.00	0.33	0.00	0.00	0.89	0.89	0.56	0.00	0	0.00	0.00	0.22
100	4.78	0.00	0.00	0.00	0.44	0.78	0.33	0.00	0	0.44	0.11	0.00
110	0.11	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.33	0.22	0.00	0.00	0	0.00	0.00	0.00
130	0.00	0.00	0.00	0.00	0.44	0.00	0.67	0.00	0	0.00	0.33	0.00
140	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0	0.00	0.00	0.00
150	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.22	0	0.00	0.00	0.00
160	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.11	0.00
					2 Metres Nozzle Height							
	1.5 Bar	**2 Bar**	**2.5 Bar**	**3 Bar**	**1.5 Bar**	**2 Bar**	**2.5 Bar**	**3 Bar**	**1.5 Bar**	**2 Bar**	**2.5 Bar**	**3 Bar**
10	52.00	107.67	360.00	250.00	71.33	169.67	123.00	2.00	0.00	211.33	47.33	50.33
20	34.33	49.44	191.00	148.11	47.67	98.78	72.78	5.22	0.00	76.22	51.11	27.00
30	20.56	32.67	73.00	72.44	27.56	38.67	13.00	2.44	0.00	10.33	7.67	7.33
40	13.89	32.00	33.67	68.44	8.00	26.56	10.11	0.00	0.00	11.33	11.89	44.11
50	4.67	15.89	21.00	24.44	8.67	35.78	4.22	0.00	0.00	5.33	3.67	15.78
60	2.89	6.44	26.00	7.33	2.22	6.89	1.78	0.33	0.00	3.57	4.78	2.33
70	4.44	2.89	10.67	4.78	2.22	11.00	0.56	0.00	0.00	0.33	0.89	0.89
80	1.44	3.22	6.67	1.00	0.33	5.33	0.89	0.00	0.00	11.20	2.00	1.00
90	1.67	2.67	0.44	4.78	1.67	1.89	1.11	0.00	0.00	0.67	0.22	0.00
100	1.22	0.78	0.00	0.11	0.33	0.78	1.44	0.00	0.00	1.50	0.00	0.78
110	0.44	0.22	0.00	0.22	0.11	1.00	0.00	0.00	0.00	0.50	0.00	0.11
120	0.11	0.11	0.00	1.00	0.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00
130	0.11	0.11	0.00	0.33	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00
140	0.33	0.00	0.00	1.44	0.00	0.22	0.11	0.11	0.00	0.00	0.00	0.00
150	1.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
160	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

When using the brown nozzle, more gemmae were dispersed with increased nozzle heights, in accordance with findings reported by Geagea (1999). However, there was no clear effect of nozzle height on the number of gemmae dispersed for the grey and blue nozzles as the results were more variable. Distances travelled by the gemmae increased with increased nozzle height. In contrast with Geagea's findings, for the blue and brown nozzles some gemmae travelled further when the nozzle was at the lower position, although the bulk of the gemmae did travel further when nozzles were higher. Steeper dispersal gradients were obtained at 2 and 2.5 bar than 3 and 1.5 bar; the exception was the 3 bar, 2m height treatment using the grey nozzle.

At the two extreme water pressures (1.5 and 3 bar), generally fewer gemmae were dispersed for all nozzles, indicating there may be an optimum combination of water pressure and nozzle for dispersing maximum gemmae numbers. This would appear to coincide with the manufacturers recommendation that 2 bar is the optimum operating pressure for this particular nozzle, as above 2.5 bar and below 1.5 bar water distribution becomes uneven, and damage may be caused to the emitters, so they fall outside the terms of the guarantee.(Anon, Undated)

The dispersal distances obtained in this experiment far exceed the distances of 2 feet (60cm) reported by Brodie (1951), however as he describes the droplets used as 'small' without giving measurements it is not possible to directly compare the results. The implications of these results are that *Marchantia polymorpha* can spread large distances – up to 1.6 m during this experiment - using these vegetative propagules.

This information could be used when developing an integrated pest management strategy, avoiding or reducing the use of overhead watering systems.

During each of these experiments, it was noticed that gemmae clump together. This affected results by making it difficult to count gemmae in Experiment 1 and causing peaks in the dispersal gradients. In experiment 2 it is not clear whether this is a mechanism to improve establishment rates. The gemmae could have been caught

within a water droplet or held together by some other means. There is a possibility that during Experiment 1, as no water was applied over the gemmae they were not dispersed, but replacements were still being produced. This could have resulted in gemmae being pushed out of the cup and away from the influence of lunularic acid, thought to prevent germination of gemmae within the cup. As the gemmae start to grow they become intertwined and locked together. Further experiments are planned to provide some clarification.

Research Plan

Fungal pathogens:

Pathogenicity tests of fungi already in culture is scheduled for year 2. This aspect of the project is already under way; a procedure is being developed for applying known concentrations of fungal spores or spore/mycelium mixtures to liverwort thalli and measuring any effects observed.

Identification of the liverwort subspecies infesting nurseries:

Molecular methods will be used to identify the *Marchantia polymorpha* samples currently being maintained, as there are three morphologically similar subspecies of this liverwort that may be prevalent in nursery situations (Bischler and Boisselier-Dubayle, 1998).

Gemma dispersal:

Experiments to determine the replenishment rate of gemmae in gemma cups, also investigating whether more gemmae are produced during cooler periods of spring and autumn. Increased production of gemma cups per unit area, and also more gemma cups on female plants than male plants on liverworts grown in short days has been observed (Voth and Hamner, 1940). This information would provide an insight into the effective use of irrigation regimes as part of an integrated pest management system.

‘Clumping’ may be a method of improving establishment rates. A laboratory experiment is planned, growing liverworts singly and in groups to investigate the effect on establishment and methods of interrupting this phenomenon.

Lunularic acid:

Lunularic acid (LNA) is produced by *Marchantia polymorpha* and is thought to control growth, arrest germination of gemmae within gemma cups and aid drought resistance. Liquid chromatography mass spectrometry (LCMS) will be used to quantify the amount of LNA in plant material of different ages, relating this information to lifecycle stage; investigate the levels of LNA present in areas of natural dieback of liverwort thallus and explore the relationship between the level of LNA and plant death.

The effect of glucosinolate bioactive products on liverworts:

Glucosides are present in members of Cruciferae and related families. They break down to form secondary metabolites that are volatile defence substances - glucosinolates, (Taiz and Zeiger, 1998). Biologically active isothiocyanates, highly toxic to pests and pathogens, are one of the products of glucosinolate degradation, released from *Brassica* tissues following cell damage. (Bones and Rossiter, 1996) The structure of glucosinolates and toxicity of isothiocyanates varies depending on plant species, resulting in variable biofumigation potentials of brassicas (Matthiessen et al., 2001).

A product, ‘AlbaGro’ is being tested in America using seed meal from *Limnanthes alba,* a waste product following oil extraction. Studies indicate that short term (30 days) control of liverwort is possible using this product (Svenson and Deuel, 2000).

Glucosinolate bioactive hydrolysis products with potential for liverwort control will be isolated and tested against optimal and sub-optimal growth conditions identified in year 1, under laboratory conditions, and the growth stage when application would produce most effective results will be determined.

Technology Transfer

An article in the HDC News announced the start of this project.

HDC Members have been contacted via the HDC News (No.11, February 2004) requesting them to contact us with details of any unusual liverwort plants and also any incidents of unexplained decline or dieback of established liverworts.

A poster was presented at the annual Post Graduate Symposium held at the Department of Agricultural Sciences, Imperial College London, Wye Campus on 20th June 2004.

An article is planned to appear in the February 2005 issue of the HDC News, giving an update on the project.

A conference is being sought for 2005, where a poster will be presented.

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