Project title:	The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS)
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Project leader:	Dr Jill England, ADAS Boxworth
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Date project commenced:	1 May 2013
Date project completed (or expected completion date):	30 April 2015

[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with 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.]

AUTHENTICATION

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

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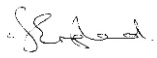
Signature  Date 28 April 2015

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GROWER SUMMARY

Headline

High light levels, vapour pressure deficit (VPD) and temperatures are potentially linked to expression of pansy mottle syndrome (PaMS) symptoms.

Background

Pansy mottle syndrome (PaMS) has been reported (though not understood) since the 1960s, and is recognised as a measureable or visible change in plant growth and function (physiological response). Typical symptoms include leaf distortion, mottling, leaf bleaching, stunting and apical blindness (**Figure 1**). The extent of PaMS may vary from year to year on nurseries; bedding plant species including *Antirrhinum*, *Gerbera*, marigold, *Petunia*, *Primula*, stocks, sweet pea and *Verbena* can display similar symptoms. Determination of the cause is complicated by the transient and intermittent nature of plant response, difficulty in replicating the symptoms and linking the cause with effect (McPherson, 2010). The condition has become more common in recent years, and this has renewed interest in identifying the causal factors.

Figure 1. PaMS symptoms recorded site A, batch 1, 2013.

Grower observation suggests that PaMS may be varietal, with incidence occurring in specific seed batches and colours. Outbreaks have also been linked to environmental factors, occurring under humid conditions including warm, wet and windy weather when glasshouse vents are shut, causing humidity to increase within the glasshouse. Plug size (greater risk of PaMS in the larger module tested), growing media, and the plant hormone methyl-salicylate (associated with plant stress) also appear to promote the incidence of PaMS. Symptoms do not appear to be directly increased by fungicide, adjuvant or plant growth regulator application, the light or irrigation regimes tested, virus (tests proved negative), low irrigation

or boron/calcium (levels confirmed adequate by plant tissue analysis) (McPherson, 2010). Although not a direct cause, pesticides, plant growth regulators or adjuvants may be involved in the development of PaMS by contributing to plant stress. PaMS does not generally appear to spread between plants (McPherson, 2010). Other research has linked growth distortion with boron deficiency under high relative humidity conditions (100%); these conditions decrease water loss via transpiration, resulting in reduced boron uptake and movement from the roots to the shoot (Krug *et al*, 2013). The precise trigger however for the expression of PaMS symptoms remains unknown. As symptoms have proven difficult to replicate both on grower holdings and in research facilities, the approach taken for this study was to collect production and environmental data from nurseries during commercial pansy production for modelling together with symptom expression to identify trigger point(s) of PaMS.

Expected deliverables

To investigate the role of selected environmental factors on the incidence of PaMS, and identify any causal relationships between the incidence of PaMS and environment through, a) monitoring the nursery environment (humidity, temperature, light) and root development within commercial bedding plant production systems and b) controlled environment cabinet experiments.

Summary of the project and main conclusions

Nursery monitoring

Data was collected from four commercial nurseries (sites A-D) located in Hertfordshire, East Yorkshire, West Sussex and Essex respectively between June and September 2014. The sites were selected to include sites with a sustained record of PaMS, and one site where PaMS does not generally occur. These sites were also selected because they grow pansies from seed, so the production process from sowing to marketing could be monitored.

Four batches of pansy were monitored at sites A, three at site B, and two batches at sites C and D. Each batch was monitored using a Tinytag Plus 2 data logger (temperature and humidity), a Watchdog 1000 series microstation data logger with an external LightScout Quantum Light 3 Sensor PAR probe (temperature, humidity and light) and a WaterScout SM100 soil moisture sensor (connected to the Watchdog 1000 data logger). Data loggers were set to record data every 15 minutes. Data loggers were pole mounted within the crop at canopy height so they recorded the environmental conditions the plants experienced. The

light sensor was positioned above the crop (**Figure 2**). Two different production systems were in use on the nurseries taking part in the monitoring: coir 'teabags' in clear green plastic trays and peat based growing medium in packs. Due to the shape of the coir 'teabags', sensors were placed horizontally through the coir, whilst in the peat based system the sensors were place vertically into the growing media (**Figure 3**).

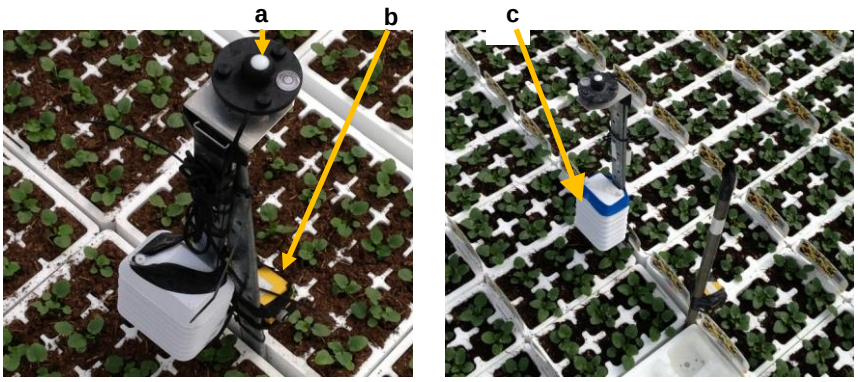

Figure 2. Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and water damage

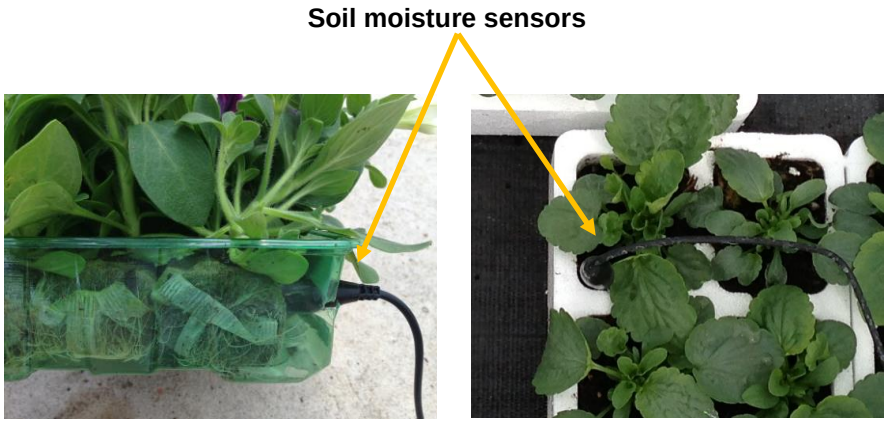

Figure 3. Positioning of SM100 Soil Moisture Sensor within a coir system, inserted horizontally (image left); and in a peat based system inserted vertically (image right) production systems

In 2013, although there was low occurrence of PaMS symptoms in the monitored batches across the four sites, a potential association was tentatively muted between environmental factors and the occurrence of PaMS symptoms. This association was derived from the observation that the VPD, temperature and PAR received by the plants in site A, batch 1 were higher than for the other batches at the same site and also for batches at other sites. It

was suggested that light levels could be a factor, in combination with high VPD and temperature that may lead to symptom development. However the sample size of one precluded any robust statistical analysis of the environmental data.

There were no significant occurrences of PaMS in the monitored batches in 2014, and consultation with the wider bedding plant sector similarly indicated few cases of PaMS within the industry. Data analysis showed that high VPD did occur in all batches on a number of occasions however, daily light integral (DLI) was generally lower across all batches, including when VPD was higher than 4 kPa. DLI was generally lower in 2014 (**Figure 12**) than 2013 (**Figure 13**). At site A in 2013, there was a sustained period (~20–50 days from sowing) when DLI was between 100-150 mol/m^2/day. In 2014, DLI generally peaked below 100 mol/m^2/day, but with a number of peaks above 150 mol/m^2/day) and sparser peaks above 150 mol/m^2/day, across the four batches. DLI was calculated per sampling period, based on a 24 hr day.

Nursery experience suggests that the absence or reduction of root hairs (water roots), as occurs when plants are grown under continually wet growing media conditions, may contribute to triggering PaMS. Root zone issues may impose additional stress on plants either because water is present within the root zone but plants are unable to take up water or nutrients because of the lack of root hairs, or there is no water present.

Recording of growing media moisture data was improved in 2014 through the use of soil moisture sensors, which provided more consistency in the data collected and indicated that none of the batches became critically dry during the monitoring period. Linking this information with the root assessments, where root hair development was good in all of the assessed batches provides an indication that roots were not critically under- nor over-watered during production.

If PaMS symptoms are associated with stress due to high water requirements under high light and temperature conditions, including for photosynthesis, then we would expect the stress to have been lower in 2014 due to the lower light levels. Lower light levels – along with less extreme temperatures and high VPD events – recorded in the nursery monitoring in 2014 would also help to explain the reduced incidence of PaMS across the industry.

Vapour pressure deficit describes the drying effect of air; high VPD occurs under high temperature, low humidity conditions, where high VPD is greater than 2.0 kPa (dry air) and low VPD is less than 0.2 kPa (humid air). Most plants grow well in the middle of this range (0.5 kPa to 0.95 kPa), with pansies performing well around 0.6-0.7 kPa. To put high VPD into context, VPD greater than >5.3 kPa is reported in the Sonoran Desert of Southern California. The data suggested a potential link between high VPD, high temperature and the development of PaMS symptoms.

Controlled environment work

Seeds of Pansy Matrix Autumn Select were sown (31 March 2014) into 288 trays (24 trays), using Bulrush growing media, at Bryants Nursery, Hertfordshire. The environment (temperature, humidity, and light) was monitored using two Tinytag data loggers and two Watchdog 1000 series data loggers with light sensors. The pansy plugs were transported to ADAS Boxworth on 14 April (cotyledon stage) where they were grown on in a glasshouse compartment, maintained between 15 and 25°C. Of the trays of plugs, six from each irrigation treatment remained in the glasshouse throughout the trial, where the environment continued to be monitored.

Irrigation treatments

Plants were grown under two irrigation regimes, wet and dry. The intention had been to provide these two different irrigation treatments (wet and dry) from sowing to encourage greater root hair development under the dry treatment, and water roots (no root hairs) under the wet treatment, but this had not been achieved. The pansies were uniform, with a similar number of root hairs visible on all plants on arrival at ADAS Boxworth, when the two irrigation regimes were applied, but although the two irrigation treatments at Boxworth did achieve greater root hair development under the dry regime, water roots were not present on the plants grown under the wet treatment (**Figure 21**). Reassessment of the roots following the cabinet treatments indicated no change in root hair development.

Two controlled environment cabinets (Sanyo Fitotron SGC097.CPX.F) were set to 35°C and 30% relative humidity and, with the addition of silica gel / cobalt chloride crystals, VPD >3 was achieved on each cabinet treatment day. The cabinet treatments ran for five consecutive days (5 – 9 May 2014) once the plants had reached 3 – 4 true leaves (**Table 1**). Plants were assessed daily for PaMS symptoms for two weeks post treatment, but no symptoms were expressed in either wet or dry treatments.

Table 1.Controlled environment treatments

Treatment no.	Location		Treatment
1a 1b	Glasshouse	Wet Dry	15-25°C
2* 3*	Cabinet Cabinet	Wet Dry	Temperature (>35ºC), VPD (>3), 6 hrs on 5 consecutive days

*Plants were returned to the glasshouse between treatments

PaMS symptoms did not occur in any of the plants subjected to the controlled environment work. A maximum instantaneous light level of 1021 µmol/m^2/s was achieved. During the 2013 monitoring, light levels reached ~1300-1400 µmol/m^2/s when high VPD conditions were experienced, and this correlated with nursery experience where more PaMS developed in glasshouses without screens, and with higher light levels. The lack of symptom development under high VPD and temperature conditions in the controlled environment work may also support the theory that high light levels in association with high VPD and temperature are required for PaMS symptoms to develop – and root development or root zone water balance may also prove to play an important role.

Financial Benefits

Published statistics (Defra, 2014) estimate pansy production in England and Wales at 9.4 million plants with a farm gate value of £2.1 million in 2004 (21p/plant); these values are likely to have increased in subsequent years. It is difficult to quantify plant losses due to PaMS for several reasons (the intermittent and variable nature of PaMS, growers rogueing distorted plants, unreported incidence, incidence identified as PaMS), however, reports have been received of 5-20% of batches on individual nurseries being affected. Based on Defra data, this would to equate to losses of £21,000 (1% of crop affected), £105,000 (5% of crop affected) or £420,000 (20% of crop affected). Additional costs are also incurred by nurseries in refilling plug trays or packs once affected plants have been discarded.

Action Points

The results of this study suggest a causal link between environmental conditions (high VPD, temperature and light) and the expression of PaMS symptoms, however, this is based on the results from a single site in year 1. The precise triggers and sequence of events that lead to

PaMS still remain to be elucidated within the current project but growers should take measures to monitor environmental conditions, and reduce plant stress:

1) Monitor VPD and temperature.
2) Ensure that during periods where extreme high temperatures are predicted measures are taken to reduce plant stress by providing shade, maximum ventilation appropriate to prevailing weather conditions and adequate irrigation. High VPD may be reduced by increasing relative humidity by, for example, path damping and use of mist irrigation where available.
3) Ensure healthy plant root development through careful application of water; over-application of water will limit root development, particularly in tray module production units.

SCIENCE SECTION

Introduction

Symptoms of pansy mottle syndrome (PaMS) have been reported since the 1960s, and have generally been considered to be a physiological response to stress. Symptoms include leaf distortion, mottling, leaf bleaching, stunting and apical blindness (**Figure 4**). Symptom expression may vary from year to year on nurseries; bedding plant species including *Antirrhinum*, marigold, *Petunia*, stocks, sweet pea, *Verbena, Gerbera* and *Primula* can display similar symptoms. Determination of the cause is complicated by the transient and intermittent nature of the symptoms, difficulty in replicating the symptoms and linking the cause with effect (McPherson, 2010). The condition has become more common in recent years, particularly under the relatively cool, wet conditions of 2012, and this has renewed interest in identifying the cause.

a) mottling and leaf bleaching b) leaf distortion

Figure 4. Pansy mottle symptoms: a) mottling and leaf bleaching and b) leaf distortion

Grower observation suggests that PaMS may be varietal, with incidence occurring in specific seed batches and colours. Outbreaks have, however, been linked to environmental factors, as symptoms have often been observed under humid conditions. These include warm, wet and windy weather when glasshouse vents are shut, causing humidity to increase within the glasshouse. Symptoms also tend to appear after transplant, although they may have been triggered earlier and have also been linked to high root-zone moisture levels. A previous HDC funded study (PC 286) included a survey of growers, 68% of whom had seen the problem on their nursery, and similar symptoms on other crops. Treatments that had some impact on symptoms included plug size, with increased risk of PaMS in the larger module tested. Growing media also had some influence, and the plant hormone methyl-salicylate appeared to be associated with symptoms, suggesting that plants were under stress. In this study, symptoms were not directly caused by fungicide, adjuvant or plant growth regulator application, the light or irrigation regimes tested, virus (tests proved negative), low irrigation

or boron/calcium (levels confirmed adequate by plant tissue analysis). Observations made at the time indicated that symptoms first appeared on the first and second true leaves, and were potentially related to initial root development; susceptibility may also be linked to cultivar. PaMS does not generally appear to spread between plants (unless by a volatile or water soluble agent). Although not a direct cause, pesticides, plant growth regulators or adjuvants may be implicated through their contribution to plant stress (McPherson, 2010).

Whipker *et al* (2000) suggest that high temperatures (29°C) and high light levels increase susceptibility to PaMS, and provide production recommendations: day temperature 13-18°C, night temperature 10-13°C, light 47.28 - 78.79 watts/m^2. Symptoms are attributed to a genetic defect rather than nutritional deficiencies, with symptoms disappearing under cool night and daytime temperatures (below 27°C), but reappearing when plants are again stressed as application of boron, iron and magnesium mask the underlying genetic problem. Hammond (2013) found no biotic cause of PaMS, and although an *ilarvirus* was found to be common to pansies from many sources, there was no correlation with PaMS. 1,3 dichlorobenzene (1,3-DCB), proposed as a potential contaminant of peat causing herbicide-like symptoms, was also discounted as symptoms could not be replicated.

Other research correlates with the use of controlled release fertilisers and high temperatures which, in well watered plants, appears to trigger the production of hormones to accelerate growth. Genetic variation within pansies is large, and off-types (<1%) are known to occur; those plants with mottling exhibit membrane proliferation (over-expression of Golgi bodies and endoplasmic reticulum), but without cell divisions. The stress is induced in young plants, before flower bud initiation (de Rooij-van der Goes, 2013).

Krug (2007) has shown that PaMS symptoms could be linked to specific environmental and production conditions. Boron deficiency symptoms are often caused by an inability to uptake boron, rather than a lack of boron in the growing media; high growing media pH reduces the availability of boron to plants. Krug *et al* (2013) linked growth distortion and boron deficiency to high relative humidity conditions (100%). Under these conditions the decrease in water loss via transpiration results in lower boron uptake, and consequently reduced boron levels in shoot tissue. Distorted growth symptoms were replicated in pansy, *Petunia* and *Gerbera* plugs grown under high relative humidity conditions. Boron deficiency symptoms include the inhibition of apical growth, terminal bud necrosis, reduced leaf expansion, upward cupping of leaves, chlorosis of upper leaves, clubbing of roots, inhibition of pollen development and

germination, brittle and fragile tissue, aborted flower initials and shedding of fruit. Although the roles of boron are not fully understood, it is a component of cell walls and is involved in membrane integrity.

While environmental conditions, plant genetics and nutrition are all implicated, the precise trigger or triggers for expression of PaMS symptoms remains unknown. Mottling symptoms have proven difficult to replicate both on grower holdings and in research facilities. For this project, data collected from nurseries during commercial pansy production and environmental data was modelled together with symptom expression to identify trigger point(s) for PaMS.

In year 1 the environmental conditions (temperature, humidity and light) and nursery production practices under which 10 batches of pansies were produced were monitored on four commercial nurseries. Symptoms developed in two of these batches from one site, one of which expressed symptoms including mottling and leaf bleaching, and the other distortion only. Analysis of the data collected suggested that high VPD (>3) and temperature (>35ºC) may be implicated in development of symptoms. Root status was suggested as another factor that could be involved, with plants grown under a wet regime developing water roots (no root hairs) preventing adequate water and nutrient uptake during stress conditions such as high VPD.

The nursery monitoring continued at the same sites in year 2, with the addition of growing media moisture monitoring using a soil moisture sensor and investigation of root development (under wet and dry growing media conditions) to help with understanding their contribution to symptom development. Further work was also carried out under controlled environment conditions to investigate symptom development under specific environmental (temperature >35ºC and VPD >3) and growing media (wet and dry) conditions.

Project objectives

Objective 1 - nursery monitoring: To monitor nursery environment (humidity, temperature, light and growing media moisture) within commercial bedding plant production systems and, using regression analysis approaches, elucidate any statistically robust causal relationships between the incidence of PaMS and environment.

Objective 2 - controlled environment cabinet trial: To carry out controlled environment cabinet experiments to determine the influence of temperature (>35ºC), VPD (>3) and root status on the development of Pansy Mottle Syndrome.

Materials and methods

Objective 1 - nursery monitoring

Data was collected from four commercial nurseries (sites A-D) located in Hertfordshire, East Yorkshire, West Sussex and Essex respectively between June and September 2014. The sites were selected to include holdings with a sustained record of PaMS, and one holding where PaMS does not generally occur. These sites were also selected as they grow pansies from seed, so the production process from sowing to marketing could be monitored.

Four pansy batches were monitored at sites A, three at site B, and two batches at sites C and D. Each batch was monitored using a Tinytag Plus 2 data logger (temperature and humidity), a Watchdog 1000 series microstation data logger with an external LightScout Quantum Light 3 Sensor PAR probe (temperature, humidity and light) and WaterScout SM100 soil moisture sensor.

Data loggers were pole mounted within the crop at canopy height so they recorded the environmental conditions the plants experienced. The light sensor was positioned above the crop (**Figure 5**). Data loggers were set to record data every 15 minutes. During the propagation stage, as the plugs cells were too small to accommodate the soil moisture sensor, an unplanted pot of growing media was placed alongside batches of plug trays to hold the moisture sensor, as a proxy. These pots were irrigated the same as the plug trays, and a correlation made between the pots of growing media and the plug trays to calculate the volume of water applied. Post-transplant, the sensors were placed into the packs, however, two different production systems were in use on the nurseries taking part in the monitoring: coir ‘teabags’ in clear green plastic trays and peat based growing medium in packs. Due to the shape of the coir ‘teabags’ sensors were placed horizontally through the coir, whilst in the peat based system the sensors were placed vertically into the growing media (**Figure 6**). Sowing, transplant and dispatch dates for the batches monitored were recorded (

Table 2).

Soil moisture sensor calibration

The SM100 Soil Moisture Sensor was calibrated for each unique growing media used in the trial. Soilless media tend to be hydrophobic, and shrink when dry, therefore the moisture content of each growing media was established by adding water to a known quantity of growing media. This was done on a mass wetness (MW) basis where mass wetness is defined as:

$$MW = 100 \times \frac{M_{water}}{2 \times M_{material}}$$

MW = target mass wetness (%)
M_{water} = mass of water needed
$M_{material}$ = total air-dry mass of sample

Samples of propagation and transplant growing media were collected from sites A, C and D in 2014 (a sample wasn't provided by site B). For each growing media sample, 18 containers (1L) were used, providing three replicates at six different water contents. Each empty pot weighed 21 g.

Approximately 3.5 L of growing media was placed into a polythene bag and weighed, six bags per growing media, one for each mass wetness. Target mass wetnesses of 0, 40, 80, 120, 160 and 200% were used. Water was added to each bag to bring the material to the desired mass wetness using the following equation:

$$M_{water} = 2 * \frac{MW}{100} * M_{material}$$

Once the water had been incorporated, the sealed bags were left for 24 hours to allow the water and material to come to equilibrium. The material was added to the 1 L container and weighed. For each container, three readings were taken using the SM100. Readings were taken perpendicular to the sides of the container. The growing media in the containers was then completely air-dried and re-weighed. The volumetric water content (VMC) for each container was calculated using the following equation:

$$VWC = \frac{M_{wet} - \left(M_{dry-only} + M_{cont}\right)}{P_w \times V_{cont}}$$

$$VWC = \frac{M_{wet\text{-}total} - (M_{dry\text{-}only} + M_{cont})}{P_w * V_{cont}}$$

VWC = Volumetric water content (%)
$M_{wet\text{-}total}$ = Total mass of container and wet material
$M_{dry\text{-}only}$ = Mass of air-dry material

M_{cont} = Mass of container
P_w = Density of water (1 g/ml)
V_{cont} = Volume of container

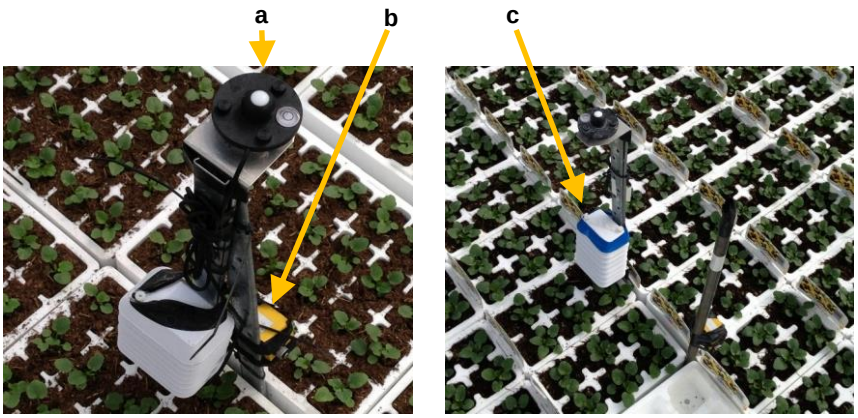

Figure 5. Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and water damage

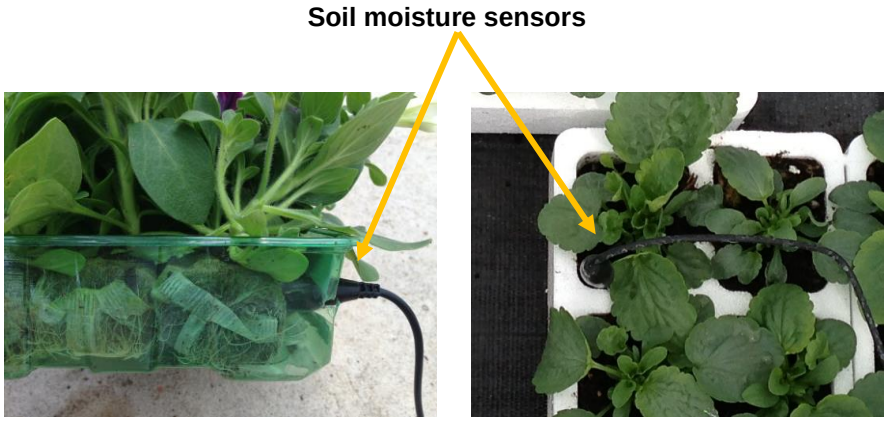

Figure 6. Positioning of SM100 Soil Moisture Sensor within a coir system, inserted horizontally (image left); and in a peat based system inserted vertically (image right) production systems

Table 2. Dates of sowing, transplanting and dispatch for each monitored batch at each site

Site	Batch no.	Sowing date	Transplant date	First symptom expression	Dispatch date
Site A	1	24.06.14	22.07.14	-	12.08.14
Site A	2	31.07.14	28.08.14	-	18.09.14
Site A	3	04.07.14	30.07.14		18.08.14
Site A	4	07.08.14	02.09.14	-	07.10.14
Site B	1	27.06.14	30.07.14	-	*
Site B	2	27.06.14	30.07.14	-	*
Site B	3	03.07.14	05.08.14		*
Site C	1	26.06.14	25.07.14	-	18.08.14
Site C	2	04.07.14	31.07.14 – 01.08.14	-	08.12.14
Site D	1	24.06.14	22.07.14	-	08.08.14
Site D	2	07.07.14	04.08.14 – 05.08.14	-	01.09.14

*These batches were produced specifically for this trial and were not dispatched for sale

Objective 2 - controlled environment cabinet trial

Site and crop details

Work was carried out between March and June 2014 at ADAS Boxworth. Seeds of Pansy Matrix Autumn Select were sown on 31 March 2014 into 288 cell trays (24 trays), using Bulrush growing media, at Bryants Nursery, Hertfordshire. Two Tinytag data loggers and two Watchdog 1000 series data loggers, with light sensors, were placed with the batch, to record temperature, humidity and light every 30 minutes from the point of sowing. All trays received the same amount of irrigation whilst at Bryants Nursery. The plants were transported to ADAS Boxworth on 14 April (cotyledon stage) where they were grown on in a glasshouse compartment, maintained between 15 and 25°C.

The trays were cut in half to allow for greater replication, and two irrigation regimes (wet and dry) applied, each to 50% of the plants (18 half-trays per treatment) (**Figure 7**). Of the trays, six from each irrigation treatment remained in the glasshouse throughout the trial (untreated control, treatments 1a and 1b, **Table 3**). The two sets of loggers (Tinytag and Watchdog 1000) monitored the glasshouse environment, one per watering regime, for the duration of the trial.

Figure 7. Layout of trays in the glasshouse, separated into two irrigation regimes (wet and dry) ADAS Boxworth

Irrigation treatment

On arrival at ADAS Boxworth, the trays of plants were weighed, watered to the point of saturation, and then re-weighed. Plants destined for the dry treatments (50% of the total) were allowed to dry down for two days, with no further water applications. Plants destined for the wet treatment were then watered daily to the point of saturation (treatment 2); and for the dry treatment, plants received between 50 ml and 100 ml (treatment 3) depending on temperature conditions (**Table 3**). All trays were weighed daily prior to watering throughout the trial. As the plug cells were too small to accommodate the soil moisture sensors, 9 cm pots were used as a proxy to estimate moisture levels. WaterScout SM100 soil moisture sensors were placed in 9 cm pots of Bulrush growing media (one per treatment) which received the same volume of water as the plants.

On the five consecutive cabinet treatment days, the wet treatment trays were watered to the point of saturation as normal, and the dry treatment were given 100 ml. The trays were watered in the afternoon, once they had been removed from the cabinets. Once the cabinet treatments had finished, the two separate watering treatments continued until the end of the trial.

Table 3.Controlled environment treatments

	Location	Treatment	
1a	Glasshouse	Wet	15-25°C
1b		Dry	
2*	Cabinet	Wet	Temperature (>35ºC), VPD (>3),
3*	Cabinet	Dry	6 hrs on 5 consecutive days

*Plants were returned to the glasshouse between treatments

Controlled environment (CE) cabinet treatments

Two CE cabinets (Sanyo Fitotron SGC097.CPX.F) were set to 35°C and 30% humidity to achieve a VPD of >3, whilst maintaining the temperature above 35°C (**Table 3**). As the introduction of plants into the cabinets increased humidity and reduced VPD trays of silica gel crystals were placed on the bottom of each cabinet. The silica gel crystals were also removed each day and placed in an oven overnight set to 80°C, so that they could dry out and be placed back in the cabinets each day. The cabinet treatments ran for five consecutive days, (5 – 9 May 2014) once the plants had reached 3 – 4 true leaves.

The plants were moved from the glasshouse to the CE cabinets on a trolley and placed in the cabinets according to the trial plan, 12 half trays per cabinet (one half tray per plot). The trays were randomised within the cabinets (**Appendix 1**), but kept separate in the two watering regimes within the glasshouse, for ease of watering (**Appendix 2**).

Environmental conditions were monitored using a Tinytag and Watchdog data logger with light sensor in each cabinet; these data loggers remained with the plants, moving in and out of the cabinets each day, and the trays were placed in the same area of the cabinet (**Figure 8**) each day. The plants were placed in the cabinets at 9 am each day, removed at 3 pm, reweighed and watered.

Figure 8. Layout of trays in the CE cabinets

Assessments

Objective 1 - nursery monitoring

Nursery staff provided production data for routine inputs: irrigation (method, volume, and source), fertiliser, crop protection and plant growth regulator application, and growing media as detailed within a monitoring template (**Appendix 3**). Plants were monitored daily for PaMS symptoms by nursery staff and the location of symptomatic plants recorded, along

with the date and time of inspection. Any symptomatic plants were to be further inspected by ADAS, to quantify the number of infected plants and their position both within the module tray and the glasshouse.

At site B, batches 1 and 2 were sown on the same day (same cultivar), and transferred to different areas within the same glasshouse post transplant to provide contrasting environmental conditions: unshaded (stressed) vs shaded (not stressed).

A root hair assessment of 20 plants per batch was carried out by nursery staff at transplant, scoring on a scale of 0-3, where 0 = no root hairs and 3 = many root hairs (**Figure 9**), using the guide provided.

Root score 0	**Root score 1**	**Root score 2**	**Root score 3**
Either no roots present, or there are water roots with no root hairs	Very few hairs present	Root hairs present	Roots are extremely hairy

Figure 9. Root assessment scores. Scale = 0-3; 0 = no root hairs and 3 = many root hairs

Objective 2 - controlled environment cabinet trial

The following assessments were carried out according to the timetable below (**Table 4**):

- Root hair assessment, five plants per tray scored on a scale of 0-3, where 0 = no root hairs and 3 = many root hairs (**Figure 9**).
- Assessment of PaMS symptoms.

Table 4. Timetable of actions and assessments

Date	Action
31.03.14	· Seeds of Pansy Matrix Autumn Select sown into 288 celltrays at Bryants Nursery. Data loggers monitoring from sowing
Early April	· Irrigation regime for the wet and dry treatments determined at ADAS Boxworth
14.04.14	· Plants arrive at ADAS · Initial root assessment · Irrigation treatments commence
Mid-April	· CE cabinets tested with plants to ensure VPD >3 achievable
02.05.14	· Root hair assessment (pre-cabinet treatments)
05.05.14	· Day 1 of cabinet treatments. Plants at 3-4 true leaf stage
09.05.14	· Day 5 of cabinet treatments. Root assessment (post cabinet treatment)
Mid May – 11.06.14	· Daily weighing, watering and monitoring for symptoms of PaMS

Results

Objective 1 - nursery monitoring

No PaMS symptoms occurred in any of the monitored batches in 2014 other than one or two plants at site A, batch 1. Stunting and distortion, but no mottling or variegation, was reported in an unmonitored batch of Pansy Frizzle-Sizzle at site C (August) that was located in an area of the glasshouse that was monitored later in the season. The possibility of using the nursery environmental monitoring system data for analysis was investigated but it did not prove possible to access the full range of data required.

Data capture

Production information provided by the nurseries (available as a separate appendix: PaMS nursery data appendix 2014) was reviewed and considered in association with environmental data.

Environmental data was recorded by both the Tinytag (temperature, humidity) and Watchdog (temperature, humidity, light and growing media moisture) data loggers for all sites and batches. The only issue arose in site B, batch 2 where there appears to have been an issue where the sensor recording zero initially, and then around 1000 micromols/m^2/s for every

sample (day and night) before resetting itself after 7 days (**Figure 11**). After this point, PAR is consistently in line with the other nurseries.

Data analysis

The data analysis component of the work was to determine any statistically robust relationship between the monitored environment variables and the occurrence of Pansy Mottle symptoms. Whilst full statistical analysis was not possible due to the lack of occurrences of symptoms in the monitored batches, exploratory data analysis focussed on identifying any differences in the environmental conditions in 2013 and 2014 that may be implicated as triggers for symptom expression.

Preliminary analysis using daily data

Initial analysis focussed on using cumulative day degrees above a threshold of 0°C to examine the consistency of the data across all sites and batches using the temperature and humidity data from the Tinytag loggers, as they were located nearest to the plant canopy and so provided a more accurate assessment of the temperature and humidity conditions experienced by the plants. Cumulative day degrees (**Figure 10**) for all batches were highly consistent across all sites. This graph also indicates that there were no differences between the batches in terms of daily temperature accumulation. We can be confident that the data is representative of the conditions experienced by the plant.

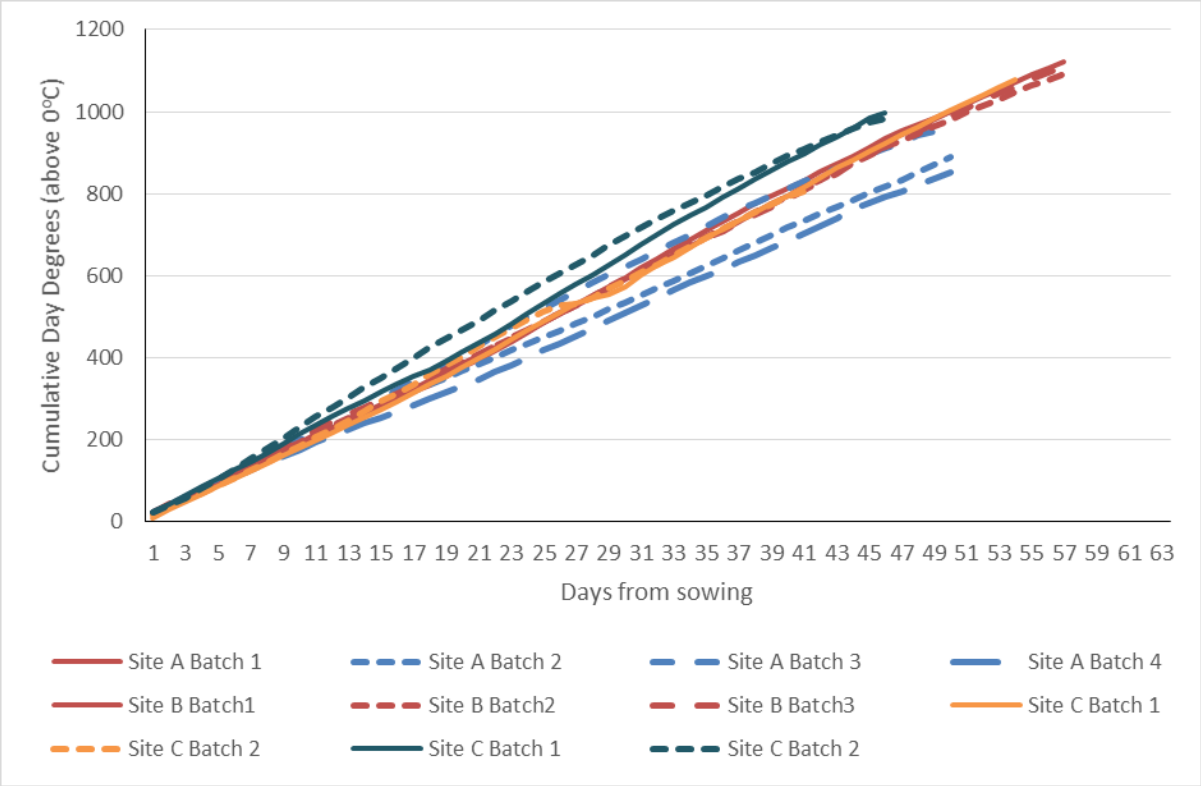

Figure 10. Cumulative Day Degrees (ºC) 2014, all batches

The cumulative photosynthetically active radiation (PAR) chart (**Figure 11**) shows a high level of consistency in the PAR received by the plants in different batches at sites A, C and D, and within batches 1 and 3 at site C. The PAR received by site B, batch 2 was noticeably higher that the other batches; there had been data logger issues up to day 7 (see comments above under 'Data capture').

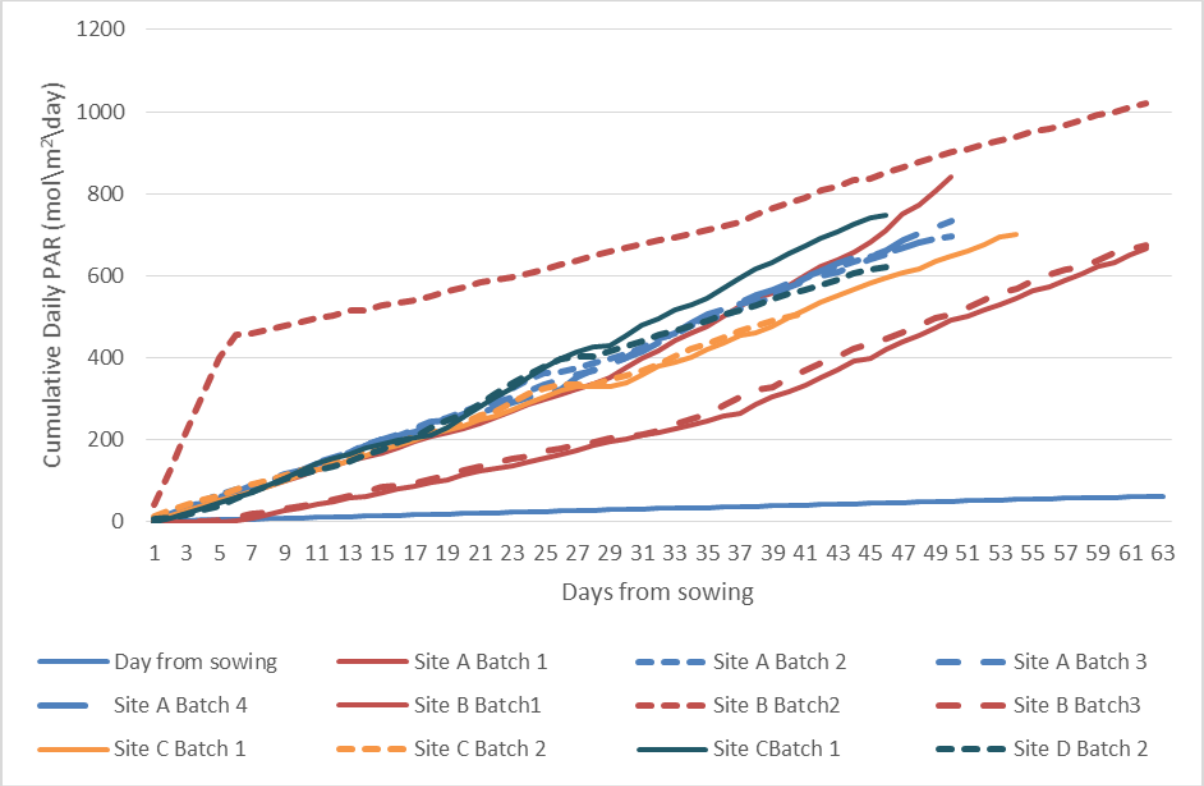

Figure 11. Cumulative daily PAR (mols/m^2/day) 2014, all batches

As PaMS symptoms only occurred on site A in 2013, light, temperature and VPD data for that site for 2013 and 2014 is discussed below (**Figure 12** to **Figure 17**). Environmental data (temperature, daily light integral (DLI) and VPD; 2014) for sites B, C and D may be found in Appendix **4**.

DLI was generally lower in 2014 (**Figure 12**) than 2013 (**Figure 13**). At site A in 2013, there was a sustained period (~20–50 days from sowing) when DLI was between 100-150 mols/m^2/day. In 2014, site A, DLI generally peaked below 100 mol/m^2/day, but with a number of peaks above 150 mol/m^2/day and sparser peaks above 150 mol/m^2/day, across the four batches. DLI was calculated per sampling period, based on a 24 hr day. VPD did exceed 4.0 kPa in 2014 (**Figure 16**) but on fewer occasions, and events were less clustered than in 2013 (**Figure 17**) when VPD exceeded 5.0 kPa. Similarly in 2014 (**Figure 14**), temperatures did not reach the high levels seen in 2013 (**Figure 15**).

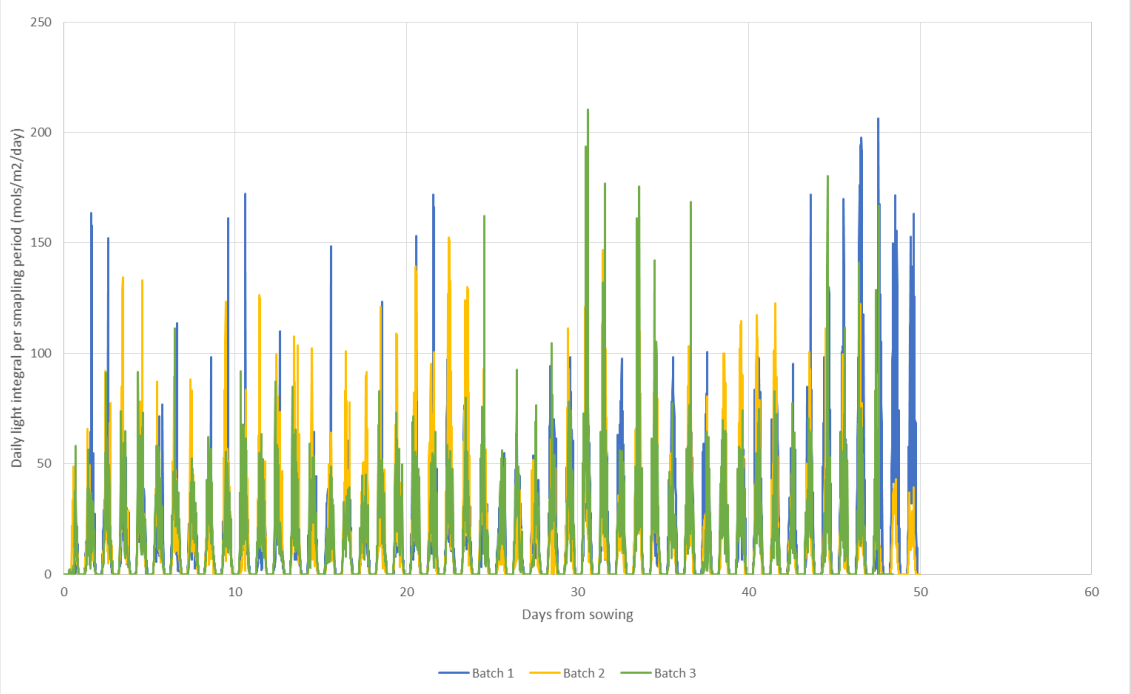

Figure 12. Daily Light Integral per sampling period (mols/m^2/day), site A all batches, 2014 (calculation based on a 24 hr day)

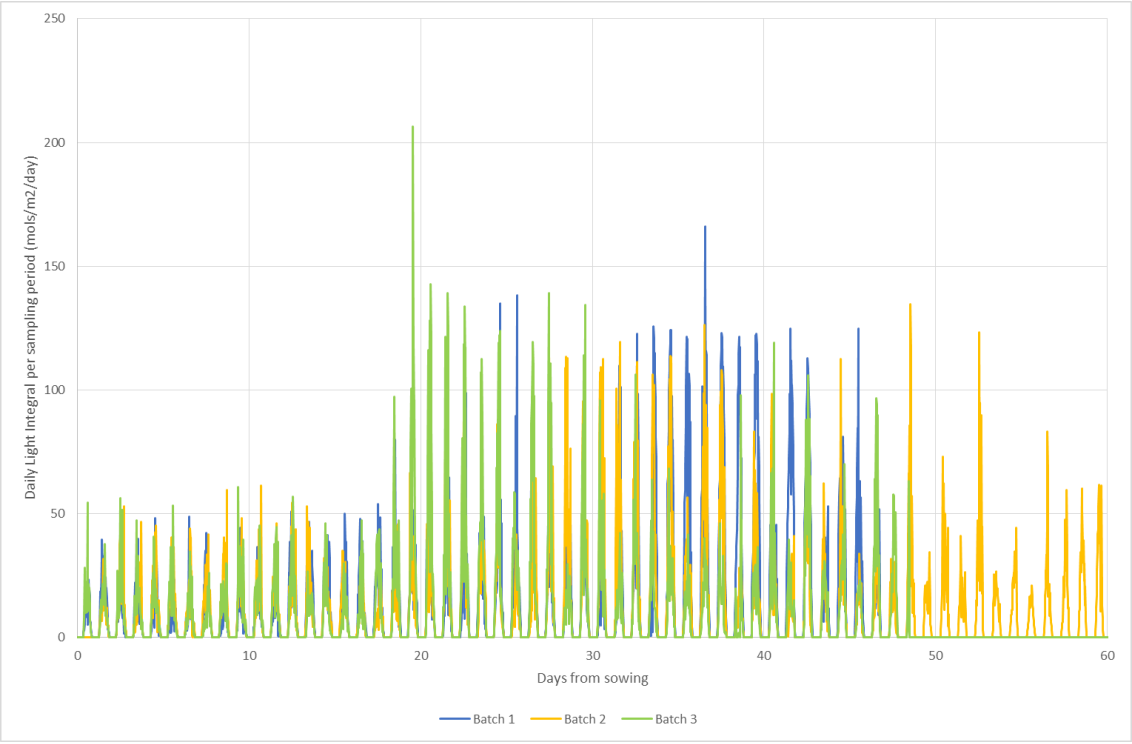

Figure 13. Daily Light Integral per sampling period (mols/m^2/day), site A all batches, 2013 (calculation based on a 24 hr day)

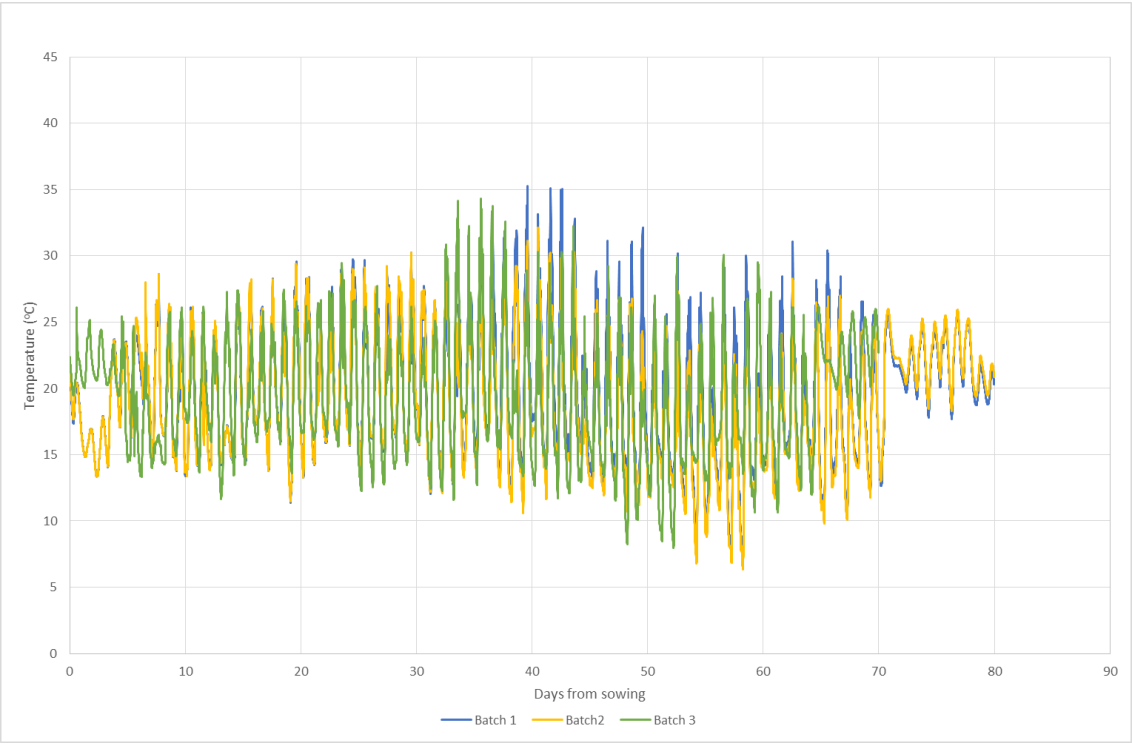

Figure 14. Site A: Temperature (°C) 2014

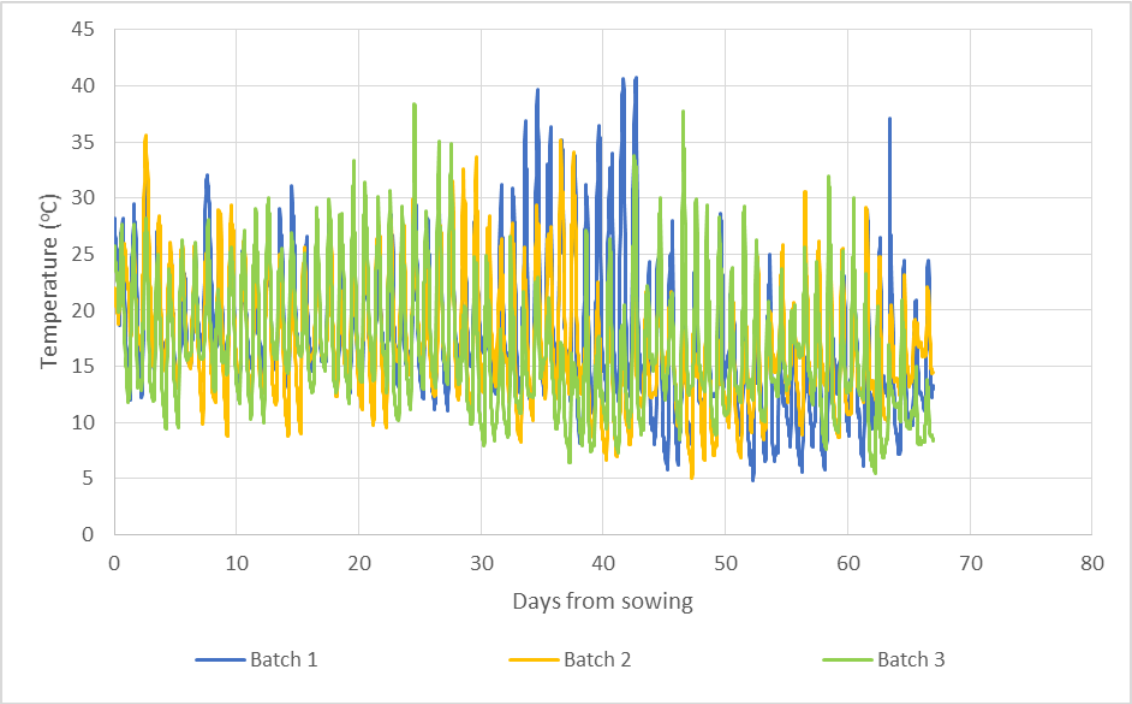

Figure 15. Site A: Temperature (°C) 2013

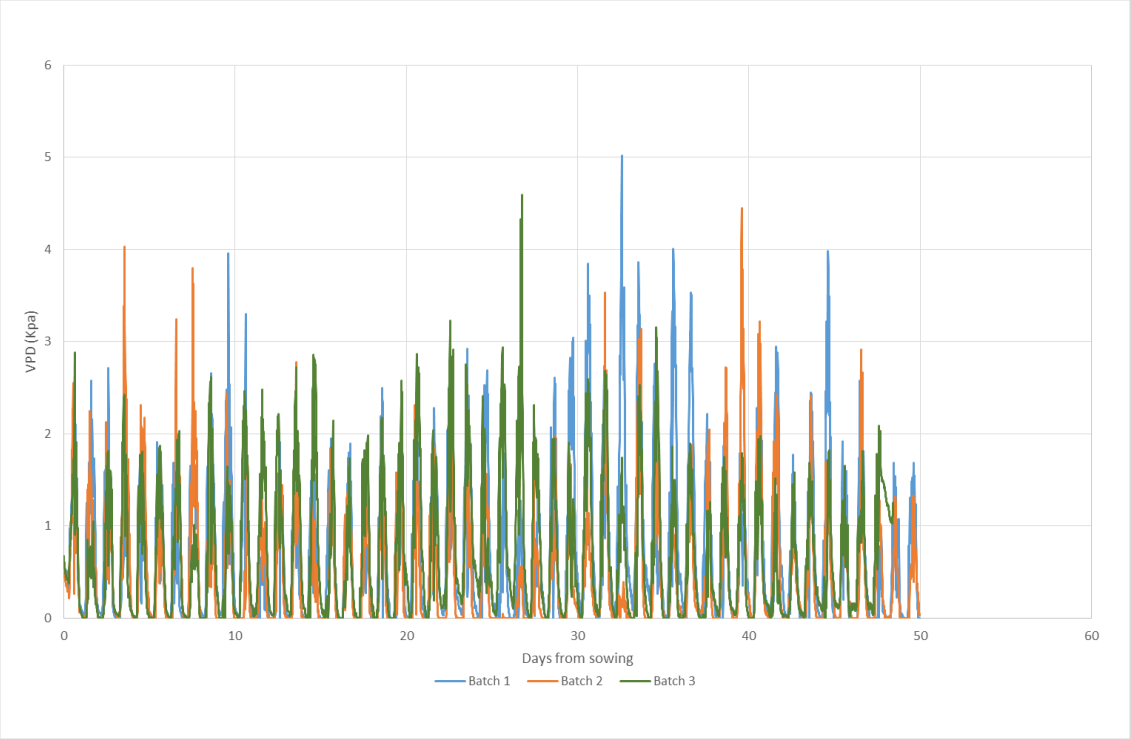

Figure 16. Site A: VPD (kPa) 2014

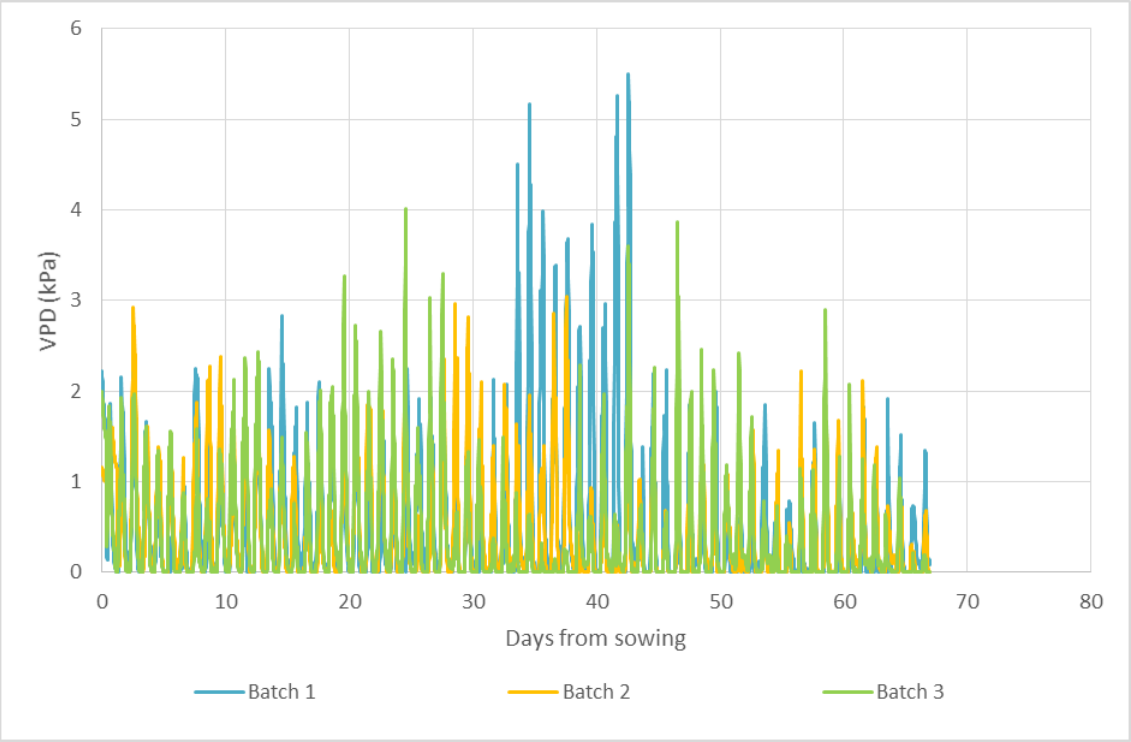

Figure 17. Site A: VPD (kPa) 2013

Root hairs assessments were carried out by nursery staff at transplant, on the premise that plants with poorly developed root systems with few root hairs would be under additional stress under high VPD conditions, when sufficient water uptake may be critical for survival.

Plants were scored on a scale of 0-3, where 0 = no root hairs and 3 = many root hairs. For all batches where an assessment was completed, the sample was assessed to have good root hair development (**Table 5**).

Table 5. Average root hair score per batch at transplant. Plants were scored on a scale of 0-3, where 0 = no root hairs and 3 = many root hairs (Figure 9)

Site	Batch	Average root score
A	1	2.00
A	2	not completed*
A	3	2.00
A	4	3.00
B	1	not completed*
B	2	not completed*
B	3	not completed*
C	1	2.65
C	2	2.25
D	1	2.75
D	2	2.65

**assessment not completed for this batch*

Growing media moisture is presented as the volumetric water content (VMC); the ratio of water to substrate expressed as a percentage. As moisture levels in the plug trays were recorded using a proxy (9 cm pot of growing media, irrigated with the same volume of water as the trays of plugs), a correlation was made between the pots of growing media and the trays of plugs to calculate the volume of water applied (**Figure 18** to **Figure 20**); data was not collected at site B. The data cannot be compared between nurseries, as differences may be due to the different substrates used which require different irrigation regimes. There were no occasions when moisture levels were low for long periods.

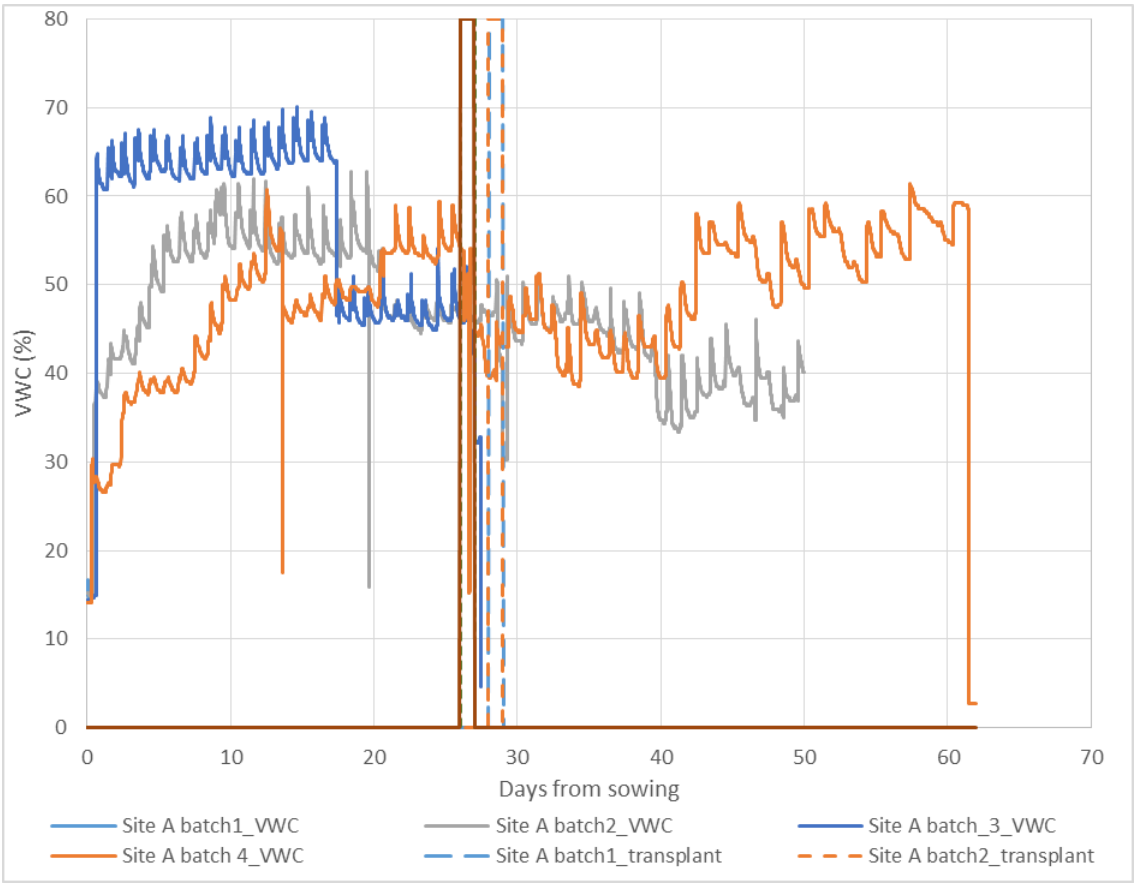

Figure 18. Volumetric water content (VWC, %): Site A

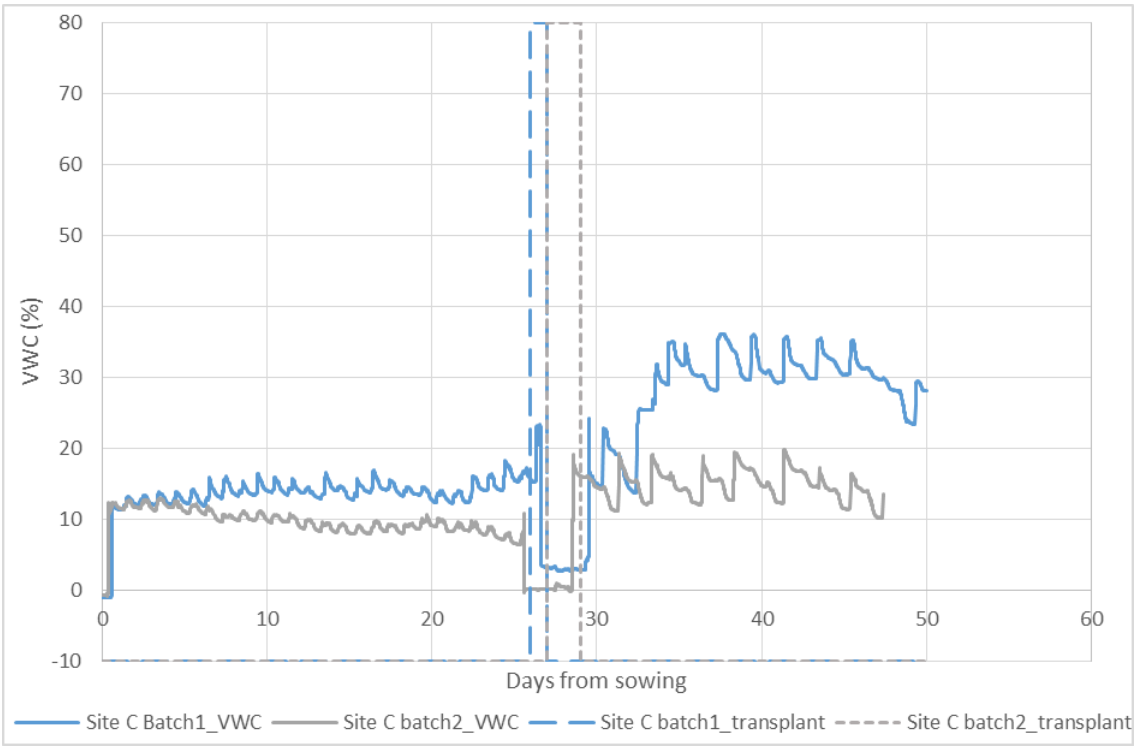

Figure 19. Volumetric water content (VWC, %): Site C

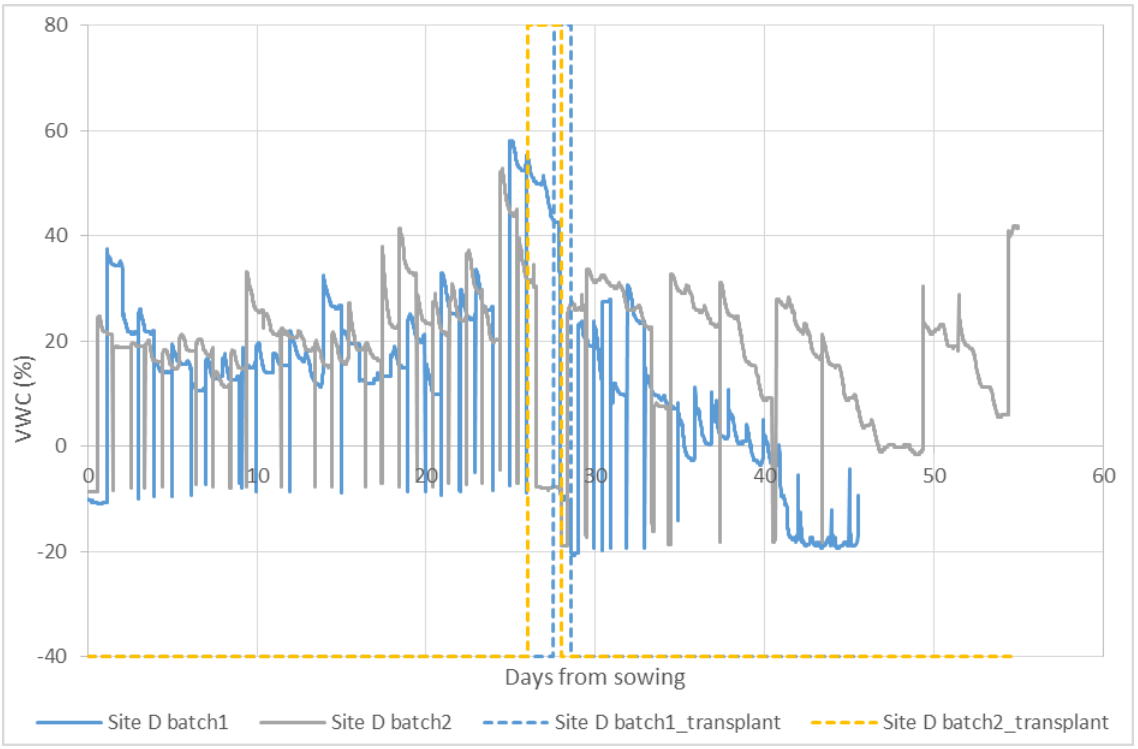

Figure 20. Volumetric water content (VWC, %): Site D

Objective 2 - controlled environment cabinet trial

Root hair assessment

On arrival at ADAS Boxworth the pansies were uniform, with a similar number of root hairs visible on all plants. The intention had been to provide two different irrigation treatments (wet and dry) from sowing to encourage greater root hair development under the dry treatment, and water roots (no root hairs) under the wet treatment. Although the two irrigation treatments at Boxworth did achieve greater root hair development under the dry regime, water roots were not present on the plants grown under the wet treatment (**Figure 21**). Reassessment of the roots following the cabinet treatments indicated no change in root hair development.

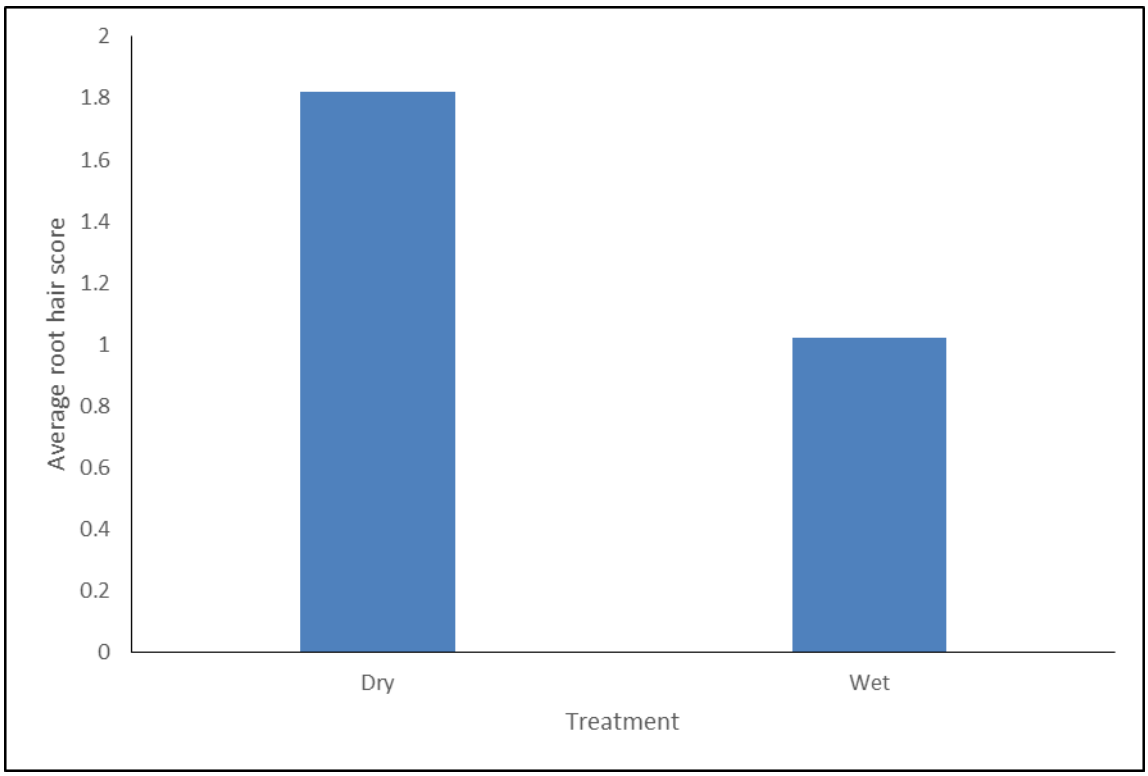

Figure 21: Average root hair assessment following water treatments, prior to cabinet treatments, on a scale of 0-3, where 0 = no root hairs and 3 = many root hairs

CE cabinet treatment

The cabinets were set to 35°C and 30% relative humidity and, with the addition of silica gel / cobalt chloride crystals, VPD >3 was achieved on each cabinet treatment day (**Figure 22**). The five vertical bars on the graph indicate when VPDs of 3 to 4.5 were achieved whilst the trays were in the cabinets. Instantaneous PAR light levels between 852 - 1021 µmol/m^2/s were achieved within the cabinets.

Plant assessment

Plants were assessed daily for PaMS symptoms for two weeks post treatment, but no symptoms were expressed in either wet or dry treatments.

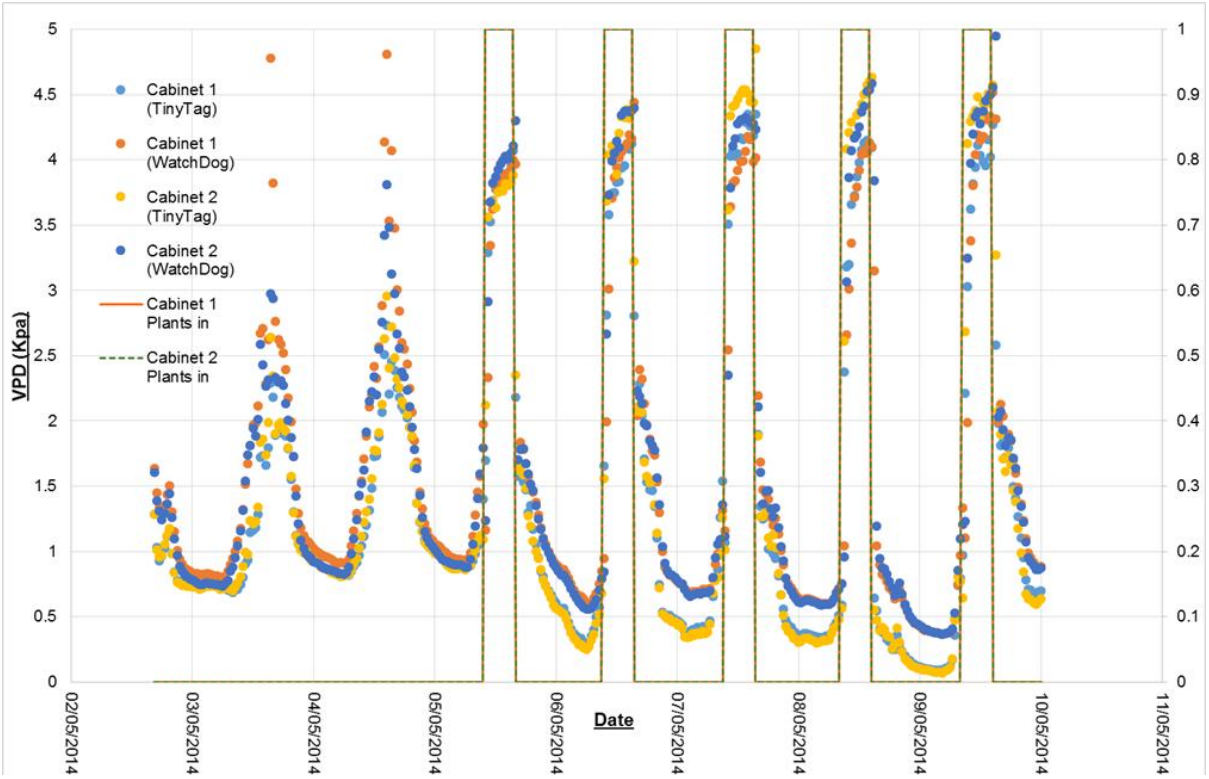

Figure 22. Calculated VPD for CE cabinet experiments

Discussion

Objective 1 - nursery monitoring

In 2013, although there was low occurrence of PaMS symptoms in the monitored batches across the four sites, a potential association was tentatively muted between environmental factors and the occurrence of PaMS symptoms. This association was derived from the observation that the VPD, temperature and PAR received by the plants in site A, batch 1 were higher than for the other batches at the same site and also for batches at other sites. It was suggested that light levels could be a factor, in combination with high VPD and temperature that may lead to symptom development. However the sample size of one precluded any robust statistical analysis of the environmental data.

In 2014, significant PaMS symptoms did not occur in any of the monitored batches across the four sites. Data analysis showed that high VPD occurred in all batches on a number of occasions in 2014. DLI was generally lower in 2014 (**Figure 12**) than 2013 (**Figure 13**), and at site A, DLI generally peaked below 100 mol/m^2/day, but with a number of peaks above 150 mol/m^2/day) across the four batches (DLI was calculated per sampling period, based on a 24 hr day).

At site A in 2013, there was a sustained period (~20–50 days from sowing) when DLI was between 100-150 mols/m^2/day. In 2014, DLI generally peaked below 100 mol/m^2/day, but with a number of peaks above 150 mol/m^2/day) and sparser peaks above 150 mol/m^2/day, across the four batches. VPD did exceed 4.0 kPa in 2014 but on fewer occasions, and events were less clustered than in 2013; temperatures did not reach the high levels seen in 2013.

Nursery experience suggests that the absence or reduction of root hairs (water roots), as occurs when plants are grown under continually wet growing media conditions may contribute to triggering PaMS. Root zone issues may impose additional stress on plants either because water is present within the root zone but plants are unable to take up water or nutrients due to the lack of root hairs, or there is no water present.

Recording of growing media moisture data was improved in 2014 through the use of soil moisture sensors, which provided more consistency in the data collected and indicated that none of the batches became critically dry during the monitoring period. Linking this information with the root assessments, where root hair development was good in all of the assessed batches provides an indication that roots were not critically under- nor over-watered during production.

Objective 2 - controlled environment cabinet trial

PaMS symptoms did not occur in any of the plants subjected to the controlled environment work. A maximum instantaneous light level of 1021 µmol/m^2/s was achieved. During the 2013 monitoring, light levels reached ~1300-1400 µmol/m^2/s when high VPD conditions were experienced, and this correlated with nursery experience where more PaMS developed in glasshouses without screens, and with higher light levels. The lack of symptom development under high VPD and temperature conditions in the controlled environment work may also support the theory that high light levels in association with high VPD and temperature are required for PaMS symptoms to develop – and root development or root zone water balance may also prove to play an important role.

Conclusions

Following the lack of symptoms expressed in monitored batches, consultation with the wider bedding plant sector indicated few cases of PaMS in the industry during 2014. If PaMS symptoms are associated with stress due to high water requirements for photosynthesis

(under high light and temperature conditions), then we would expect the stress to have been lower in 2014 due to the lower light levels. Lower light levels – along with less extreme temperatures and high VPD events – recorded in the nursery monitoring in 2014 would also help to explain the reduced incidence of PaMS across the industry.

Further nursery monitoring will be carried out during 2015, to further examine the relationship between VPD, temperature, light levels and root status, and their effect on the development of PaMS.

Knowledge and Technology Transfer

An informal briefing has been presented to the industry representatives to provide updates on the controlled environment work and the second year of nursery monitoring. An HDC News article was published in October 2014.

Acknowledgements

We would like to acknowledge the work of Chris Need, Fay Richardson, Mike Smith, Caroline Shove, Russ Woodcock and the staff of Roundstone Nurseries, Coletta & Tyson, W.D. Smith & Son and Bryants Nurseries in developing this project and providing the data used in this study.

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Appendix 1. Controlled environment cabinet trial plan

Cabinet 1

Back of cabinet

Shelf 1

Block	1	2	1	2	4	3
Plot	1	5	2	6	10	8
Treatment	**2**	**3**	**2**	**3**	**3**	**2**
Block	4	3	5	6	6	5
Plot	12	9	13	16	17	15
Treatment	**3**	**2**	**2**	**3**	**3**	**2**

1

Front of cabinet

Cabinet 2

Back of cabinet

Shelf 1

Block	7	8	8	7	10	9
Plot	20	22	24	21	29	25
Treatment	**2**	**3**	**3**	**2**	**3**	**2**
Block	9	10	11	12	12	11
Plot	26	30	31	34	35	33
Treatment	**2**	**3**	**2**	**3**	**3**	**2**

2

Front of cabinet

Location of loggers which move from the glasshouse to the cabinets during cabinet treatment

Appendix 2. Glasshouse trial plan

Block	1	1	1		2	2	2
Plot	1	2	3		4	5	6
Treatment	2	2	1a		1b	3	3
Block	3	3	3		4	4	4
Plot	7	8	9	1	10	11	12
Treatment	1a	2	2		3	1b	3
Block	5	5	5		6	6	6
Plot	13	14	15		16	17	18
Treatment	2	1a	2		3	3	1b
Block	7	7	7		8	8	8
Plot	19	20	21		22	23	24
Treatment	1a	2	2		3	1b	3
Block	9	9	9		10	10	10
Plot	25	26	27	2	28	29	30
Treatment	2	2	1a		1b	3	3
Block	11	11	11		12	12	12
Plot	31	32	33		34	35	36
Treatment	2	1a	2		3	3	1b

Treatment no.	**Watering**
1a	Wet control
1b	Dry control
2	Wet cabinet
3	Dry cabinet

Only treatment 2 and 3 move into the CE cabinets

Location of loggers that follow the batch from sowing and remain in the glasshouse at Boxworth

Location of loggers which move from the glasshouse to the cabinets during cabinet treatment

Appendix 3. Grower monitoring template

Project title:	The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS) 2014	
ADAS:	HDC: PO 016	
Date	Comment	Initials

Production information	
Seed details	
Breeder/ supplier:	
Cultivar, genetics (F_1):	
Seed treatment:	
Storage Location (cold room, fridge):	
Storage Temperature:	
Germination/propagation information	
Date of sowing/batch number:	
Sowing method:	
Location within Nursery:	
Floor, bench, stillage? (Include construction details (open mesh, polystyrene, concrete floor):	
Position within location (e.g. any doors/vents nearby):	
Date covered (note if not milky plastic):	
Date cover removed:	
Module (cell number):	
Module (cell volume):	
Growing media (product, specification, additives e.g. wetters). Obtain sample.	
Movement information:	
Transport method:	
Route (outdoors, indoors etc):	
Duration:	

Covered?	
Growing on information:	
Date of transplanting:	
Growth stage at transplanting (no. of leaves)	
Location within Nursery:	
Floor, bench, stillage? (Include construction details (open mesh, polystyrene, concrete floor):	
Position of monitors within location (within 5 m of a doorway/vent/fans):	
Module (cell number):	
Module (cell volume):	
Growing media (product, specification, additives e.g. wetters). Obtain sample.	

Irrigation application:				
Date	**Stage of production**	**Volume**	**Method of application**	**Source (mains/reservoir/borehole)**

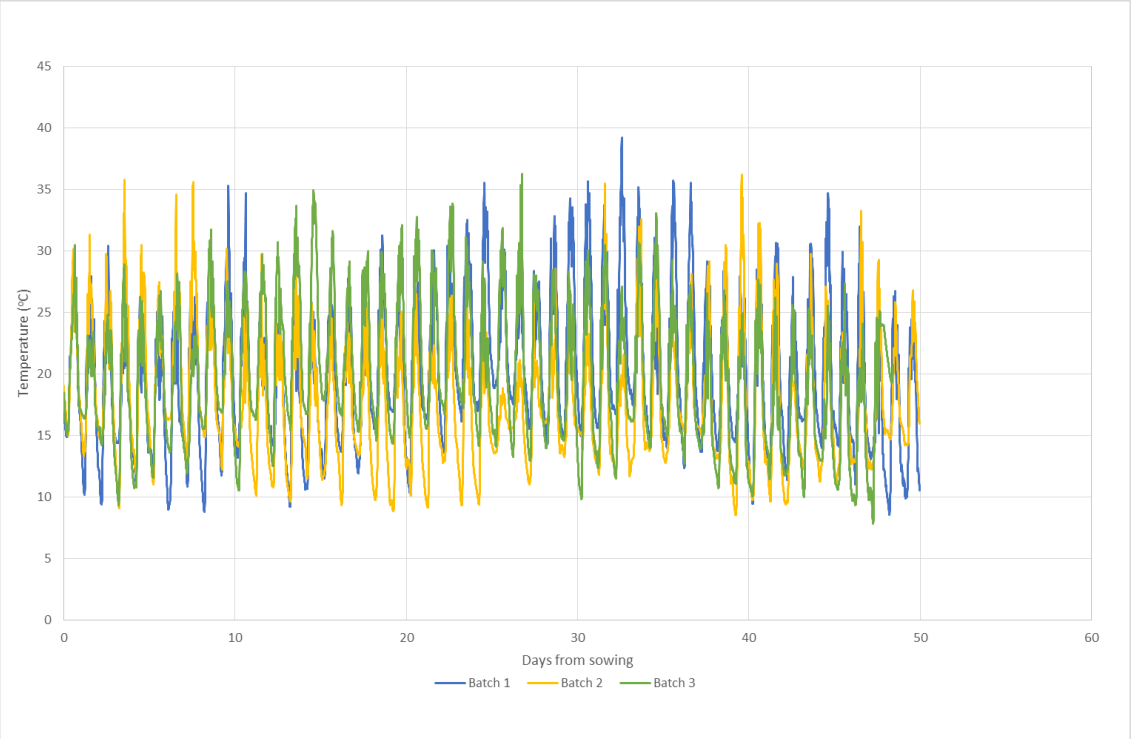
Fertiliser application:					
Date	**Stage of production**	**Product**	**NPK content**	**Method of application**	**Concentration (g/l)**

Crop protection and PGR application:					
Date	**Input type**	**Dose rate/water volume**	**Product name**	**Active ingredient**	**Application Method**

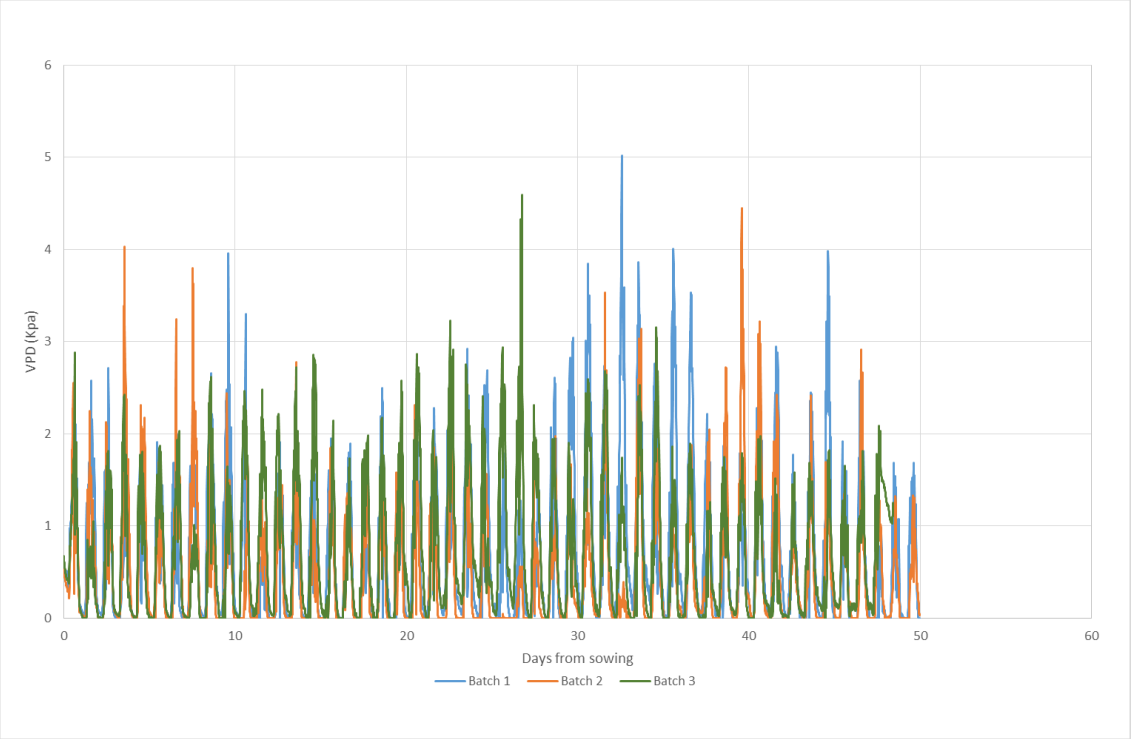
Root hair assessment at transplant		**Date:**			
Pansy batch / sowing date:		**Growth stage (no of leaves):**			
Plant no.	**Root hair score (0-3 scale)**	**Comments**	**Plant no.**	**Root hair score (0-3 scale)**	**Comments**
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

Pansy Mottle Syndrome incidence					
Date	**Time**	**Sowing batch**	**Tray number in batch**	**Number of plants affected**	**Growth Stage**

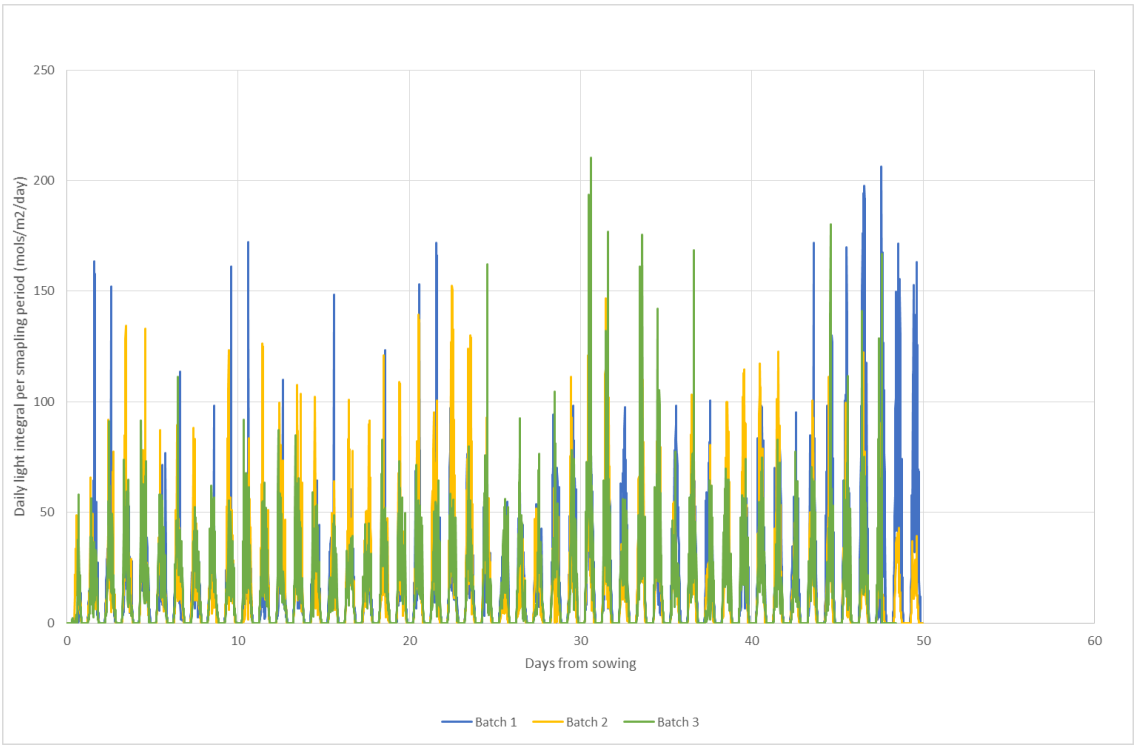
Appendix 4. Site environmental data



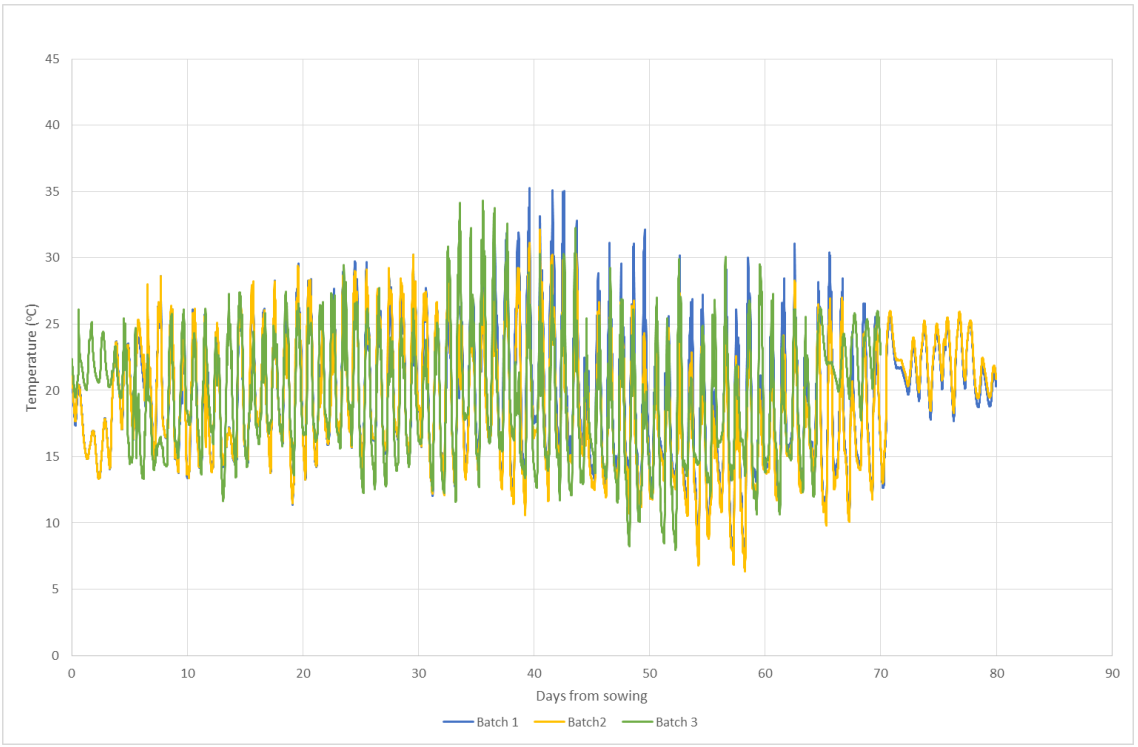
Temperature (°C): site A, all batches, 2014



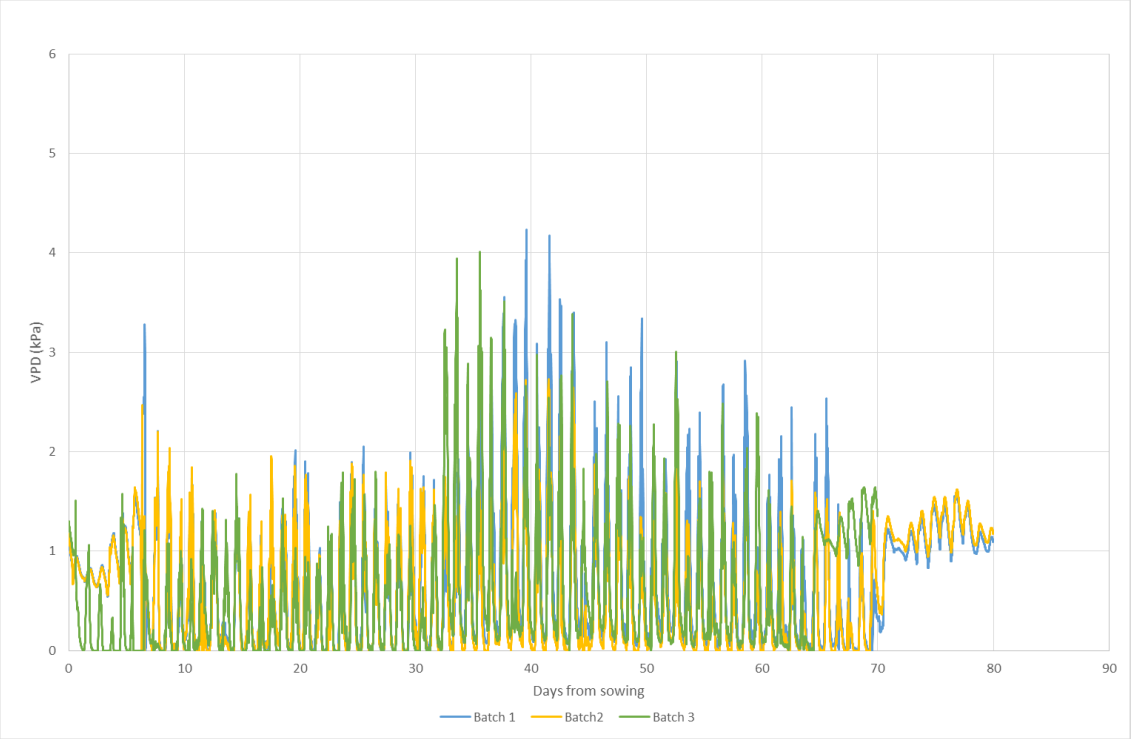
VPD (kPa): site A, all batches, 2014



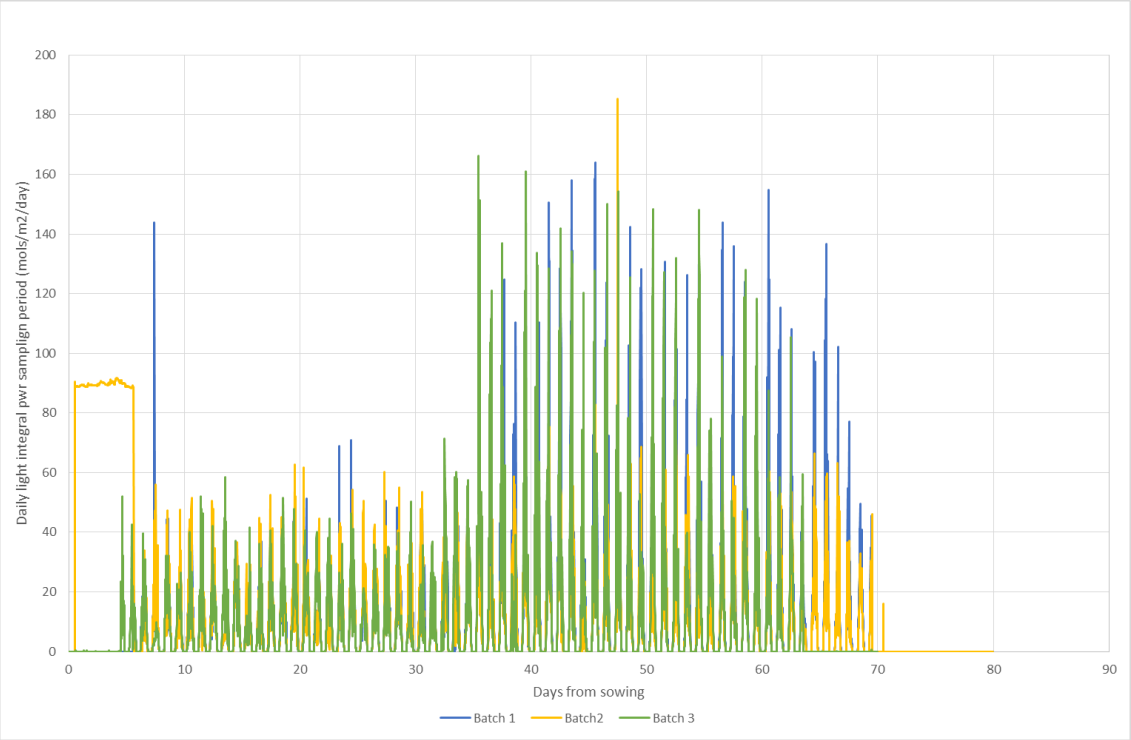
Daily Light Integral per sampling period (mols/m^2/day), site A all batches, 2014 (calculation based on a 24 hr day)



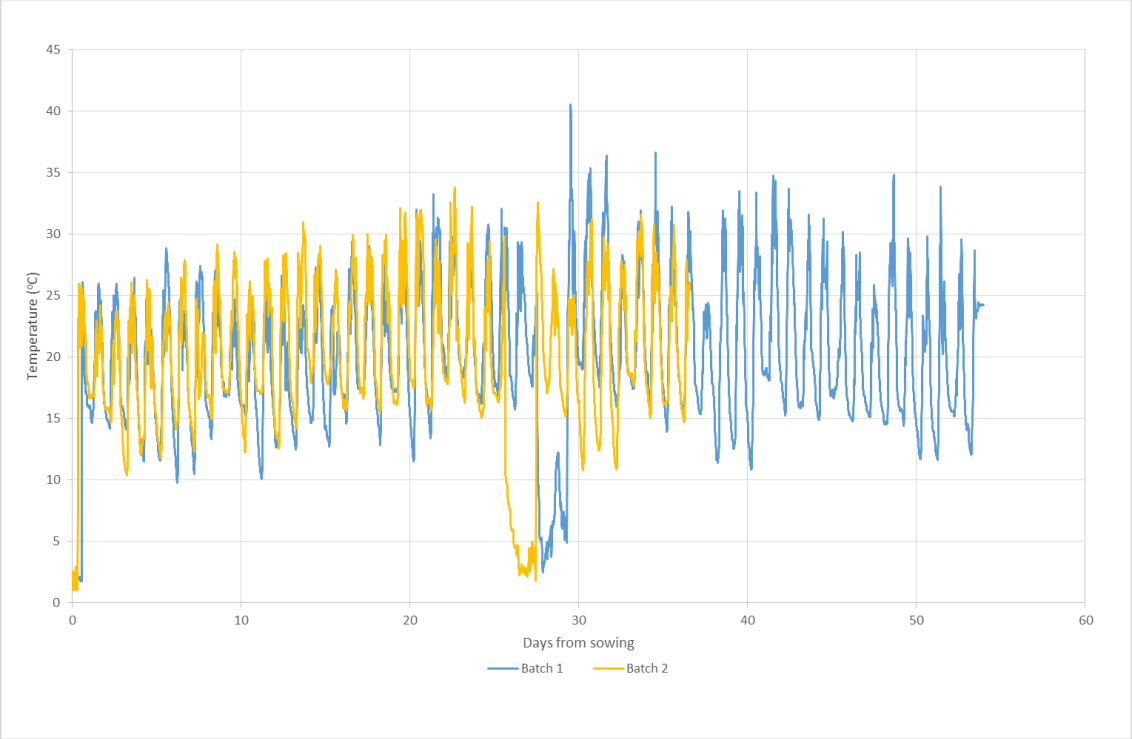
Temperature (°C): site B, all batches, 2014



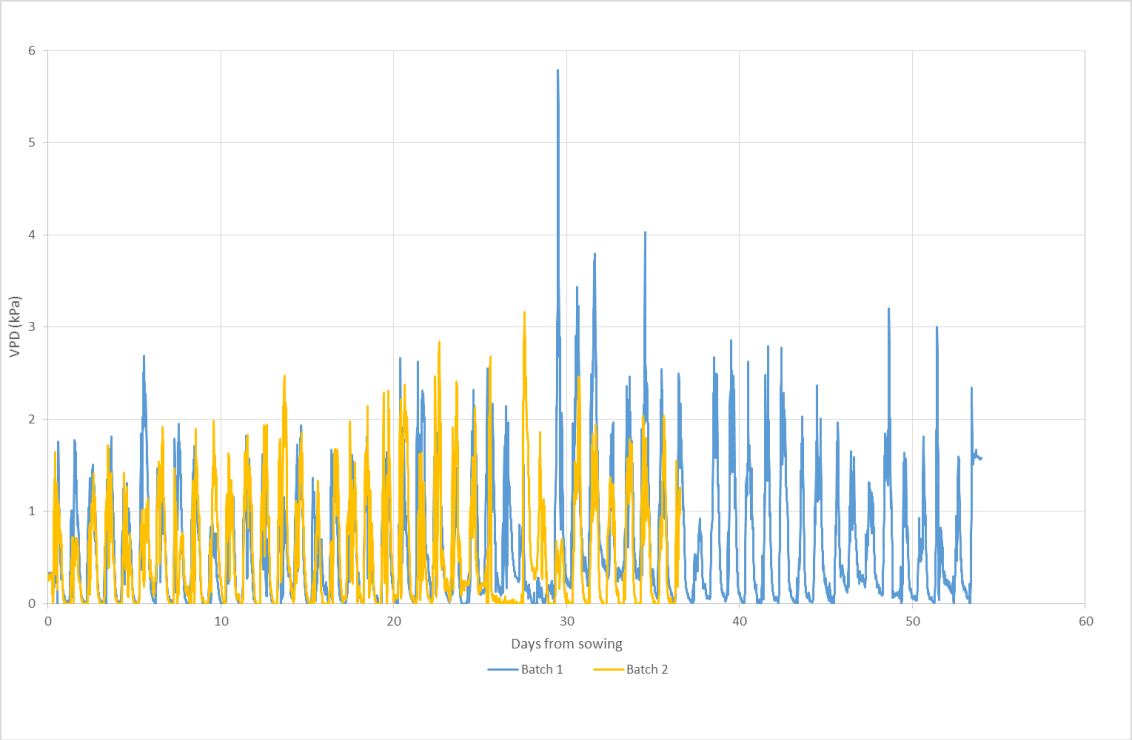
VPD (kPa): site B, all batches, 2014



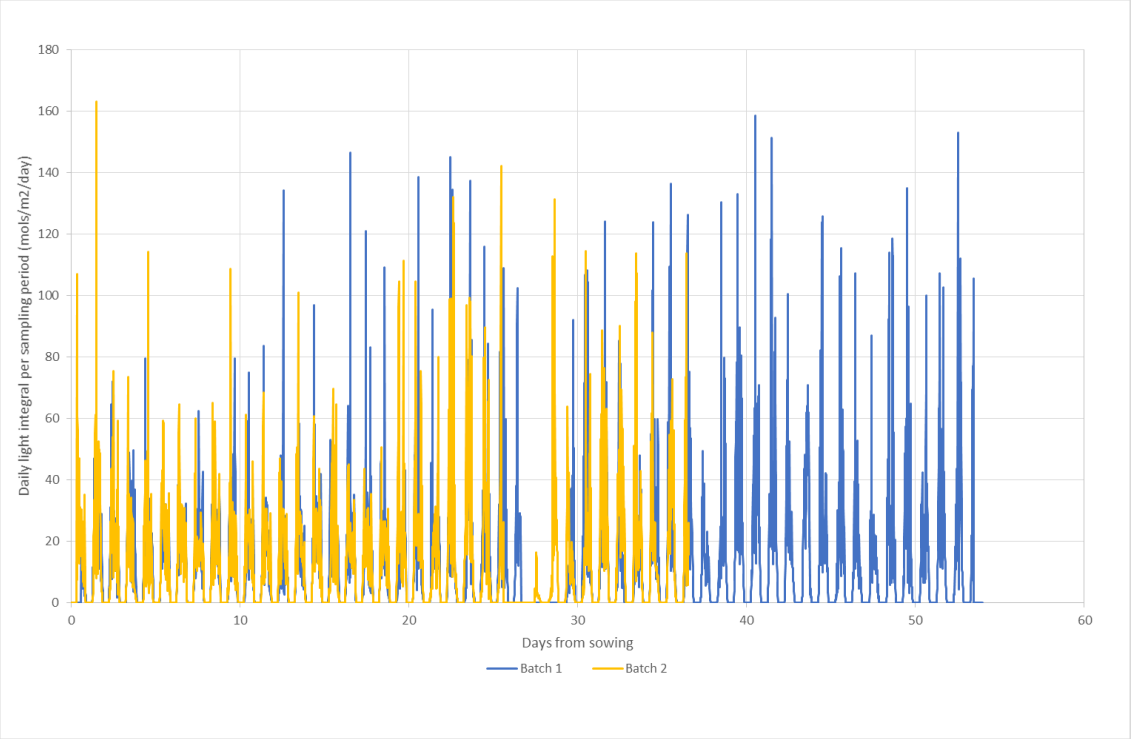
Daily Light Integral per sampling period (mols/m^2/day), site B all batches, 2014 (calculation based on a 24 hr day)



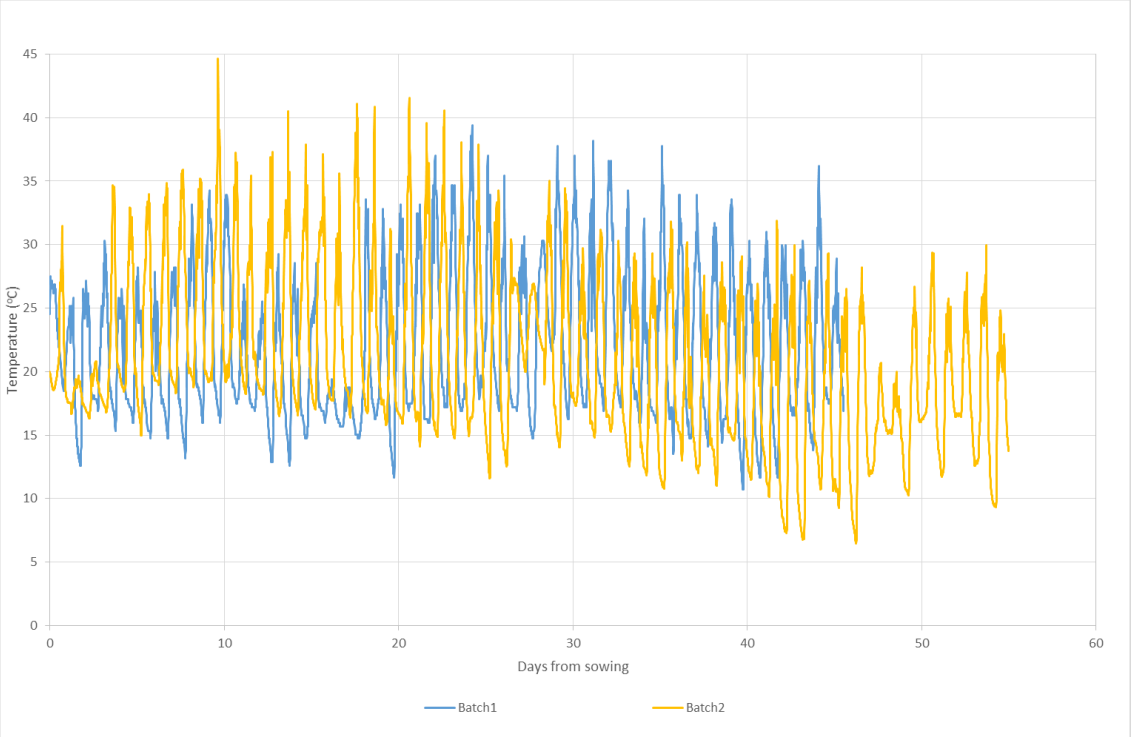
Temperature (°C): site C, all batches, 2014



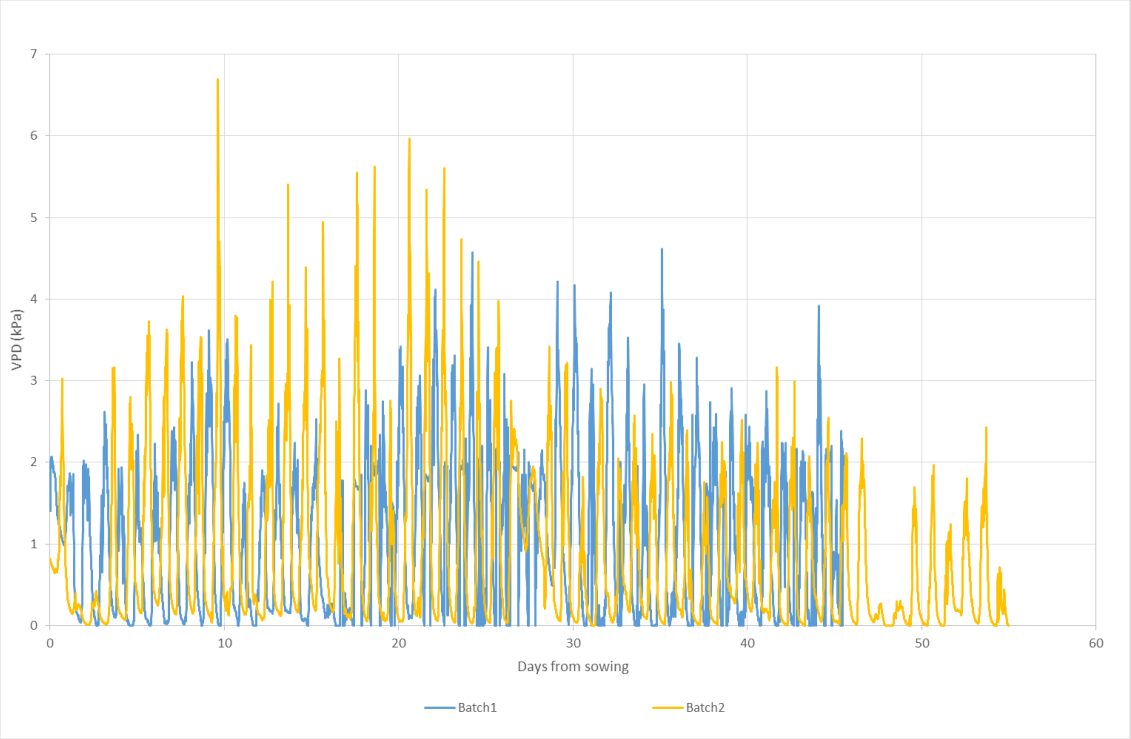
VPD (kPa): site C, all batches, 2014



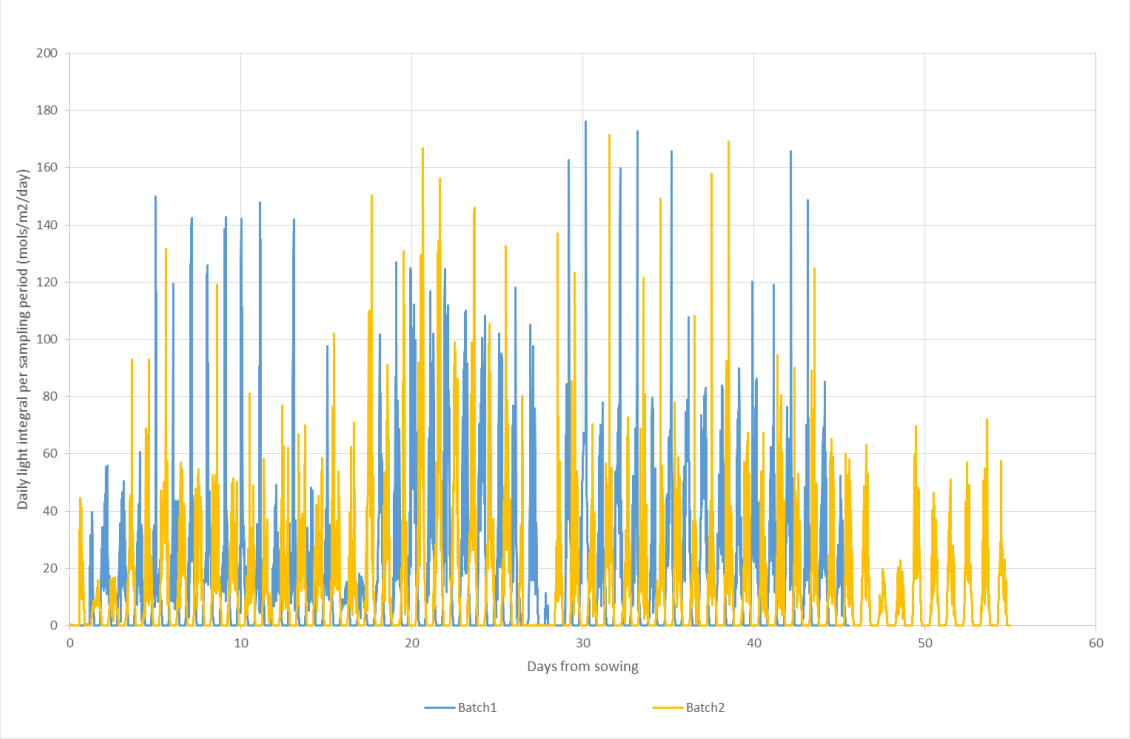
Daily Light Integral per sampling period (mols/m^2/day), site C all batches, 2014 (calculation based on a 24 hr day)



Temperature (°C): site D, all batches, 2014



VPD (kPa): site D, all batches, 2014



Daily Light Integral per sampling period (mols/m^2/day), site D all batches, 2014 (calculation based on a 24 hr day)