| | |
|---|---|
| Project title: | The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS) |
| Project number: | PO 016 |
| Project leader: | Dr Jill England, ADAS Boxworth |
| Report: | Year 1 report, April 2014 |
| Key staff: | Dr Jill England, Project Leader<br>Dr Dave Skirvin (ADAS), Senior Research Consultant<br>Chloe Whiteside (ADAS), Horticulture Consultant |
| Location of project: | Commercial growers |
| Industry Representative: | Chris Need, Roundstone Nurseries, Pagham Rd, Lagness, Chichester, W. Sussex PO20 1LL.<br>Fay Richardson, Coletta & Tyson, 324 Hull Rd, Woodmansey, Beverley, E. Yorks HU17 0RU<br>Mike Smith, W.D. Smith & Son, Grange Nurseries, Woodham Rd, Battlesbridge, Essex SS11 7QU |
| Date project commenced: | 1 May 2013 |
| Date project completed<br>(or expected completion date): | 30 April 2014 |

[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.

Dr Jill England
Horticulture Consultant
ADAS

Signature ..... Date .....

Dr Dave Skirvin
Senior Environmental Modeller
ADAS

Signature ..... Date .....

Chloe Whiteside
Horticulture Consultant
ADAS

Signature ..... Date .....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature ..... Date .....

CONTENTS

# GROWER SUMMARY

## Headline

High 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. 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 also 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 cause.

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, specifically to monitor nursery environment (humidity, temperature, light) within commercial bedding plant production systems and identify any causal relationships between the incidence of PaMS and environment.

## Summary of the project and main conclusions

Data were collected from four commercial nurseries (sites A-D) located in Hertfordshire, East Yorkshire, West Sussex and Essex respectively between July and October 2013. The sites selected included grower holdings with a record of PaMS and which produced pansies from seed to enable the entire production process be monitored. Three pansy batches were monitored at sites A and B, and two batches at sites C and D. Each batch was monitored using a Tinytag Plus 2 data logger (temperature and humidity), and a Watchdog 1000 series microstation data logger (temperature, humidity, and light). 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 1). Sowing, transplant and dispatch dates for the batches monitored were recorded along with production data for routine inputs: irrigation (method, volume, and source), fertiliser, crop protection and plant growth regulator application, and growing media was also collected. Batches were monitored daily for PaMS symptoms and the location of symptomatic plants recorded, along with the date and time of inspection.

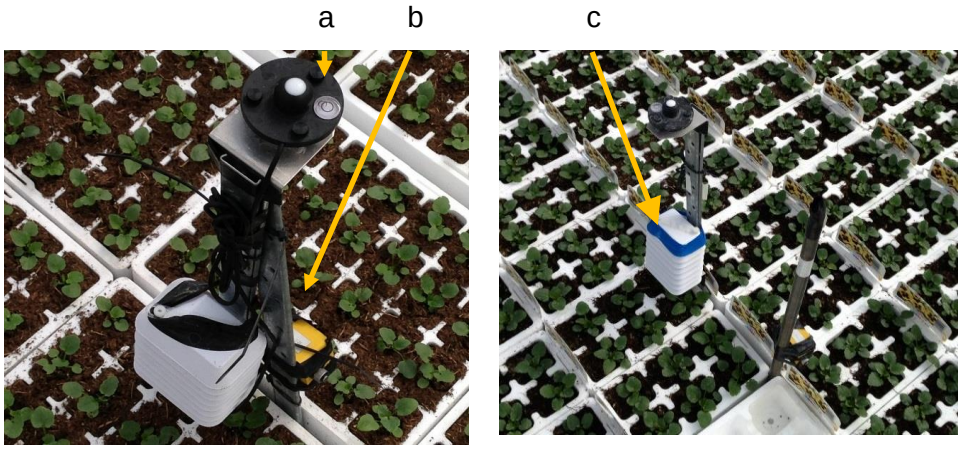


**Figure 1.** Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and water damage

 

Of the crops monitored, PaMS symptoms, including variegation and leaf distortion, developed in a single batch (site A, batch 1) of Pansy 'Autumn Mixed'. Approximately 10-20% of the batch showed the full range typical PaMS symptoms, i.e. leaf distortion, mottling, leaf bleaching, stunting and apical blindness (Figure 2). Symptoms were first noted on 9th September, two weeks post transplanting. In another batch (site A, batch 2) approximately 10-20% of the plants showed some leaf distortion, but the variegation was not present. These symptoms were first observed on 25th September.

**Figure 2.** PaMS symptoms recorded site A, batch 1, 2013.

**Data analysis**

Initial data analysis examining cumulative day degrees above a threshold of 0°C determined that for all batches data were highly consistent across the sites. For light, despite the partial loss of data due to logger failure, there was generally a high level of consistency in cumulative photosynthetically active radiation (PAR) received by the plants in different batches and sites. The PAR received by site A, batch 1, where full range of PaMS symptoms were observed, was noticeably higher than that received by all other monitored batches on all sites from around 700 day degrees after sowing (38 days after sowing). The second batch in which symptoms were observed (site A, batch 2) showed slightly higher cumulative PAR compared to the other batches (excluding site A, batch 1). However, due to losses of some of the light data, it was not possible to determine absolutely whether the high values for PAR in site A, batches 1 and 2 were unusual compared with all batches monitored, linked to the occurrence of PaMS symptoms, or part of the general variation in conditions across batches and sites.

The data showed a lack of consistency between holdings in the volume of water applied, although this may be linked to the way area irrigated was reported for each batch. The data for site A, where PaMS symptoms were observed, indicated that batch 3 received less water than batches 1 and 2 (that expressed symptoms of PaMS), particularly during the early growth stages.

 

*Site A, batch 1 (PaMS – full range of symptoms includ*ing *leaf distortion, mottling, leaf bleaching, stunting and apical blindness observed)*

Based on the observation of PaMS (site A, batch 1), a more detailed assessment of the data was undertaken focussing on vapour pressure deficit (VPD) and temperature. 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 occurrence of PaMS symptoms (in contrast to reported observations) (Figure 3). The data for site A, batch 1 showed that in the two weeks prior to the occurrence of PaMS symptoms, the plants experienced a VPD greater than 4 KPa and a temperature greater than 35°C for over 1 hour on 6 days over a 10 day period, with over 4 hours exposure for both VPD and temperature on two consecutive days (4th and 5th September). This level of exposure and the clustering of events into a short period does not occur in any other batches.

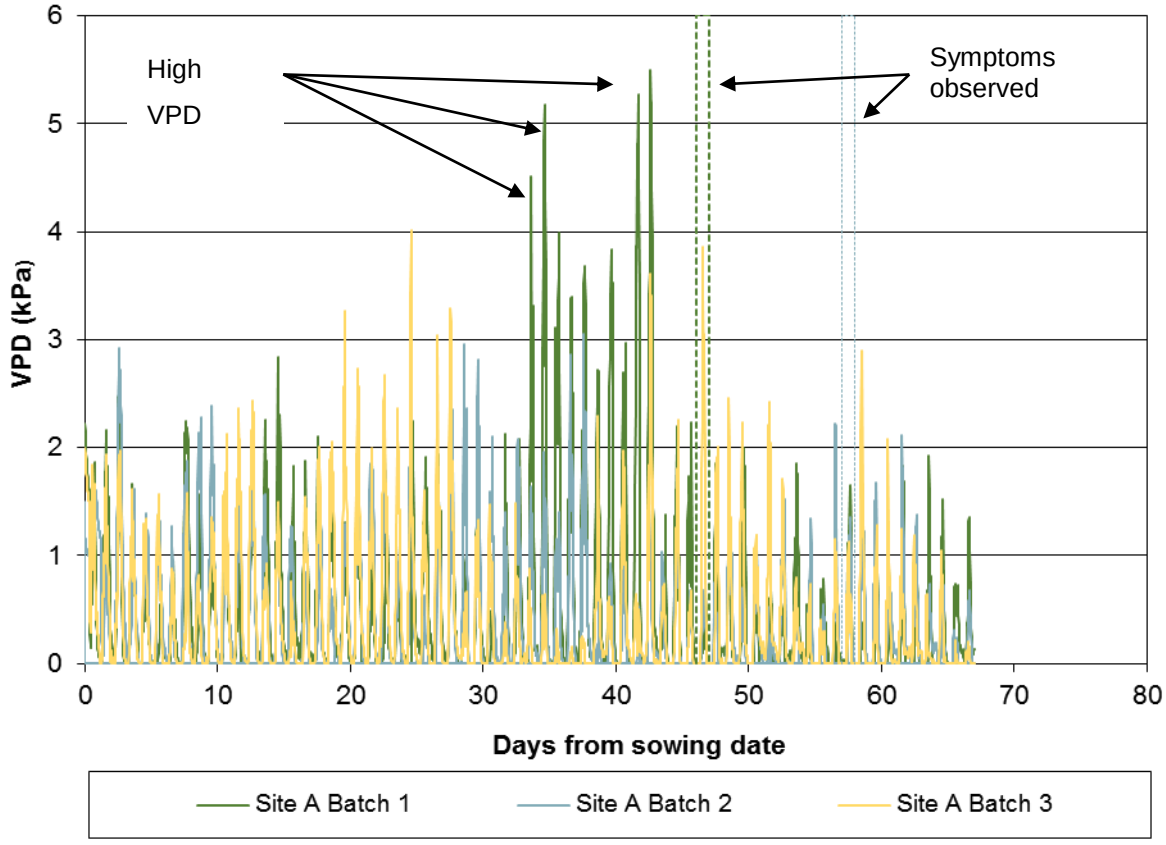


**Figure 3.** The vapour pressure deficit (VPD) for all batches on site A (the dashed lines show the day on which PaMS symptoms were observed for batches 1 and 2).

 

Site A, batch 1 was grown on in a polytunnel post transplanting rather than a glasshouse, hence there would have been less control over the environmental conditions. Given that this batch did show the variegation, then the expression of this variegation and leaf distortion could potentially be a response to stress associated with more extreme high VPD and temperature conditions in the polytunnel. It is also possible that the observed PaMS symptoms were related to the generally elevated temperatures in the polytunnel rather than specific high VPD and temperature events. As the polytunnel did not have shading, this would explain the higher light levels and daily light integral experienced by the plants after transplanting, and could be linked to the expression of PaMS symptoms.

*Site A, batch 2 (PaMS symptoms observed, excluding mottling or bleaching)*

Site A, batch 2, which was grown on in a glasshouse post transplanting, was observed to have leaf distortion, which was recorded as PaMS symptoms, but no association with high VPD and temperature events could be found. Assuming the high VPD and high temperature conditions are linked to PaMS, there are two possible explanations for this:

a) PaMS occurrence is driven by more than just high VPD and temperature events
b) The symptoms observed in site A, batch 2 were not PaMS symptoms

It is not possible to determine which of these explanations is more plausible using the evidence currently available from this project.

*Sites B, C and D (no PaMS symptoms observed)*

For site B there were no spikes of high VPD or temperature in any of the batches and no PaMS symptoms were observed on this nursery. At sites C and D there were occasional spikes of high VPD and temperature in both batches at each site, but the spikes were not as prolonged or as clustered as in site A, batch 1; no PaMS symptoms were observed

*Analysis of additional data from site B*

Although batches monitored at site B did not show any observable PaMS symptoms, symptoms had been observed on other batches within the same growing location earlier in the season. To cross check whether high VPD and temperature were associated with the occurrence of these symptoms, data from the Priva environmental monitoring system was analysed and used to determine the VPD for the five days prior to the occurrence of PaMS symptoms. Vapour pressure deficit was shown to exceed values of 3KPa for a minimum of 3 hours on 3 dates, 2 of which were consecutive, and to exceed 4KPa for at least 0.5 hours

 

on each of these days, with one day having 2 hours with VPD greater than 4KPa. All of the events where VPD exceeded 3 KPa were also associated with periods when the temperature at canopy level was predicted to exceed 35°C. These results show that elevated temperatures and VPD occurred prior to observations of PaMS symptoms, providing further evidence for a potential link between these adverse environmental conditions and the occurrence of PaMS symptoms.

Despite the low occurrence of PaMS symptoms in the monitored batches across the four sites, it was possible to identify a potential association between environmental factors and the occurrence of PaMS symptoms. This association was derived from the observation that the VPD, temperature and daily light integral in Batch 1 at site A were higher than for the other batches at the same site and also for batches at other sites. It must be stressed that this association is extremely tentative due to the sample size of one, which has precluded any robust statistical analysis of the environmental data and the different symptoms and environmental factors associated with Batch 2, site A. Further data is required to confirm that high VPD and temperature events are associated with the occurrence of PaMS symptoms and the role that light levels may play in expression of symptoms.

## 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 the first year of this study suggested a causal link between high VPD and high temperature and the expression of PaMS symptoms. The precise triggers and sequence of events that lead to PaMS still remain to be elucidated within the current project but growers should:

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. The results of this study will feed into future studies and provide grower advice to prevent PaMS occurring.

## Materials and methods

Data was collected from four commercial nurseries (sites A-D) located in Hertfordshire, East Yorkshire, West Sussex and Essex respectively between July and October 2013. 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 because they grow pansies from seed, so the production process from sowing to marketing could be monitored.

Three batches of pansy were monitored at sites A and B, and two batches at sites C and D. Each batch was monitored using a Tinytag Plus 2 data logger (temperature and humidity) and a Watchdog 1000 series microstation data logger with an external Light Scout Quantum Light 3 Sensor PAR probe (temperature, humidity and light). Data loggers were set to record data every 30 minutes on sites A, C and D, and every 5 minutes at site B. This difference allowed comparisons to be made between logging intervals in relation to capturing the effect of short term changes in the environment on symptom development. Sowing, transplant and dispatch dates for the batches monitored were recorded (Table 1). 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).

 

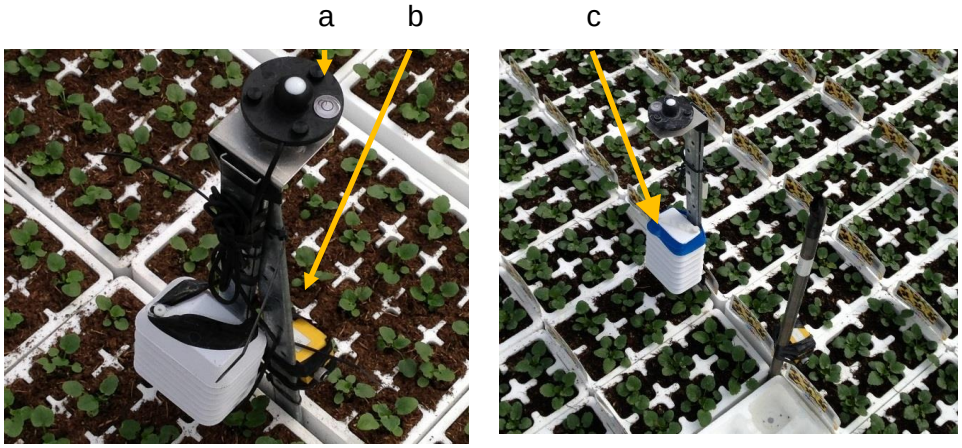


**Figure 5.** Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and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

**Table 1. 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 | Batch 1 | 25.07.13 | 27.08.13 | 09.09.13 | 03.10.13 |
| Site A | Batch 2 | 30.07.13 | 09.09.13 | 25.09.13 | 14.10.13 |
| Site A | Batch 3 | 09.08.13 | 16.09.13 | - | 23.10.13 |
| Site B | Batch 1 | 01.08.13 | 5/6.09.13 | - | 20.10.13 |
| Site B | Batch 2 | 01.08.13 | 05.09.13 | - | 7.10.13 |
| Site B | Batch 3 | 08.08.13 | 12.09.13 | - | 20.10.13 |
| Site C | Batch 1 | 24.07.13 | 22.08.13 | - | 30.09.13 |
| Site C | Batch 2 | 30.07.13 | 27.08.13 | - | 30.09.13 |
| Site D | Batch 1 | 23.07.13 | 21.08.13 | - | 11.09.13 |
| Site D | Batch 2 | 30.07.13 | 27.08.13 | - | 27.09.13 |

## Assessments

Batches were monitored daily for PaMS symptoms by nursery staff and the location of symptomatic plants recorded, along with the date and time of inspection. Symptomatic plants were then further inspected by ADAS, to quantify the number of infected plants and their position both within the module tray and the glasshouse. 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 1).

 

At site B, batches 1 and 2 were sown on the same day (same cultivar), and then separated at transplanting stage and transported to different growing locations, which were of different construction and age, providing different environmental conditions. Growing location 1 had boom irrigation, whilst at growing location 2, the plants were watered by a combination of hand and overhead irrigation. Monitoring plants from the same batch at different locations enabled observation of the effect of the different environmental conditions on incidence of PaMS.

## Results

Of the crops monitored, classic PaMS symptoms (variegation and leaf distortion) developed in a single batch (site A, batch 1) of pansy 'Autumn Mixed'. Approximately 10-20% of the batch showed symptoms typical of PaMS (Figure 6). Symptoms were first noted on 9th September, two weeks post transplanting. In another batch (site A, batch 2) approximately 10-20% of the plants showed some leaf distortion, but the variegation was not present. These symptoms were first observed on 25th September.

**Figure 6. PaMS symptoms seen in site A, batch 1, 2013.**

## Environmental Data Capture

Temperature and humidity data recorded by the TinyTag data loggers was captured for all sites and batches. For the Watchdog data loggers, however, data did not cover the entire growing period for all batches due to some loggers failing to start or restart as programmed following data download, leading to a loss of Watchdog temperature, humidity and light data for some batches at some sites. Total loss of Watchdog data occurred at site C, batch 1; partial loss of data occurred at site A, batch 3 (from 27/9/13), and site B in all 3 batches (from 8/9/2013). The Tinytag loggers did provide temperature and humidity data for the periods when the Watchdog loggers failed to record. Logger checks indicated that the Watchdog loggers had not completely failed.

 

## Data Analysis

The data analysis component of the work aimed to determine whether there was a statistically robust relationship between the monitored environment variables and the occurrence of Pansy Mottle symptoms. Due to insufficient occurrences of symptoms in the monitored batches for statistical analysis, only exploratory data analysis could be undertaken. This section of the report describes the analysis of the data and the results obtained from this analysis.

## 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. This analysis used the temperature and humidity data from the TinyTag loggers as full datasets (covering the entire production period of all batches on each site) were available from these loggers; these loggers were also located nearest to the plant canopy and so provided a more accurate assessment of the temperature and humidity conditions experienced by the plants. The cumulative day degrees, PAR (photosynthetically active radiation) and irrigation experienced by each batch of plants is shown below (Figures 7, 8 and 9).

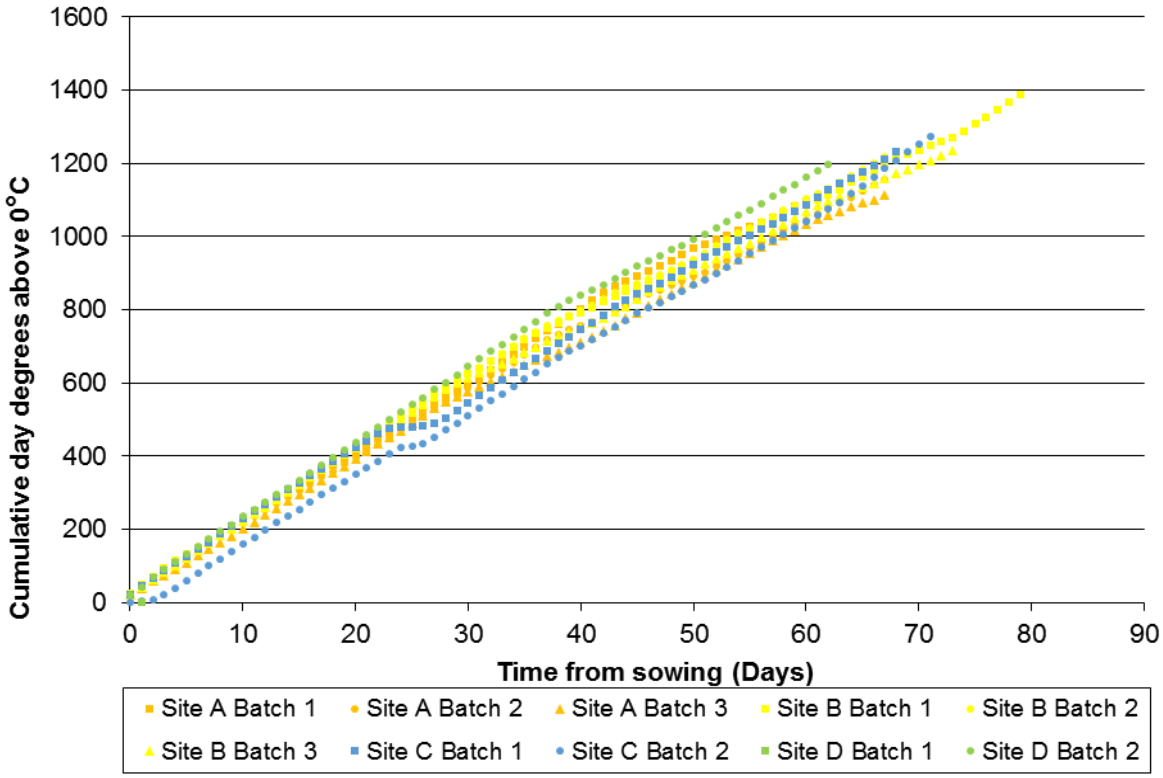


**Figure 7. Cumulative day degrees above 0°C for all batches at all sites**

Cumulative day degrees (Figure 7) for all batches were highly consistent across the sites. For site C, the storage of sown seeds in a cold store at the start of production, and prior to

 

transplanting are easily identified as flat spots starting at 0 and 25 days from sowing. 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, and that the 30 minute logging interval was sufficient to capture the main dynamics of the environment al conditions as there were no differences between data collected at site B (data collected at five minute intervals) and sites A, C and D (data collected at 30 minute intervals).

The cumulative photosynthetically active radiation (PAR) chart (Figure 8) clearly shows where there was full or partial loss of data. Despite this, there was a high level of consistency in the PAR received by the plants in different batches and sites. The PAR received by site A, batch 1 was noticeably higher that the other batches from around 700 day degrees (38 days) after sowing; 'classic' PaMS symptoms were observed in this batch. The other batch in which symptoms were observed (site A, batch 2) showed slightly elevated cumulative PAR compared to the other batches (excluding site A, batch 1), being higher than batches at other sites and also site A, batch 3. However, due to the full loss of data from site C (and partial loss of data from sites B and D) it was not possible to determine absolutely whether the high values for cumulative PAR in batches 1 and 2, site A were unusual, linked to the occurrence of PaMS symptoms, or part of the general variation in conditions across batches and sites. Nevertheless, this does suggest that monitoring PAR is important as further data may help to elucidate any association that PAR may have with PaMS symptom occurrence.

 

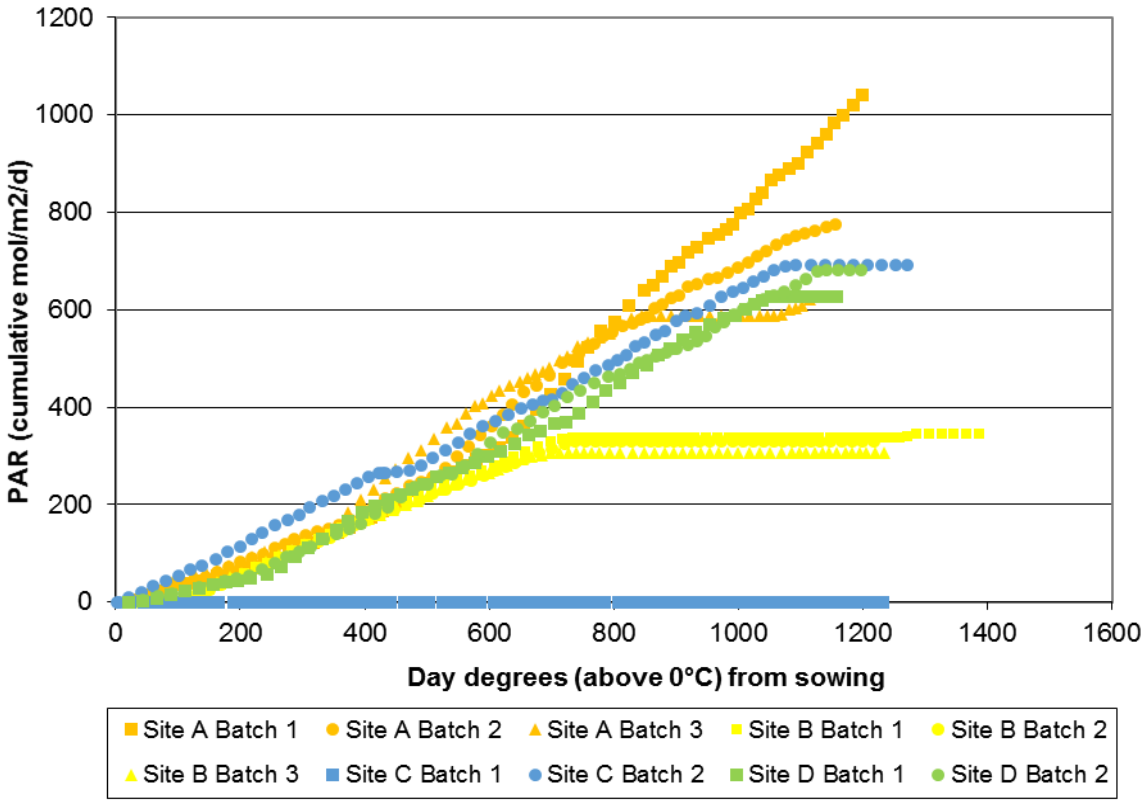


**Figure 8. Cumulative photosynthetically active radiation (PAR) experienced by each batch**

The cumulative irrigation charts (Figure 9a, b and c) show less consistency between batches and sites, although this may be linked to the reporting of the area within each batch to which irrigation was applied. No data was available for the volume of irrigation applied at site B, and the volumes reported for site D were an order of magnitude lower than those reported for sites A and C. Despite these issues, the irrigation volumes reported within sites were consistent, and a similar volume of water appears to have been used at sites A and C. However, without further information on the area to which the volumes were applied (to allow appropriate scaling and adjustment of irrigation to the same units on all sites), it is not possible to draw any firm conclusions about irrigation and its association with PaMS symptoms. The data for site A, where PaMS symptoms were observed, indicate that a different irrigation profile occurred for batch 3 compared to batches 1 and 2 (that expressed symptoms of PaMS); batch 3 received less water than batches 1 and 2, particularly during the early growth stage (Figure 9c).

 

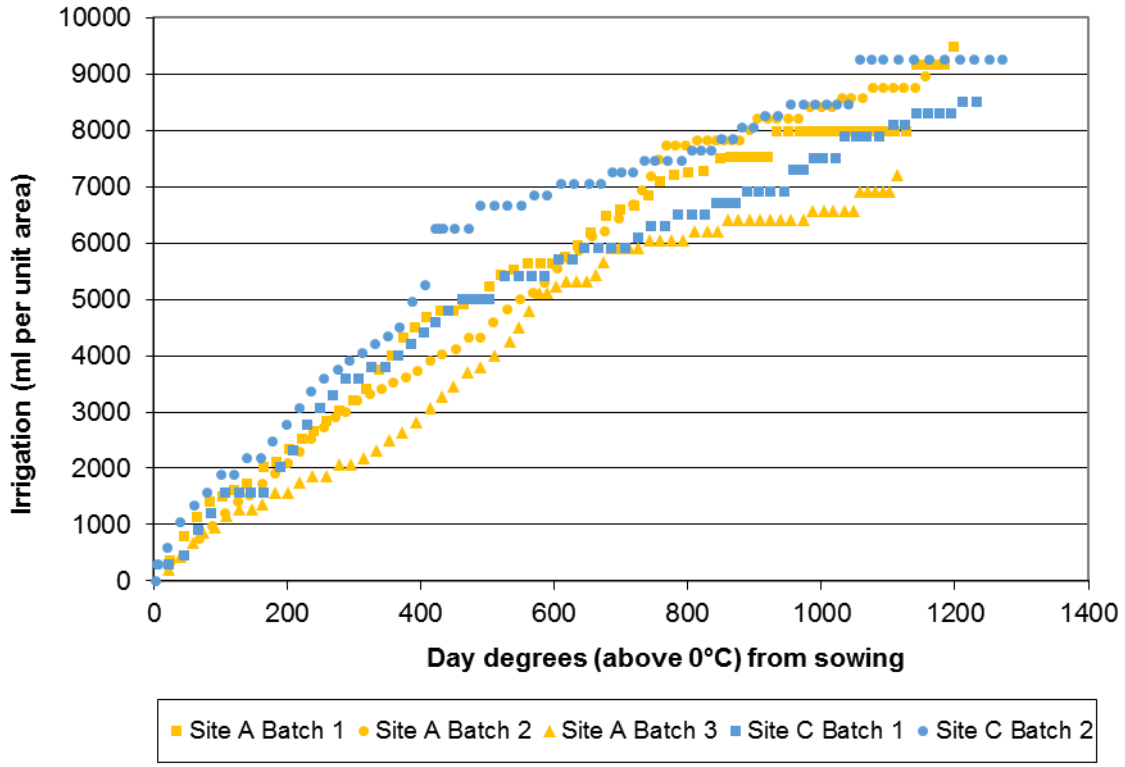


**Figure 9a. Cumulative irrigation of batches at sites A and C**

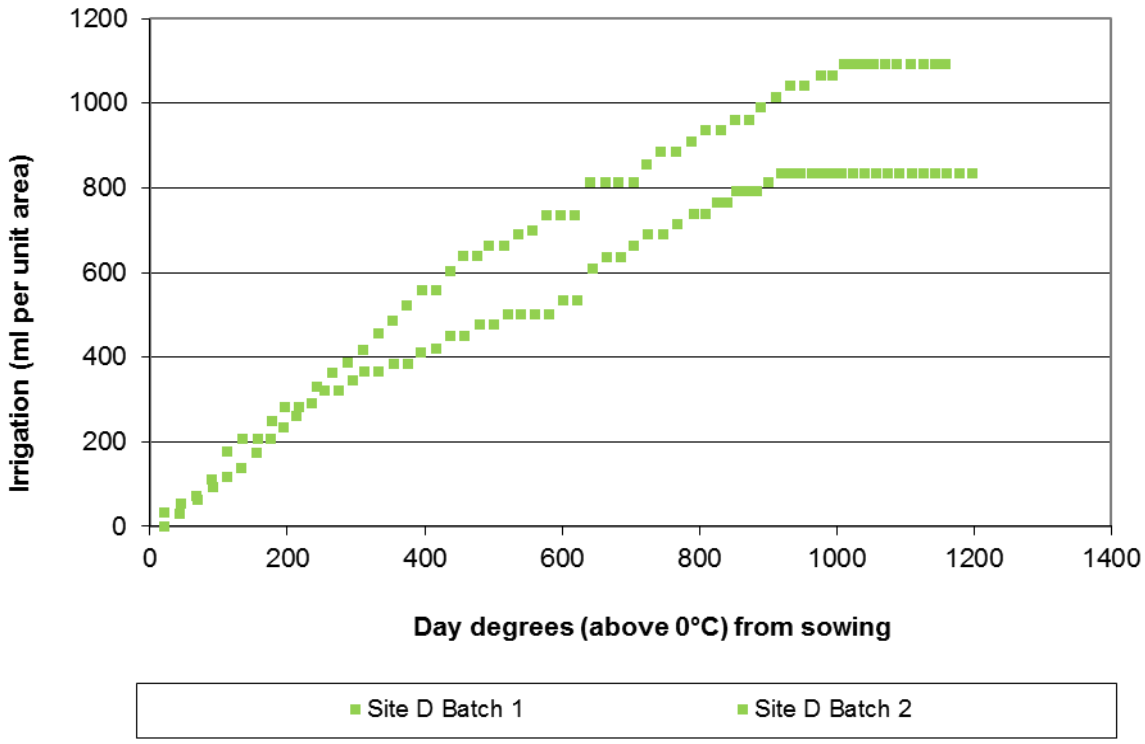


**Figure 9b.** Cumulative irrigation of batches at site D

 

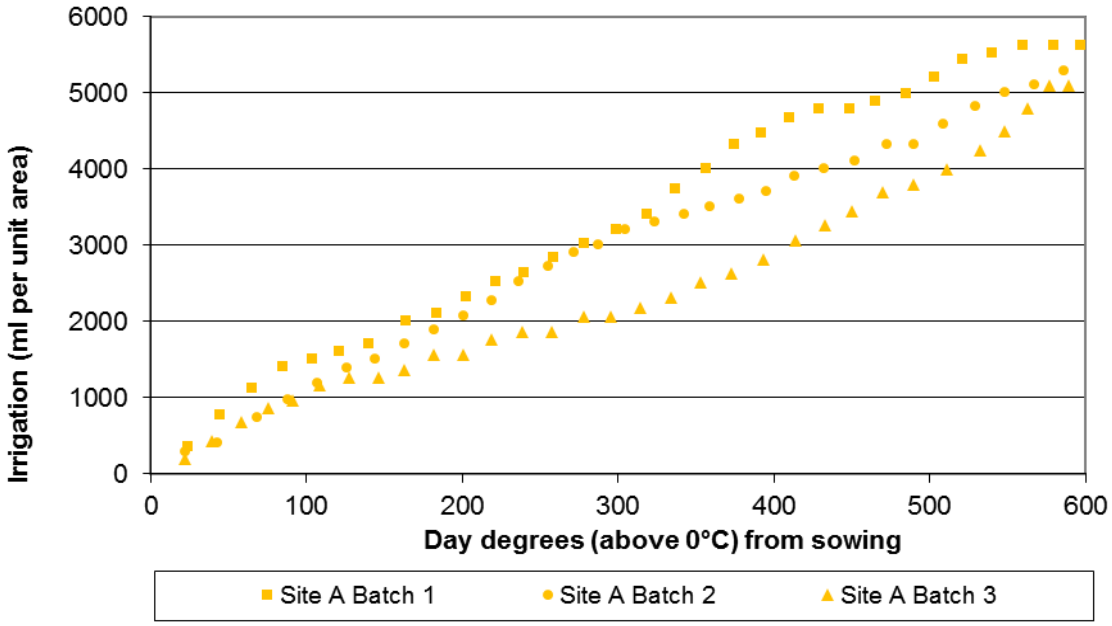


**Figure 9c.** Cumulative irrigation, site A, in early growth stages, prior to transplanting.(600 day degrees)

### *Detailed data analysis*

As the preliminary analysis did not reveal any clear evidence of a link between environmental factors and PaMS symptoms, more detailed analysis was undertaken using both hourly and daily averaged data, to determine whether there were differences in the accumulation of thermal time, vapour pressure deficit (VPD), daily light integral and irrigation volume across different time periods close to transplanting (Table 2); periods close to transplanting were selected as the PaMS the symptoms observed (site A, batches 1 and 2) occurred within 14 days of transplanting. Vapour pressure deficit describes the drying effect of air; high VPD occurs under high temperature, low humidity conditions.

Table 2 indicates that for site A, batch 1, where PaMS symptoms were observed, the cumulative daily light integral, vapour pressure deficit (VPD) and irrigation after transplanting appear to be higher than those for other batches, either on the same site or on a different site. Although site A, batch 2 was also reported as having symptoms, these were not “classic” PaMS symptoms as no variegation of the leaves was present, and apart from a high cumulative irrigation in the 7 days prior to transplanting, the environmental factors shown in Table 2 were similar to those of other batches.

 

**Table 2.** Observed values for key environmental variables prior to or just after transplanting

| | Site A | | | Site B | | | Site C | | Site D | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Batch 1 | Batch 2 | Batch 3 | Batch 1 | Batch 2 | Batch 3 | Batch 1 | Batch2 | Batch 1 | Batch 2 |
| PaMS observed | Y | Y | N | N | N | N | N | N | N | N |
| Transplant - days from sowing | 33.0 | 41.0 | 38.0 | 35.0 | 28.0 | 35.0 | 34.0 | 28.0 | 28.0 | 29.0 |
| Cumulative irrigation (ml/unit) | | | | | | | | | | |
| 7 days prior to transplanting | 960.0 | 1860.0 | 800.0 | * | * | * | 600.0 | 1900.0 | 150.0 | 149.0 |
| 7 days after transplanting | 1290.0 | 340.0 | 400.0 | * | * | * | 900.0 | 800.0 | 122.0 | 188.0 |
| 10 days after transplanting | 1560.0 | 730.0 | 550.0 | * | * | * | 1100.0 | 800.0 | 147.0 | 238.0 |
| 14 days after transplanting | 1560.0 | 730.0 | 750.0 | * | * | * | 1500.0 | 1200.0 | 227.0 | 190.0 |
| Cumulative daily light integral | | | | | | | | | | |
| 7 days prior to transplanting | 118.3 | 124.5 | 71.9 | 66.7 | 76.2 | 48.5 | * | 52.8 | 102.2 | 132.5 |
| 7 days after transplanting | 238.3 | 79.7 | 82.7 | * | * | * | * | 104.1 | 124.9 | 131.4 |
| 10 days after transplanting | 311.8 | 105.4 | 119.4 | * | * | * | * | 137.0 | 181.6 | 163.5 |
| 14 days after transplanting | 380.1 | 142.1 | 119.4 | * | * | * | * | 179.9 | 244.1 | 200.4 |
| Cumulative hour degrees above zero | | | | | | | | | | |
| 7 days prior to transplanting | 3666.3 | 3147.0 | 2603.1 | 3508.9 | 3610.4 | 2879.8 | 2008.3 | 2908.0 | 4058.2 | 3961.0 |
| 7 days after transplanting | 3961.1 | 2619.2 | 2866.1 | 2880.5 | 3090.0 | 2631.3 | 3878.7 | 3806.8 | 4058.2 | 3963.5 |
| 10 days after transplanting | 5464.3 | 3522.9 | 4122.6 | 3857.1 | 4110.3 | 3891.9 | 5317.2 | 5261.4 | 5582.0 | 5455.4 |
| 14 days after transplanting | 6797.6 | 5029.8 | 5653.5 | 5102.5 | 5328.6 | 5459.7 | 7290.5 | 6806.3 | 7520.1 | 6868.9 |
| Cumulative vapour pressure deficit | | | | | | | | | | |
| 7 days prior to transplanting | 195.2 | 148.5 | 52.8 | 183.9 | 193.1 | 106.1 | 112.3 | 222.2 | 324.9 | 284.6 |
| 7 days after transplanting | 415.9 | 92.0 | 142.1 | 110.6 | 159.5 | 100.9 | 279.7 | 320.0 | 304.2 | 384.9 |
| 10 days after transplanting | 581.3 | 121.6 | 221.4 | 144.9 | 194.6 | 184.4 | 410.4 | 427.0 | 444.0 | 495.1 |
| 14 days after transplanting | 661.5 | 168.2 | 316.0 | 193.0 | 255.4 | 250.0 | 550.6 | 526.8 | 591.6 | 574.3 |

 

Based on the observation of PaMS (site A, batch 1), a more detailed assessment of the data was undertaken focussing on VPD and temperature. Temperature and humidity for each 30 minute monitoring interval were plotted. From the graphs of temperature and VPD (Figures 10-17 inclusive), there appears to be a link between spikes of high VPD and temperature and the occurrence of PaMS symptoms (site A, batch 1, (Figure 10 andFigure 11 respectively), with spikes of VPD greater than 3 KPa (although many spikes exceeded 4 KPa VPD) occurring up to 10 days prior to the occurrence of PaMS symptoms. Such spikes were not as evident for site A, batch 2.

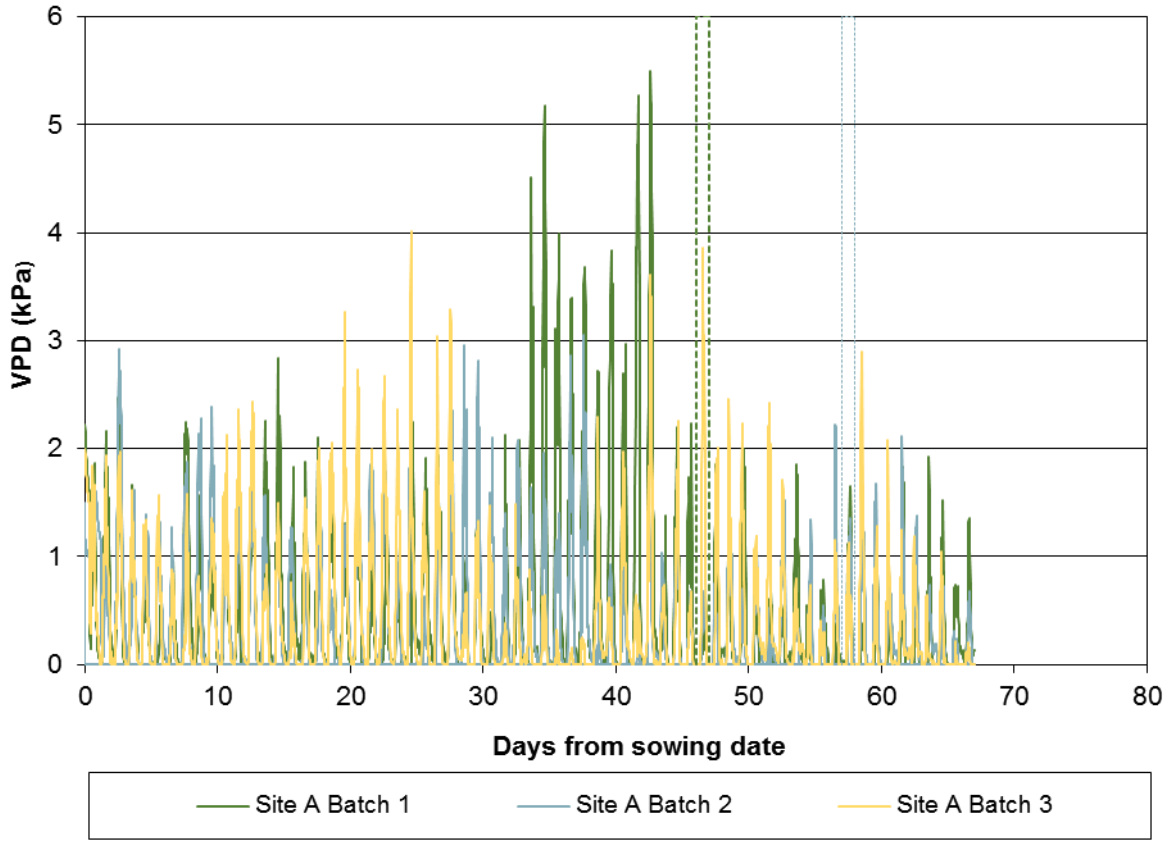


**Figure 10.** The vapour pressure deficit (VPD) for all batches on site A (the dashed lines show the day on which PaMS symptoms were observed for batches 1 and 2).

 

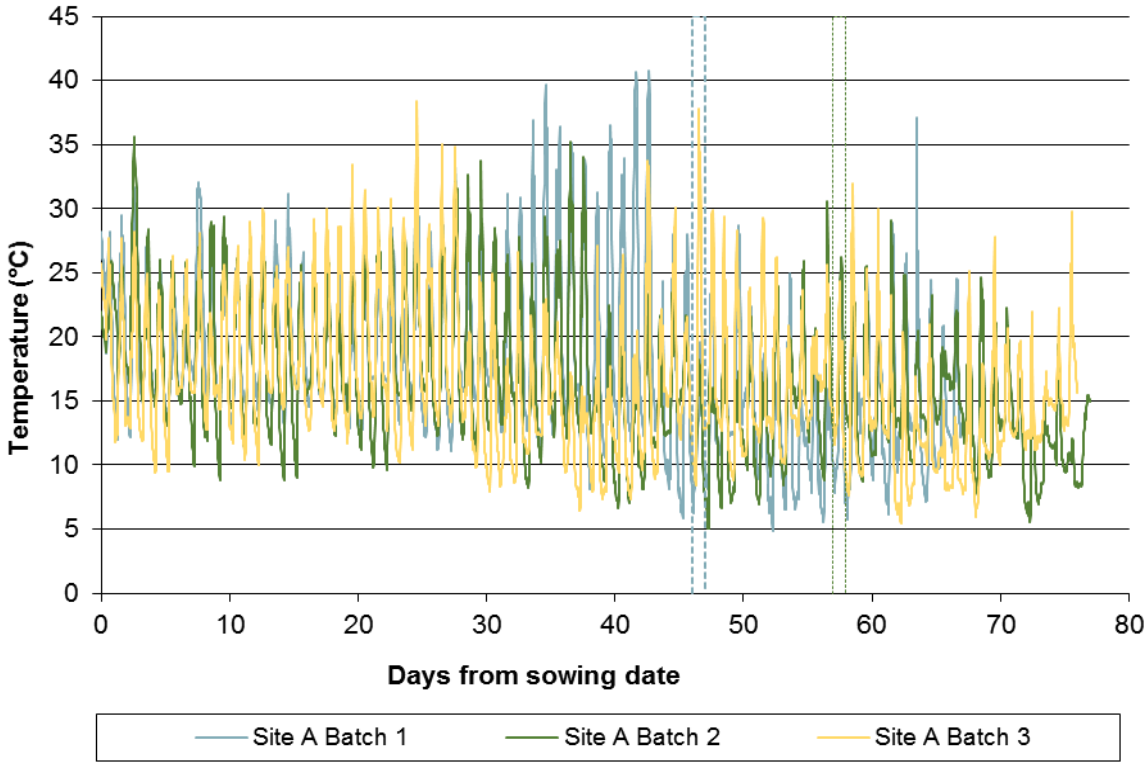


**Figure 11.** Temperature profile over time for all batches on site A (the dashed lines show the day on which PaMS symptoms were observed for batches 1 and 2).

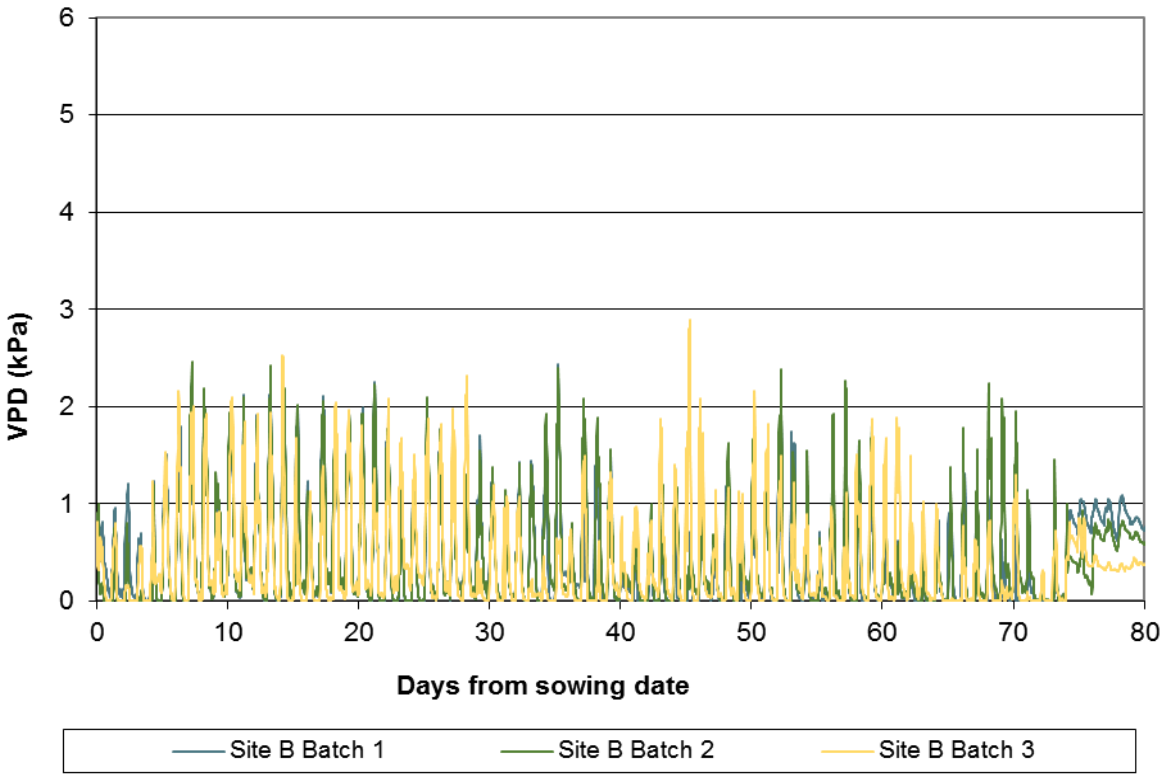


**Figure 12.** VPD profile over time for all batches at site B

 

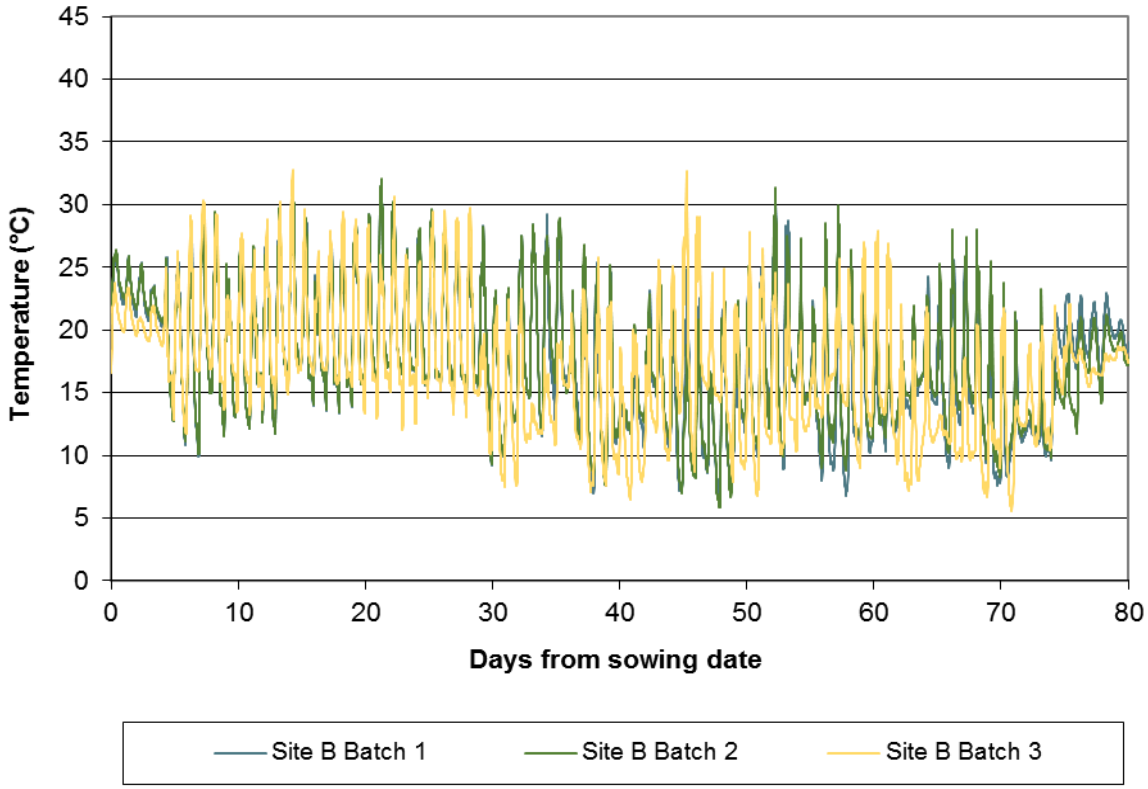


**Figure 13.** Temperature profile over time for all batches at site B

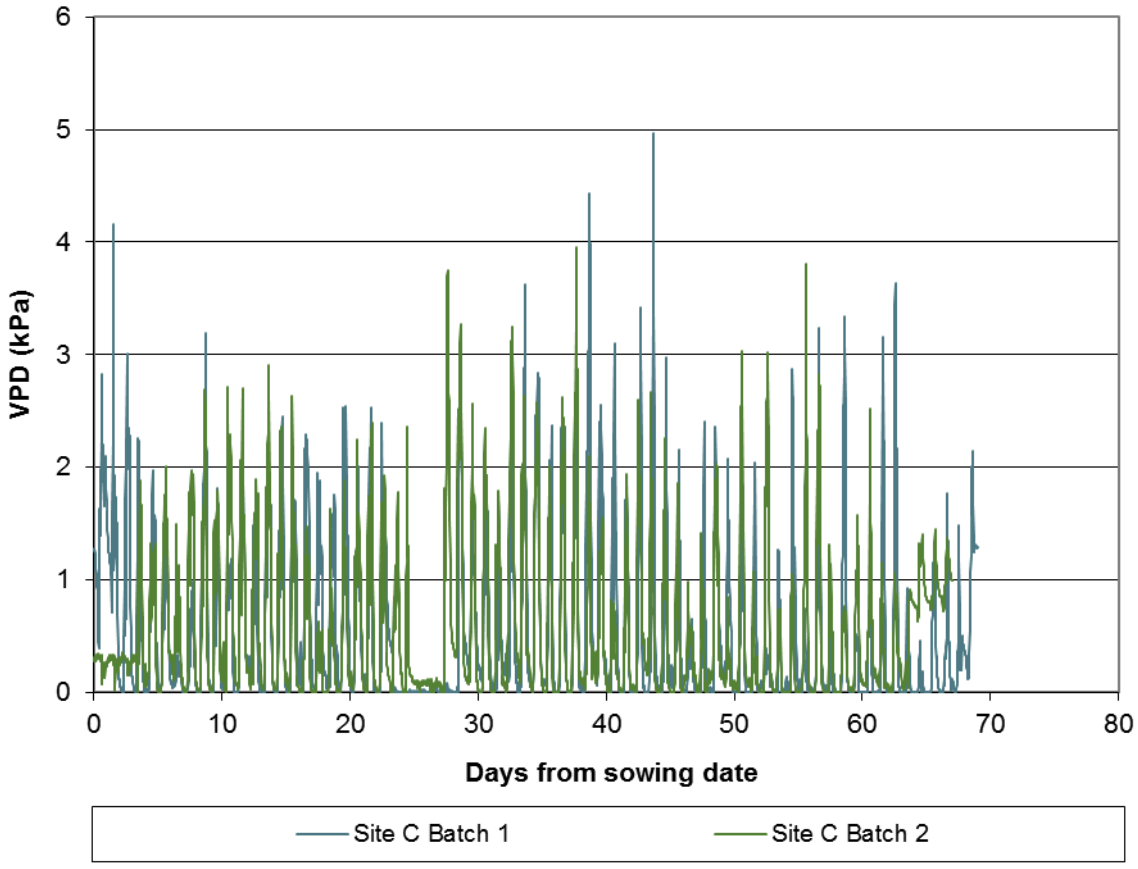


**Figure 14.** VPD profile over time for all batches at site C

 

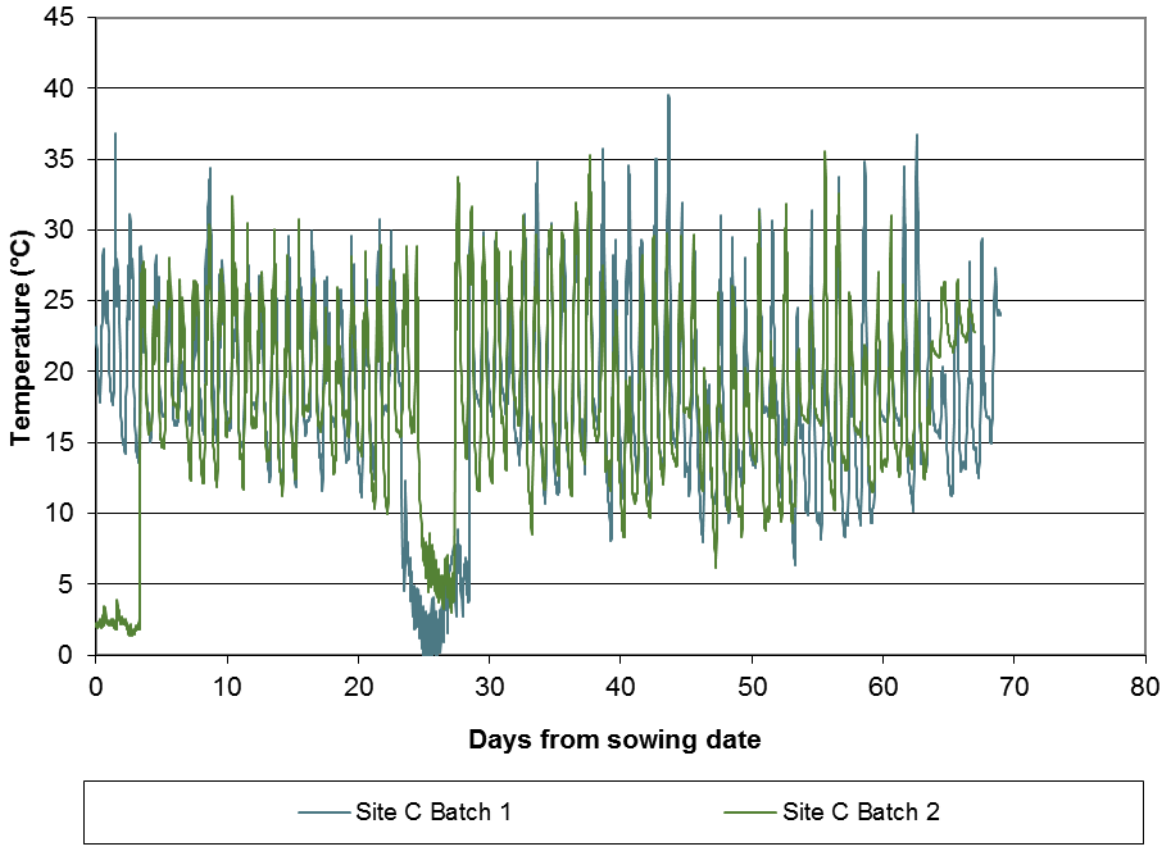


**Figure 15.** Temperature profile over time for all batches at site C

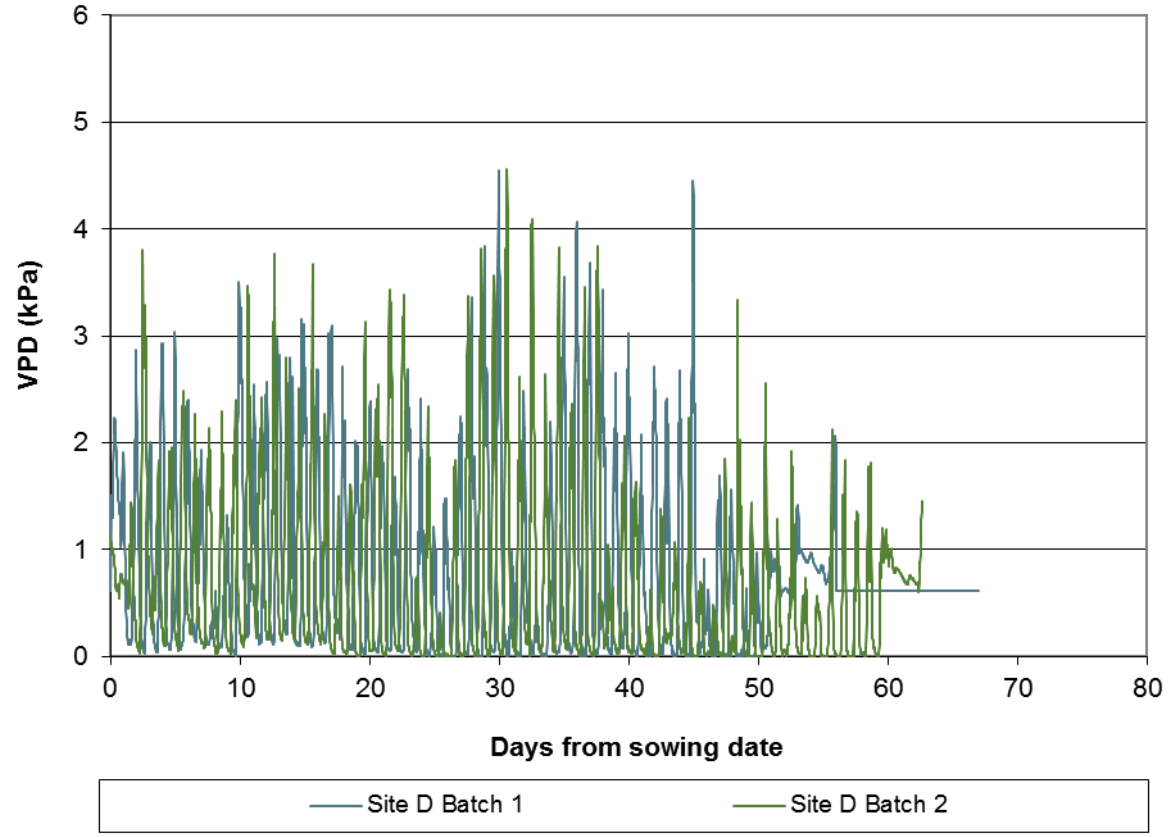


**Figure 16.** VPD profile over time for all batches at site D

 

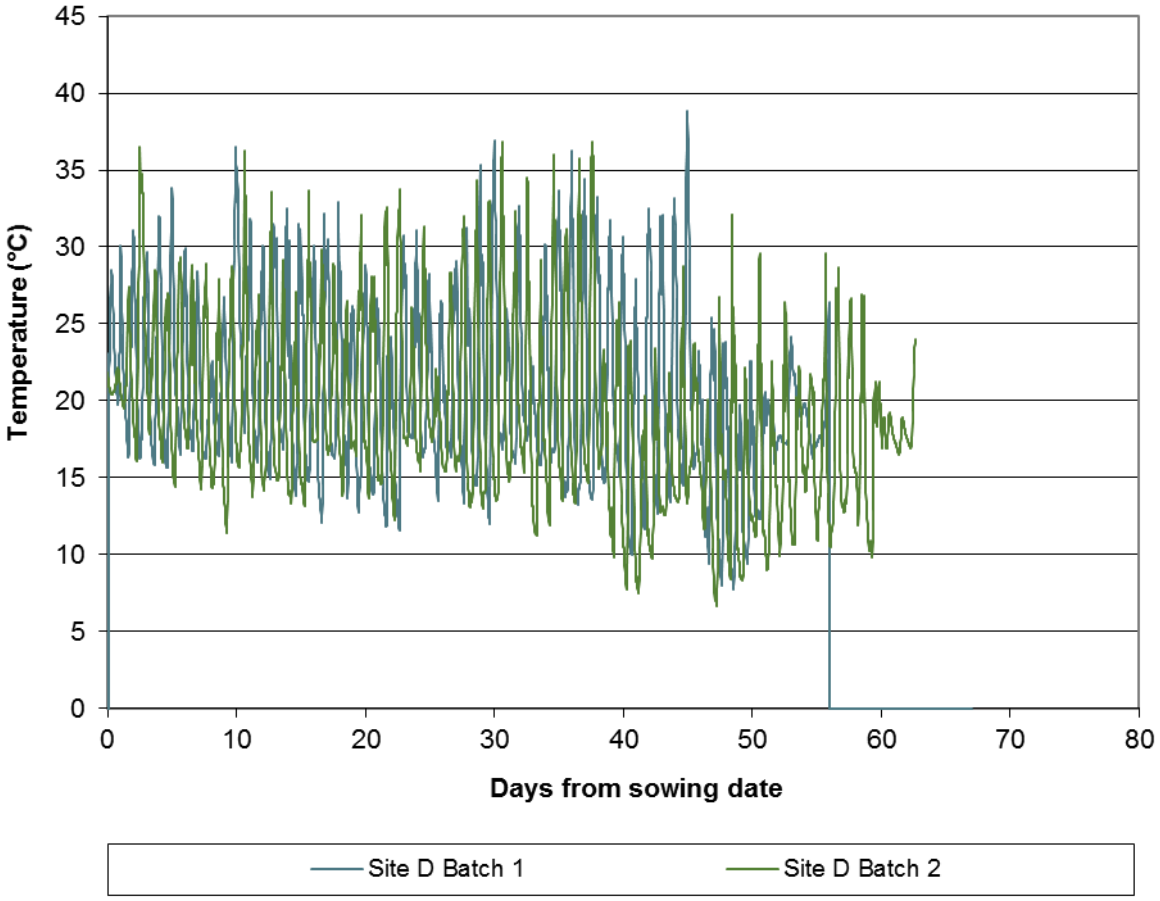


**Figure 17.** Temperature profile over time for all batches at site D

For site B, the graphs (Figure 12 and Figure 13) show that there were no spikes in VPD or temperature in any of the batches and no PaMS symptoms were observed on this nursery. At sites C (Figure 14 andFigure 15) and D (**Error! Reference source not found.** andFigure 17), there were occasional spikes of VPD and temperature in both batches at each site, but the spikes were not as prolonged or as clustered as in site A, batch 1.

Overall, these graphs suggest that there may be a potential link between high VPD, high temperature and the occurrence of PaMS symptoms. The data for site A, batch 1 show that in the two weeks prior to the occurrence of PaMS symptoms, the plants experienced a VPD greater than 4 KPa and a temperature greater than 35°C for over 1 hour on 6 days over a 10 day period, with over 4 hours exposure for both VPD and temperature on two consecutive days (4th and 5th September). This level of exposure and the clustering of events into a short period does not occur for any other batches. For site A, batch 2, which had some leaf deformity, the VPD and temperature do not reach the levels seen for batch 1, suggesting that either other factors contributed to causing the symptoms observed in batch 2, or that

 

the symptoms in batch 2 may be something other than “classic” PaMS, the latter supported by the lack of variegation.

## Analysis of additional data from site B

Although batches monitored at site B did not show any observable PaMS symptoms, symptoms were observed in other batches within the same growing location earlier in the season. To cross check whether high VPD and temperature were associated with the occurrence of these symptoms, data from the Priva environmental monitoring system were analysed and used to determine the VPD for the five days prior to the occurrence of PaMS symptoms.

*Derivation of the relationship between site B monitoring data and ADAS monitoring data*

The first stage in the analysis was to examine the raw data supplied. Temperature and relative humidity measurements were supplied for each half hour within the time period 07:00 to 19:00 on 22nd – 26th August inclusive. From this data, the saturated vapour pressure (SVP) was calculated from the temperature data using the following equation (Murray, 1967; Monteith & Unsworth, 1990):

$$SVP = 610.7 \times 10^{\frac{7.5 \times T}{237.3+T}}$$

where SVP is saturation vapour pressure (pascals, Pa) and T is temperature ($^{o}$C).

The vapour pressure deficit (VPD) was then calculated using the formula:

$$VPD = \left(\frac{(100 - RH)}{100}\right) * SVP$$

where VPD is vapour pressure deficit (Pa), RH is relative humidity and SVP is saturation vapour pressure (Pa).

Within the five day period of data supplied, the VPD shows spikes on the 22nd, 23rd and 26th August, with the VPD on these dates almost twice that on the 24th and 25th, suggesting that high VPDs may have been experienced by the plants.

*Calibration of Priva data to match ADAS TinyTag data*

In order to compare the VPD spikes in the Priva data with the thresholds found during the ADAS monitoring on the nurseries, it was necessary to convert the temperature and relative

 

humidity data from the Priva system to those that would have been expected at canopy level (since the ADAS monitoring data recorded temperature and humidity at canopy level using TinyTags was placed just above the plant canopy).

ADAS monitored a batch of pansies at site B during September 2013. Priva monitoring data (at half hourly intervals) for the growing location was provided for the period 6th to 12th September inclusive. The data from the two sources was compared and analysed (Excel trendline) to derive regression equations to convert the monitored temperature and relative humidity to equivalent plant canopy level temperature and relative humidity.

For temperature, the equation is:

$$T_t = 0.0288\,T_P^2 + 0.3255\,T_P + 4.5166 \qquad (R^2 = 0.97,\ n = 336)$$

where $T_t$ is the temperature at canopy level (as recorded by the ADAS TinyTag data loggers) and $T_P$ is the temperature recorded by the Priva system.

For relative humidity (RH), the equation is:

$$RH_t = 1.0655\,RH_P - 1.6153 \qquad (R^2 = 0.96,\ n = 336)$$

where $RH_t$ is the RH at canopy level (as recorded by the ADAS TinyTag data loggers) and $RH_P$ is the temperature recorded by the Priva system.

The $R^2$ values for the equations were greater than 0.95, therefore over 95% of the variation in the data is accounted for by the regression equation and we can be confident that the equations used to derive the expected temperatures and relative humidities at plant canopy level from the Priva system data are robust and accurate.

*Calculation of VPD using predicted canopy level temperatures and humidities*

The final stage in the analysis was to calculate the VPD experienced at plant canopy level based on predicted canopy level temperatures and relative humidities using the equations described above. Expected canopy level temperatures and humidities were calculated from the Priva data for the period 22nd – 26th August. The SVP and VPD were then calculated.

 

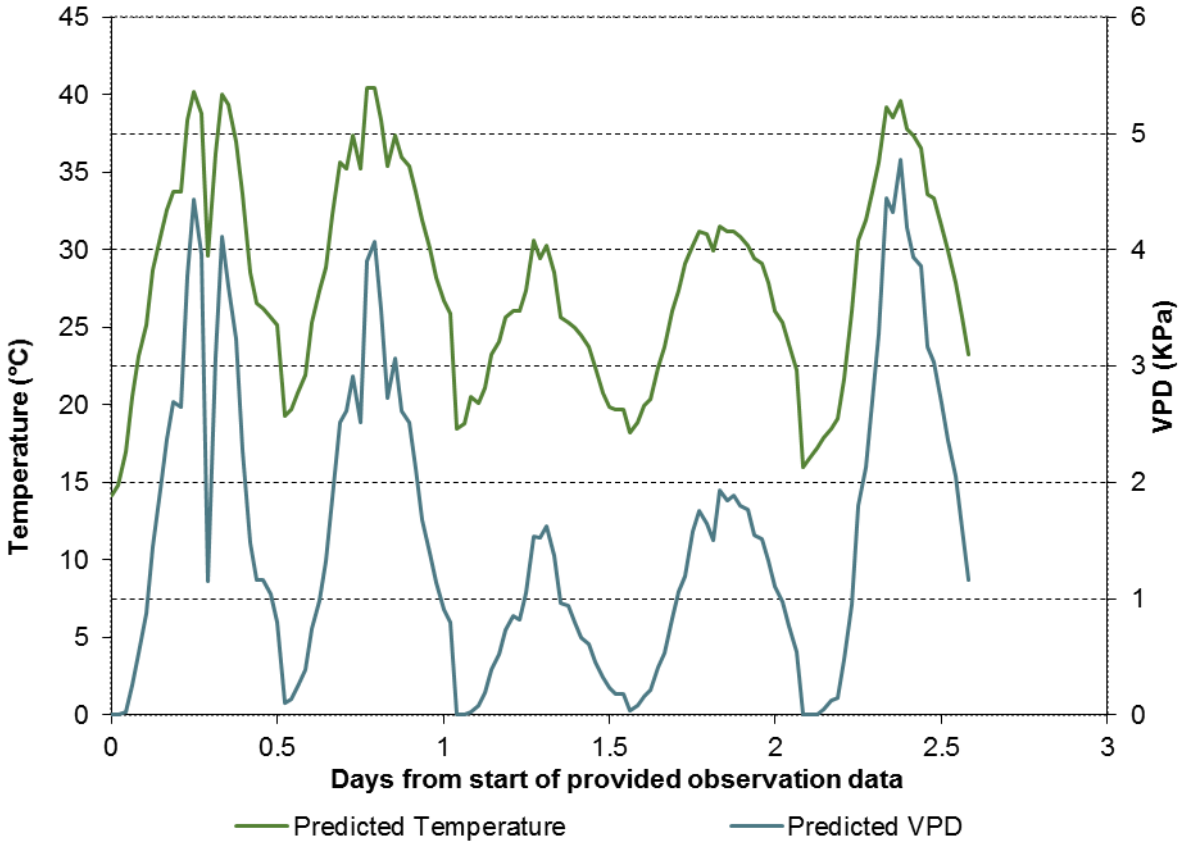


**Figure 18.** Predicted VPD and temperature from the Priva monitoring data provided by site B for a period of five days prior to observation of PaMS symptoms in a batch at site B

Vapour pressure deficit was calculated from the predicted canopy level temperatures and relative humidities, and was shown to exceed values of 3KPa for a minimum of 3 hours on 3 dates, 2 of which were consecutive, and to exceed 4KPa for at least 0.5 hours on each of these days, with one day having 2 hours with VPD greater than 4KPa. All of the events where VPD exceeded 3 KPa were also associated with periods when the temperature at canopy level was predicted to exceed 35°C (Figure 18).

The results from the analysis of the Priva monitoring data provided for site B does show elevated temperatures and VPD occurring prior to observations of PaMS symptoms. This provides further evidence for a potential link between these adverse environmental conditions and the occurrence of PaMS symptoms.

## Discussion

Despite the low occurrence of PaMS symptoms in the monitored batches across the four sites, it has been possible to identify a potential association between environmental factors and the occurrence of PaMS symptoms. This association is derived from the observation that the VPD, temperature and daily light integral in Batch 1 at site A were higher than for

 

the other batches at the same site and also for batches at other sites. It must be stressed that this association is extremely tentative due to the sample size of one, which has precluded any robust statistical analysis of the environmental data.

The analysis of additional data supplied by site B has shown that an occurrence of PaMS symptoms in a batch that was not monitored as part of this project was potentially associated with high temperatures and VPD prior to the occurrence of symptoms. This analysis was based on predictive modelling of the temperatures and relative humidities that would have been recorded by the ADAS monitoring had it been used within the batch. There are therefore a number of uncertainties and errors associated with this approach, particularly with respect to the accuracy of the models predicting temperature and relative humidity. However, these models are empirical regressions and, according to the $R^2$ statistic, they capture the majority of the variation in the data and so can be considered as good fits to the data. We can therefore be confident that the predicted temperature and humidities are reasonably accurate.

Site A, batch 2 was observed to have leaf distortion, which was recorded as PaMS symptoms, but no association with high VPD and temperature events could be found. Assuming the high VPD and high temperature conditions are linked to PaMS, there are two possible explanations for this:

a) PaMS occurrence is driven by more than just high VPD and temperature events
b) The symptoms observed in site A, batch 2 were not PaMS symptoms

It is not possible to determine which of these explanations is more plausible with the current evidence available from this project. The daily light integral experience by batch 1 at site A is more than twice that experienced by batch 2 at site A (Table 2). This suggests that light levels may be involved as a plant receiving high light levels would be transpiring more actively than a plant at lower light levels due to the need for water as part of photosynthesis. As PaMS appears to be a form of stress response, then it is possible that light levels could be an additional factor that, combined with high VPD and temperature, will lead to development of the symptoms

In support of the second explanation (b), there were no signs of variegation in batch 2, something that is commonly ascribed to PaMS. However, it is by no means certain that variegation is the defining symptom for PaMS. The lack of clarity over symptoms does potentially mean that a large number of stress responses are being reported as PaMS,

 

meaning that finding a clear cause may be difficult, as the response could be triggered by a number of factors, any adverse combination of which might lead to symptom expression.

Site A, batch 1 was grown in a polytunnel after transplanting rather than a glasshouse, hence there would have been less control over the environmental conditions than in a glasshouse. Given that this batch did show the variegation, then the expression of this variegation could be a response to stress associated with high VPD and temperature conditions in the polytunnel. It is also possible that the observed PaMS symptoms were related to the generally elevated temperatures in the polytunnel rather than specific high VPD and temperature events. As the polytunnel did not have shading, this would explain the higher light levels (PAR, Figure 5; and daily light integral, Table 2) experienced by the plants after transplanting, and this could be linked to the expression of PaMS symptoms.

Further data is required to confirm that high VPD and temperature events are associated with the occurrence of PaMS symptoms and the role that light levels may play in expression of symptoms. This data will hopefully be obtained through focussed monitoring in the second year of this project. The monitoring will be more intensive at selected sites where PaMS symptoms occurred or are known to occur to increase the chances of capturing data related to observation of PaMS symptoms. Some revision to the environmental monitoring is required to improve our understanding of the environmental factors that are associated with PaMS occurrence. An area of particular importance is the irrigation and water balance of the growing media during production. If high VPD and temperature are associated with PaMS occurrence, then this would suggest that uptake of water may be a factor since a high VPD implies that the plants will need to draw more water from their roots. If plants are unable to take up the water due either to a lack of water or to poor root structure (e.g. lack of root hairs), then plants would experience stress, and potentially some nutrient deficiencies (which would correspond with previous work and the similarity of PaMS symptoms with those of some micronutrient deficiencies). For future monitoring, the soil moisture content will be monitored to provide additional information about the water relations experienced by plants and the occurrence of PaMS, with root development assessments at the transplant stage to determine any the impact on transpiration potential.

## Conclusions

Where PaMS symptoms (with variegation) were observed, plants had experienced prolonged high VPD and temperature, suggesting a link with these environmental factors. Whilst this suggests a causal link for a particular type of PaMS, further experimentation and monitoring is required to develop a robust evidence base to identify the precise combination

 

of factors that trigger the onset of PaMS; for example plants also experienced high light levels around the time the symptoms occurred, so a link with light levels may have to be investigated. Furthermore, where PaMS symptoms occurred but without variegation, there was no association with high VPD, temperature and light conditions.

Further monitoring and experimentation is required to determine whether high VPD and temperature are triggers for PaMS symptoms and whether high light levels are required for these factors to be triggers of PaMS symptoms.

## Knowledge and Technology Transfer

A presentation and informal briefing have been presented to the industry representatives to provide updates on the first year of monitoring and the analysis of additional data from site B respectively.

## 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.

## References

de Rooij-van der Goes, I. J. (2013) Technical Manager, Syngenta Flowers. Personal communication.

Defra (2014) Basic horticultural statistics – datasets. [Online] Available from: https://www.gov.uk/government/statistics/basic-horticultural-statistics-2014.

Hammond, J. (2013) Research Plant Pathologist, Floral and Nursery Plants Research Unit, USDA-ARS, United States National Arboretum. Personal communication.

Krug, B. (2007) Physiological and environmental factors affecting shoot tissue boron concentration of pansy, petunia and gerbera plugs. *PhD Thesis, North Caroline Institute, USA*.

 

Krug, B., Whipker, B. E., McCall, I., Frantz, J. (2013) Elevated relavated humidity increases the incidence of distrorted growth and boron deficiency in bedding plant plugs. HortScience 48(3):311-313.

References

Murray FW (1967) On the computation of saturation vapor pressure. J. Appl. Meteorol. 6: 203-204.

McPherson, G. M. (2010) PS 286. Bedding plants: investigating the cause and prevention of pansy mottle syndrome. Horticultural Development Company.

Monteith JL, Unsworth MH (1990) Principles of environmental physics. Arnold

Whipker, B. E., Gibson, J. L., Pitchay, D. S., Nelson, P. V., Baker, J. R., Faust, J. E., Thomas, P. A., Benson, M., Cavins, T. J., Williams-Woodward, J. L. (2000) Diagnosing Common Pansy Problems. Greenhouse Product News. Available at: http://www.gpnmag.com/diagnosing-common-pansy-problems.

 

## **Appendix 1.** Grower monitoring template

| Project title: | The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS) | |
|---|---|---|
| ADAS: XBM5577 | 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 | |
| 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 |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

 

| Pansy Mottle Syndrome incidence | | | | |
|---|---|---|---|---|
| Date | Time | Sowing Batch | Tray number in batch | Number of plan |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

 