Project title:	The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS)
Project number:	PO 016a
Project leader:	Dr Jill England, ADAS Boxworth
Report:	Final report, April 2016
Key staff:	Dr Jill England (ADAS), Project Leader Dr Dave Skirvin (ADAS), Principal Environmental Modeller Chloe Whiteside (ADAS), Horticulture Consultant
Location of project:	Commercial growers
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Date project commenced:	1 May 2015
Date project completed (or expected completion date):	30 April 2016

[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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GROWER SUMMARY

Headline

- Monitoring of 40 batches of plants in commercial production across 4 sites from 2013 to 2015 has been unable to provide conclusive evidence of the incidence of PaMS and environmental conditions. Tentative links with high light levels, high vapour pressure deficit (VPD, >3 kPa) and high temperature (>35^{0}C) identified on one batch in 2013 were not consistently associated with symptoms in 2015.
- *Viola* white distortion–associated virus (VWDaV) was detected in samples both with and without 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 appeared to be becoming more common before the start of this project, particularly under the cool, wet conditions of 2012, and this renewed interest in identifying the cause.

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 through their contribution 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.

Previous work investigating the role of an ilarvirus in the development of PaMS symptoms concluded that although an ilarvirus was found to be common to pansies from many sources, there was no correlation with PaMS (Hammond, 2013). Subsequently, a research group from Turin, Italy studying viola plants showing leaf symptoms of white mosaic and distortion discovered a virus that showed greatest similarity to the ilarvirus, *Prune dwarf virus*. The biological and molecular differences were sufficiently distinct to describe it as a new ilarvirus species for which they proposed the name '*Viola* white distortion–associated virus' (VWDaV) (Cuiffo *et al*., 2014). In 2015, at the Fera laboratory in York, a sample of symptomatic pansies, sent in from a Plant Health and Seeds Inspector, was found to have the same newly described virus, *Viola* white distortion–associated virus. A Fera TaqMan® PCR test was subsequently designed to VWDaV from the Next Generation sequencing data and used to test pansy/viola samples from UK nurseries to investigate if the distorted and bleached leaf symptoms seen on pansy plants under production could be due to *Viola* white distortion–associated virus.

Summary of the project and main conclusions

Nursery environment monitoring

In 2015 data was collected from four commercial nurseries (sites A-D) located in Hertfordshire, East Yorkshire, West Sussex and Essex respectively between May and September 2015. The sites included three 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.

A total of 19 Pansy batches were monitored across the four sites: seven batches at site A, six at site B, two at site C and four batches at site D. Batches were monitored from the point of sowing until 'pack cover + 1 week stage', and if no PaMS symptoms had developed by that time the loggers were used to monitor a fresh batch of pansies. 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) set to record data at 15 minute intervals. 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**).

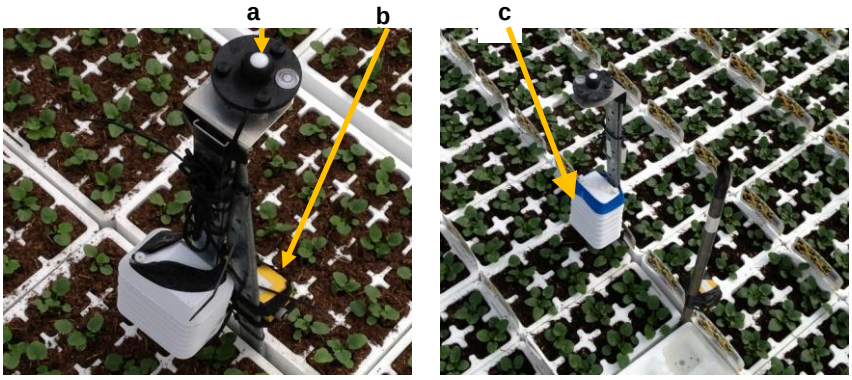

Figure 2. Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and water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

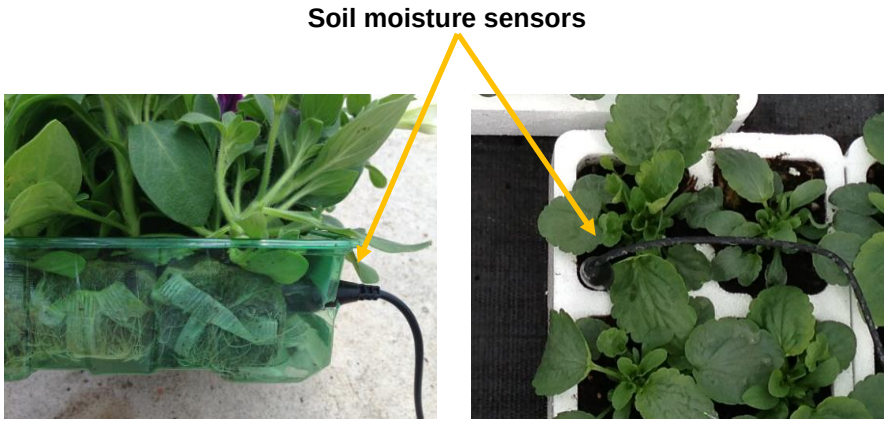

Figure 3. Positioning of SM100 Soil Moisture Sensor within a coir system, inserted horizontally (image left); and in a peat based system inserted vertically (image right) production systems

In **2013**, although there was low occurrence of PaMS symptoms in the monitored batches across the four sites, a potential association was muted between environmental factors and the occurrence of PaMS symptoms. This association was derived from the observation that the vapour pressure deficit (VPD), temperature and PAR received by the plants at site A, batch 1 were higher than for the other batches at the same site and also higher than 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. 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-0.95 kPa), with pansies performing well around 0.6-0.7 kPa.

In **2014** there were no significant occurrences of PaMS in the monitored batches and this reflected the position experienced by the wider bedding plant sector in that year. Batches of monitored plants experienced high VPD on a number of occasions, however daily light integral (DLI, calculated per 24 hr day sampling period) was generally lower across all batches than in 2013, including when VPD was higher than 4 kPa.

In **2015**, possible PaMS symptoms were seen in one batch in nursery A (distortion only) and in all batches in nursery B (distortion in all batches; distortion plus mottling/bleaching in batch 2), but the overall incidence of symptoms was low (less than 1%). The same approach as for the analysis in 2013 (no symptoms being recorded in 2014) was taken where the time series

of vapour pressure deficit (VPD), temperature, humidity and light levels were determined for each batch. The time series were examined to identify time intervals when the VPD and or temperature exceeded the thresholds identified in 2013 (>3 kPa for VPD, >35^{o}C for temperature) and there were spikes of high light intensity.

The monitoring from 2015 proved to be inconclusive as although there were environmental conditions similar to those observed in 2013 that were thought to trigger PaMS in most of the batches in which symptoms were observed in 2015, there were two batches where there were no obvious adverse or stressful environmental conditions. In addition, adverse conditions were seen in batches where no symptoms were observed. This lack of consistency would indicate that either the environmental conditions identified in 2013 were not the conditions that trigger PaMS, and were specific to the batch that developed symptoms in 2013, or there were other factors involved that we have not been able to identify from the nursery monitoring to date.

Over the three years of monitoring, PaMS has been observed in eight out of 40 batches that were monitored. Eight batches is a small sample given the variation that occurs in the environmental conditions from year to year, between sites within a year and between batches within sites. Combining the data from all batches at all sites in all years is unlikely to provide any further information as the variation in the data would act to mask the effects of any possible correlation between environmental trigger conditions that may be present and the presence of PaMS symptoms. Whilst further monitoring might eventually aid the identification of a set of environmental trigger conditions, a large effort would need to be put into the sampling in order to achieve a sufficiently large set of positive samples to increase the probability of finding a set of environmental trigger conditions.

Virus testing for an association between PaMS symptoms and VWDaV

Samples of pansies in each of three categories: plants with leaf distortion only (no bleaching or mottling); plants with white bleaching/mottling on the leaves; and plants with no symptoms were collected from various nurseries across the country by ADAS, and passed to Fera for Taqman® testing for *Viola* white distortion-associated virus. 254 samples were tested: 93 distorted, without mottling; 109 with mottling / bleaching; and 52 with no symptoms. The results were split into two groups: those from populations with a low prevalence of symptoms (1%) and those from a population with a high prevalence of symptoms (75%). VWDaV was found to be present in samples from all three symptom categories (distortion only, distortion with bleaching and no symptoms).

Statistical analysis carried out on the data resulting from the virus testing estimated the prevalence of the virus in symptomatic and asymptomatic plants and the potential reduction in symptomatic plants should the virus be removed if it is indeed a causal agent for the symptoms. In plants taken from batches with low levels of symptoms (1%) it was estimated that there was a higher incidence of PaMS symptoms among plants in which the virus was present (range of **1.08** to **1.53%)** than among plants in which the virus was absent (range of **0.332** to **0.797%**). If the virus is a causal agent for the symptoms, then it was estimated that this accounts for about half (range of **20.3** to **66.8%)** of the 1% of symptomatic plants observed in the population, i.e. there is the potential to reduce symptoms by an estimated 50% by removing the virus, if the virus is proved to cause PaMS symptoms. No association between the virus and symptoms was found in the batch with high (75%) incidence 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 2014 (21p/plant). 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.

In populations where 1% of the crop expresses PaMS symptoms, removal of the VWDaV virus may potentially reduce PaMS symptoms by 50%, which equates to £10,500 (50% of 1% of the crop value of £2.1M).

Action Points

The results from 2013 of this study indicated the possibility of a link between environmental conditions (high VPD, temperature and light) and the expression of PaMS symptoms, however, this was based on the results from a single site in 2013. Inconclusive results in 2015 have not been able to confirm or disprove this possibility as the adverse environmental conditions identified in 2013 were seen in batches with and without symptoms in 2015. Therefore, the precise triggers and sequence of events that lead to PaMS remain unclear but even so, growers should take measures to monitor environmental conditions, and reduce plant stress:

- Monitor VPD and temperature.
- 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.
- Ensure healthy plant root development through careful application of water; over-application of water will limit root development, particularly in tray module production units.
- As VWDaV is mechanically transmissible e.g. handling and pruning where sap may be transferred by contact with contaminated plants or plant debris, tools, or workers, and this would facilitate spread in a production unit. There is no cure for viruses, but measures that will help to keep them in check include destroying badly affected plant material and good nursery hygiene e.g. disinfecting tools with a disinfectant that is effective against viruses e.g. Unifect-G, Menno Florades or Jet 5. For further information refer to HDC Factsheet 03/14: Use of chemical disinfectants in protected ornamental plant production.

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 appeared to be becoming more common before the start of this project, particularly under the cool, wet conditions of 2012, and this 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. 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.

Virus research

Work carried out in the US investigating the role of an *ilarvirus* in the development of PaMS symptoms concluded that although an *ilarvirus* was found to be common to pansies from many sources, there was no correlation with PaMS (Hammond, 2013). Subsequently, a research group from Turin, Italy had been studying viola plants showing leaf symptoms of white mosaic and distortion. These scientists mechanically transmitted an infectious agent from *Viola* spp. to *Nicotiana benthamiana*.

A virus was subsequently discovered that after further molecular analysis showed that specific dsRNA bands found in the inoculated plants were not present in non-inoculated plants. The subsequent sequence data showed greatest similarity to the ilarvirus, *Prune dwarf virus* but the biological and molecular differences were sufficiently distinct to describe it as new ilarvirus species. In 2014 the Italian scientists proposed the name for this new virus as *Viola* white distortion–associated virus (VWDaV) (Cuiffo *et al*., 2014).

In 2015, at the Fera laboratory in York, a sample of symptomatic pansies, sent in from a Plant Health and Seeds Inspector, was screened for potential virus by Next Generation Sequencing. Results showed the sample had the same newly described virus, *Viola* white distortion-associated virus. A Fera TaqMan® PCR test was subsequently designed to VWDaV from the Next Generation sequencing data and used to test pansy/viola samples from UK nurseries to investigate if the distorted and bleached leaf symptoms seen on pansy plants under production could be due to *Viola* white distortion–associated virus.

Summary of previous work

In **year 1 (2013)** 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 kPa) 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.

In **year two (2014)**, the nursery monitoring continued at the same sites as in year 1, 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. 11 batches were monitored between June and September 2014, with four batches monitored at site A, as this was where the PaMS had occurred in monitored batches during 2013. No PaMS symptoms occurred in any of the monitored batches in 2014, and reports of the problem in the wider industry were low. Data analysis of the monitored batches showed that high VPD occurred in all batches on a number of occasions. However, DLI was lower (<25 mol/m^2/day and sometimes <15 mol/m^2/day) across the batches, including when VPD was higher than 4 kPa. This was different to 2013, where the high VPD was associated with a DLI greater than 25 mol/m^2/day, in batches 1 and 2 at site A, where symptoms developed.

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. However, 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.

In **year 3 (2015)** the nursery monitoring continued at the same four sites as in year 1, but was managed to enable an increased number of batches to be monitored on each of the nurseries involved in the project. In addition to this, virus testing was carried out; plants with distortion, distortion plus mottling/bleaching and no symptoms were collected and passed to Fera for virus testing.

Project objectives

Objective 1 – environmental 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 – association between symptoms of pansy mottle syndrome and VWDaV: To carry out Pansy sample analysis to identify any association of *Viola* white distortion-associated virus (VWDaV) with PaMS symptoms.

Materials and methods

Objective 1 - environmental monitoring:

Data was collected from four commercial nurseries (sites A-D) located in Hertfordshire, East Yorkshire, West Sussex and Essex respectively between May and September 2015. 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. Batches were monitored from the point of sowing, until 'pack cover + 1 week stage' and if no PaMS symptoms had developed by that time, the loggers were used to monitor a fresh batch of pansies (**Table 1**). 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 1**).

Table 1. Dates of sowing, transplanting and dispatch for each monitored batch at each site

Nursery	Batch	Sowing	Transplant	Monitoring end date
Site A	1	11/06/2015	13/07/2015	30/07/2015
	2*	24/06/2015	22/07/2015	05/08/2015
	3	03/07/2015	30/07/2015	21/08/2015
	4	10/07/2015	05/08/2015	28/08/2015
	5	12/08/2015	20/10/2015	19/11/2015
	6	18/08/2015	21/09/2015	27/10/2015
	7	25/08/2015	20/10/2015	19/11/2015
Site B	1*	12/06/2015	w/c 06/07/2015	19/08/2015
	2*	19/06/2015	24/07/2015	21/08/2015
	3*	26/06/2015	29/07/2015	Est. 25/08/2015
	4*	28/07/2015	27/08/2015	21/08/2015
	5*	03/09/2015	06/10/2015	16/11/2015
	6*	10/09/2015	13/10/2015	16/11/2015
Site C	1	29/05/2015	w/c 22/06/2015	w/c 20/07/2015
	2	09/06/2015	08/07/2015	Est. 05/08/2015
Site D	1	29/05/2015	26/06/2015	17/07/2015
	2	11/06/2015	15/07/2015	07/08/2015
	3	17/07/2015	11/08/2015	10/09/2015
	4	10/08/2015	02/09/2015	10/10/2015

*Batches with PaMS symptoms.

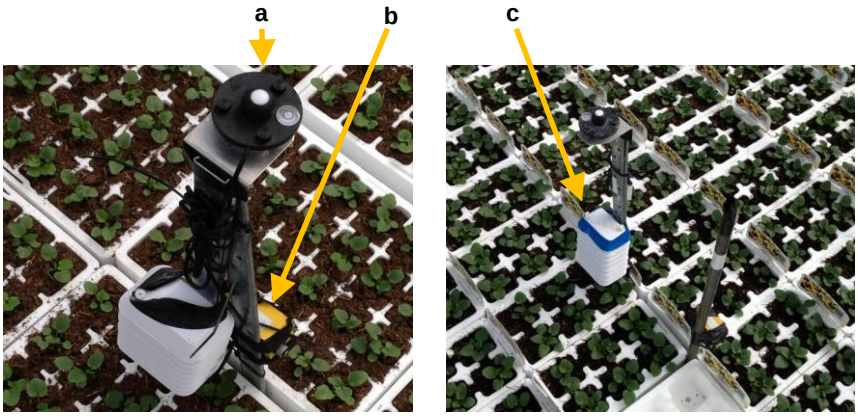

Figure 5. Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and water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

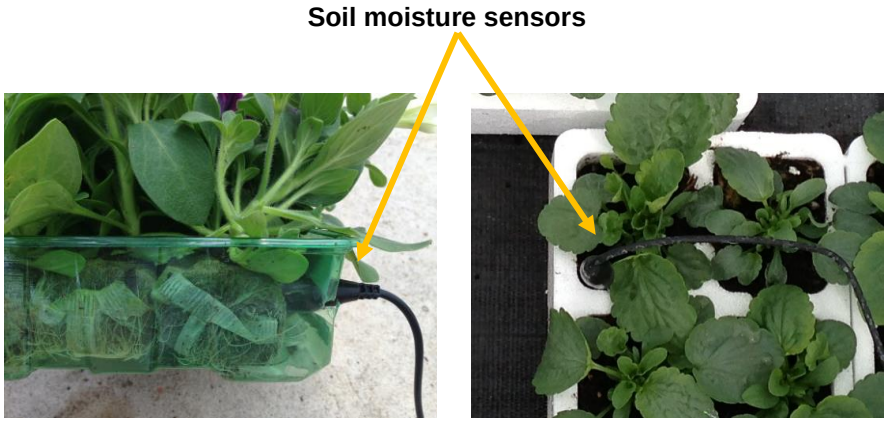

Figure 6. Positioning of SM100 Soil Moisture Sensor within a coir system, inserted horizontally (image left); and in a peat based system inserted vertically (image right) production systems

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) and all sites in 2015. For each growing media sample, 18 containers (1 L) 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 wetness's 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

Assessments

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. Grower monitoring template**). 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.

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 7**), 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 7. Root assessment scores. Scale = 0-3; 0 = no root hairs and 3 = many root hairs

Objective 2 – association between symptoms of pansy mottle syndrome and VWDaV: Samples of pansies in each of three categories: plants with leaf distortion only (no bleaching or mottling) (**Figure 8**); plants with white bleaching/mottling on the leaves (**Figure 9**); and plants with no symptoms (**Figure 10**) were collected from various nurseries across the country by ADAS, and passed to Fera for Taqman® testing for Viola white distortion-associated virus. Samples were labelled and stored at -80°C prior to testing.

Symptoms

Figure 8. Small pansy plants with leaf distortion (left) compared with symptomless plants (right)

Figure 9. Pansy plants with white bleaching symptom (left) and a close up of the white bleaching symptom

Figure 10. Symptomless plants

Laboratory Testing

Project samples were tested by real-time Taqman® PCR. Details of the general testing method are as follows:

Nucleic acid extraction

The extraction of viral RNA was performed using the in-house Fera magnetic bead method.

Real-time PCR assay design

The Fera assay for VWDaV was designed using ABI Primer Express software, using sequences obtained from the NCBI database (www.ncbi.nlm.nih.gov).

Real-time PCR

Real-time PCR (TaqMan®) was performed using generic conditions, essentially as described previously (Mumford et al., 2000), using iTaq Universal Probes One-Step Kits (Bio-Rad; Cat. No. 1725141). Primers were used at a working concentration of 375 nM and probes at 125 nM, in each 20 µl reaction. Assays were run on Applied Biosystems (ABI) 7900, 7500, and ViiA7 machines.

Each sample was also tested by TaqMan ® PCR using an internal Cox control. The Cox result was used to check the quality of the nucleic acid prior to virus testing for VWDaV. Where the Cox testing failed, the sample in question was re-extracted and retested.

Machine program for a RNA template:
10 min at 50°C, 2 min at 95°C, then 40 cycles of 95°C for 15 sec and 60°C for 1 min.

Results

Objective 1 - environmental monitoring

A total of 19 Pansy batches were monitored across the four sites. Suspected PaMS symptoms occurred in seven batches, and of these, distortion occurred in all batches and mottling/bleaching symptoms occurred in three batches (**Table 2**).

Table 2. PaMS symptoms reported

Nursery	Batch	Symptoms	Nursery	Batch	Symptoms
Site A	1	None	**Site B**	1	Distortion
	2	Distortion		2	Distortion and mottling
	3	None		3	Distortion
	4	None		4	Distortion
	5	None		5	Distortion
	6	None		6	Distortion
	7	None			
Site C	1	None	**Site D**	1	None
	2	None		2	None
				3	None
				4	None

Data capture

Production information provided by the nurseries (available as a separate appendix: PaMS nursery data appendix 2015) 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.

Data analysis

The data analysis component of the work aimed to determine any statistically robust relationship between the monitored environment variables and the occurrence of Pansy Mottle symptoms.

Preliminary analysis using daily data

Initial analysis focussed on using cumulative day degrees above a threshold of 0ºC 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 11**) for all batches were highly consistent across all sites. The graph shows that there was some deviation in day degree accumulation for the different batches at site B, but this variation was consistent with the observed cumulative day degree accumulation at other sites. We can therefore be confident that the data is representative of the conditions experienced by the plant.

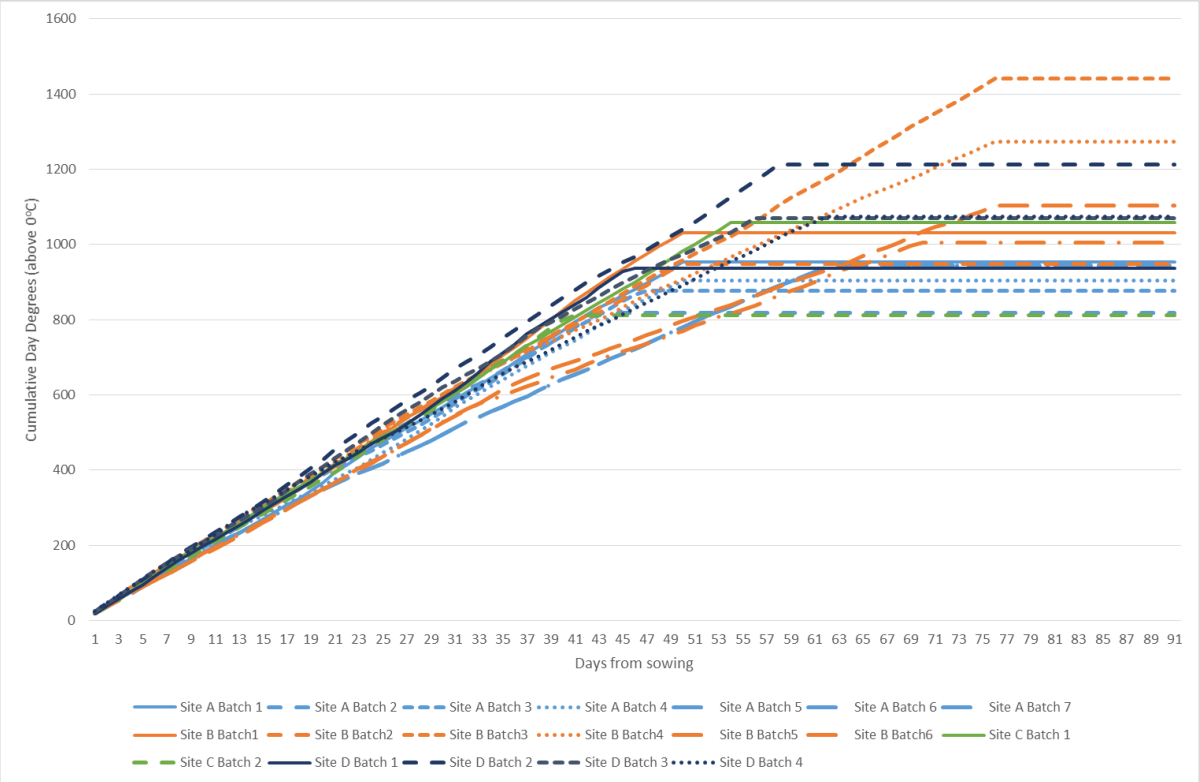

Figure 11: Cumulate day degrees above 0ºC for all batches at all sites

Cumulative daily PAR for all batches at all sites is shown in **Figure 12**. For PAR there is more variation between sites, but with reasonable consistency between batches within sites. Periods when there was a change in light accumulation can be seen from the lines, particularly for batches 1 to 3 at site A and for batch 1 and site B. The line of batch 2 at site D follows a

different trajectory to the other batches at this site due to the fact that the logger failed to record light data during the early part of the monitoring of this batch resulting in missing data.

Overall, based on both the day degree and PAR accumulation, we can have confidence that the data from the dataloggers is providing an accurate and consistent reflection of the conditions experienced by the plants in the monitored batches at all sites.

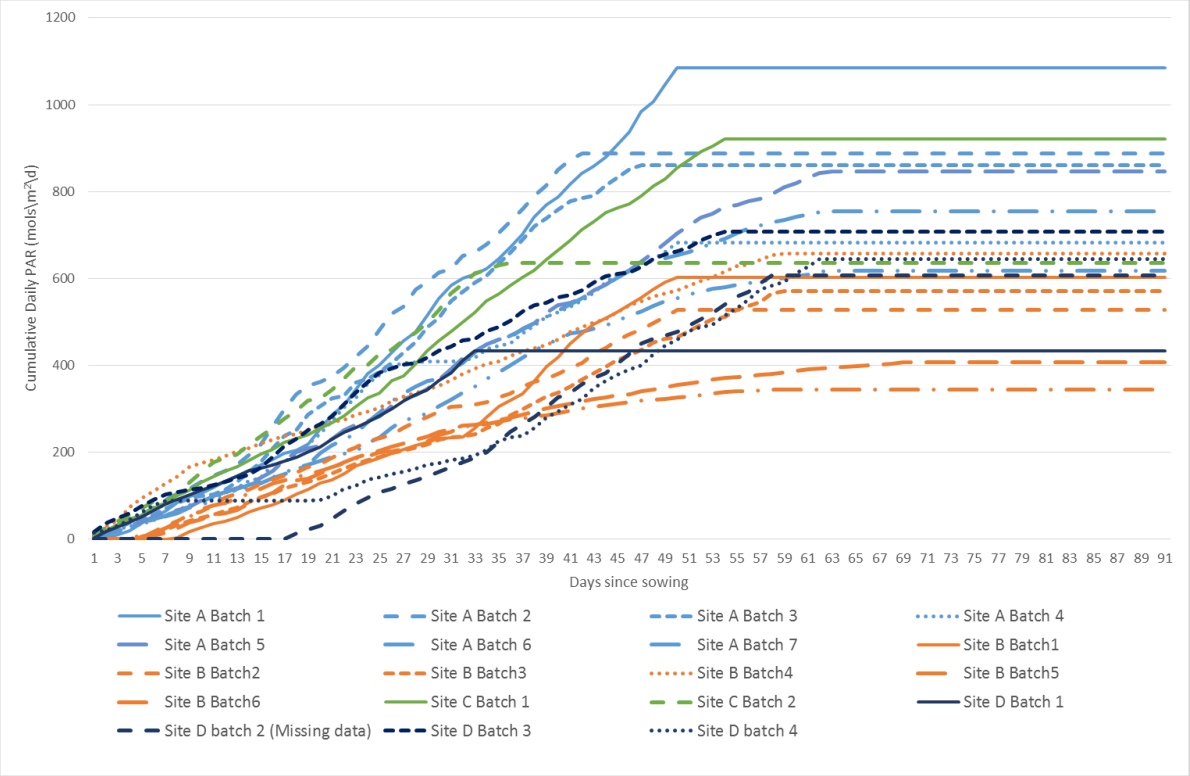

Figure 12: Cumulative daily PAR (mol/m^2/day) for all batches at all sites

In the monitoring for 2015, possible PaMS symptoms were seen in one batch in nursery A and in all batches in nursery B, but the overall incidence of symptoms was low (less than 1%). The same approach as for the analysis in 2013 (no symptoms being recorded in 2014) was taken where the time series of vapour pressure deficit (VPD), temperature, humidity and light levels were determined for each batch. The time series were examined to identify time intervals when the VPD and or temperature exceeded the thresholds identified in 2013 (3 kPa for VPD, 35^{o}C for temperature) and there were spikes of high light intensity.

For site A, the graph showing VPD through the first four batches is shown in **Figure 13**. Comparing across batches 1-4, it can be seen that the VPD is similar for all batches and rarely exceeded 2.5 to 3 kPa for any significant length of time. For batch 2, which developed distortion symptoms (first noticed at transplanting), a spike of VPD occurred on days 7 and 8, 3 weeks ahead of transplanting on day 29. This high VPD was accompanied by temperatures

greater than 30ºC (**Figure 14**). However, the high VPD and temperature were not associated with particularly high light levels. **Figure 15** shows the volumetric water content (VWC) that has been calculated for the plants in batch 2 at site A based on measurements made at the site and the calibration curve for the growing media used at site A. The VWC shows a significant dip at around days 7 and 8, which is coincident with the high VPD and temperature. However, the VWC results prior to transplanting need to be treated with a degree of caution as the measurements were carried out using small pots as the sensors were too large to fit in the plug trays, so the measurements may not accurately reflect the situation in the plug trays. The monitored VPD and temperatures were consistent with those seen in 2013 that were thought to be associated with the occurrence of PaMS. However, similar conditions were also seen in other batches on the same nursery, albeit at different time point, but still prior to transplanting when the symptoms in batch 2 were first observed, but no symptoms were seen in these batches.

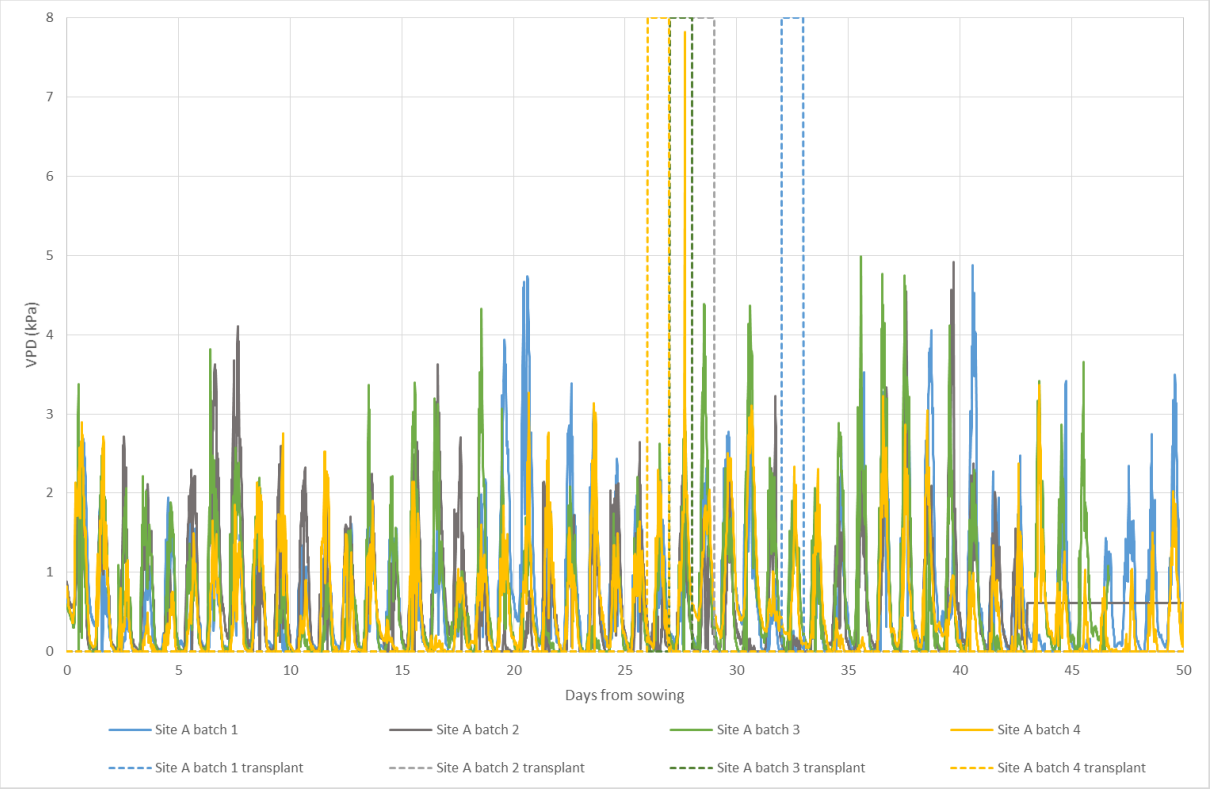

Figure 13. The VPD calculated from the measurements made at Site A in batches 1 – 4. The day on which plugs were transplanted is shown using the dashed lines

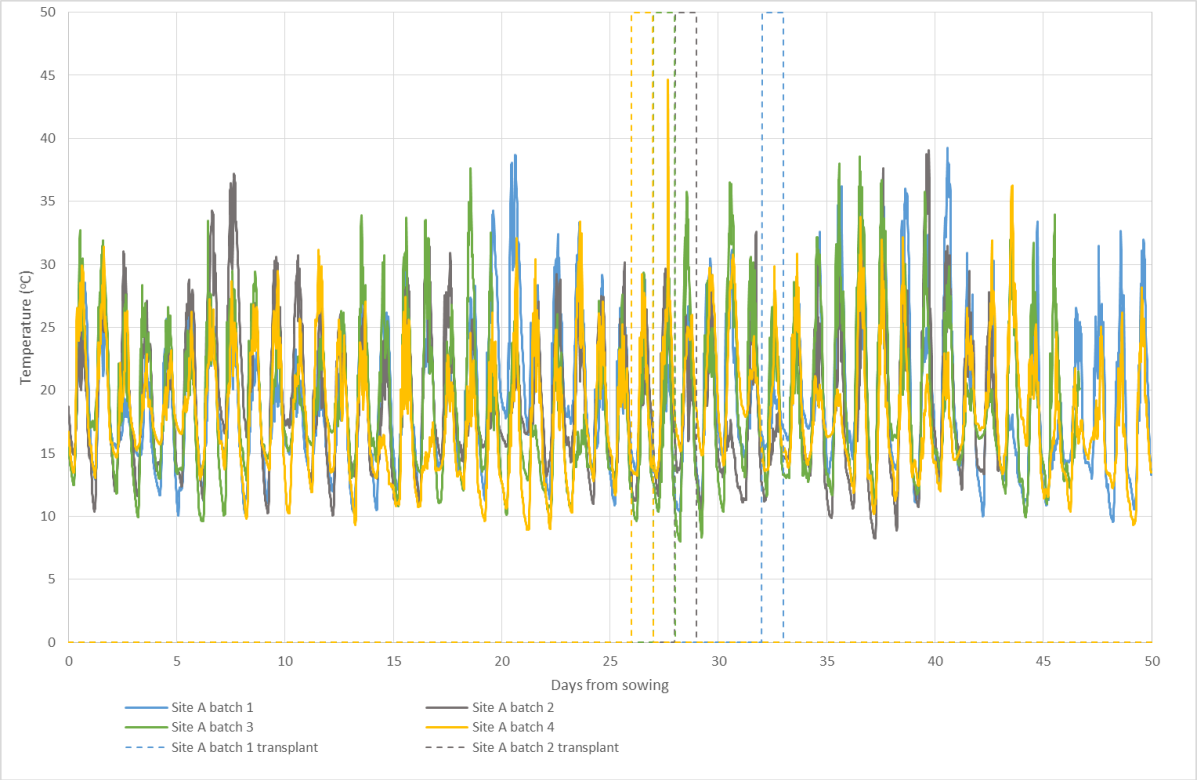

Figure 14. The measured temperature at Site A in batches 1 – 4. The day on which plugs were transplanted is shown using the dashed lines

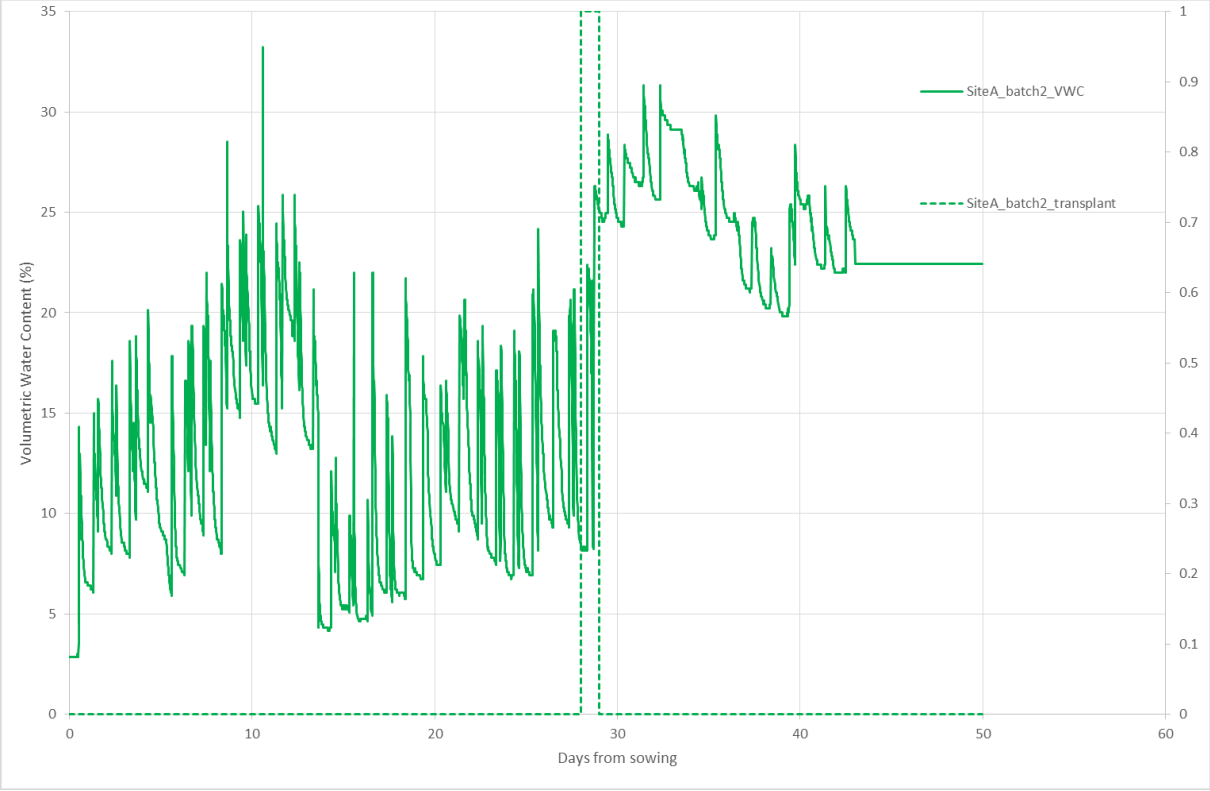

Figure 15. Volumetric water content for the plants in batch 2 at site A

For site B, the first observations of PaMS occurred around 3 weeks after sowing in all batches, throughout the season. **Figure 16** and **Figure 17** show the VPD for all six batches at site B. From these graphs it can be seen that the VPD was occasionally above the threshold of 3 kPa in the first few weeks after sowing for batches 1 to 3. Batch 4 showed significant spikes of VPD, greater than 4 kPa in the first 10 days after sowing, but batches 5 and 6 showed no such spikes and the VPD in these batches remained below 3 kPa throughout the growing season.

The plots for temperature (**Figure 18** and **Figure 19**) show that prior to PaMS symptoms occurring in batches 1 to 3, the temperature was only greater than 35^{o}C for a short period on one or two days in each batch. For batch 4, temperatures in the first 10 days after sowing exceeded 35^{o}C on 6 occasions and exceeded 40^{o}C on two of these six occasions. For batches 5 and 6, the temperatures were consistently below 30^{o}C for the entire growing season. There were no obvious spikes in light level for all batches, except for batch 4, where some high light levels occurred in the first 10 days after sowing (>1.4 micromols per m^2 per 15 minute sampling interval).

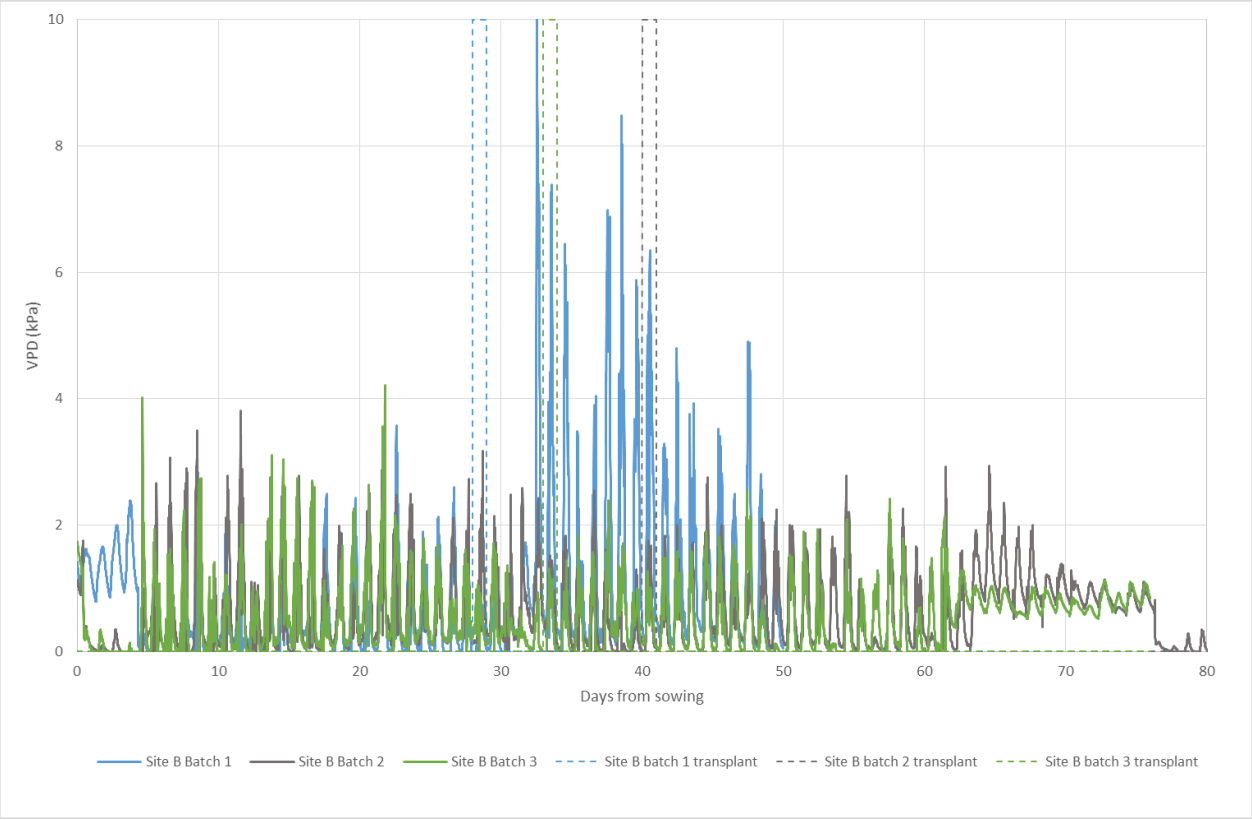

Figure 16. Vapour pressure deficit calculated from measurements at Site B for batches 1 to 3

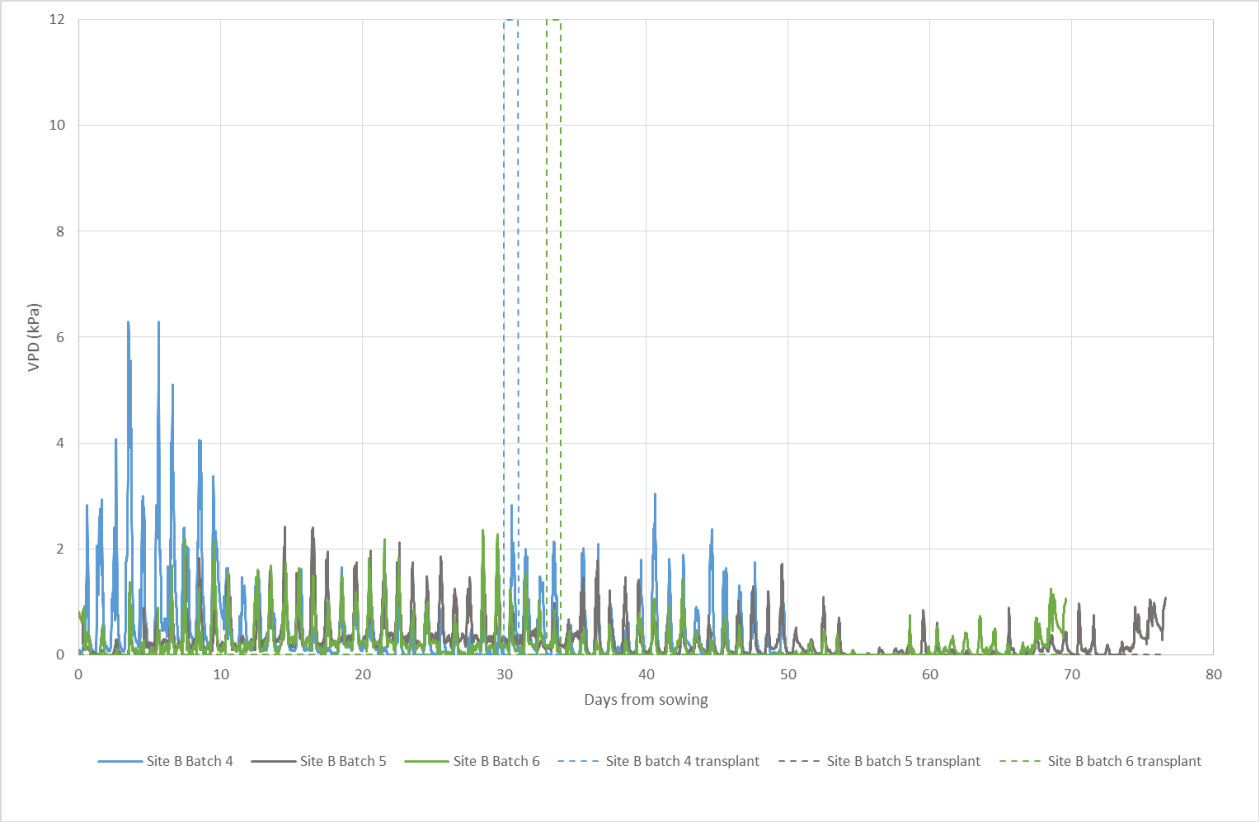

Figure 17. Vapour pressure deficit calculated from measurements at Site B for batches 4 to 6

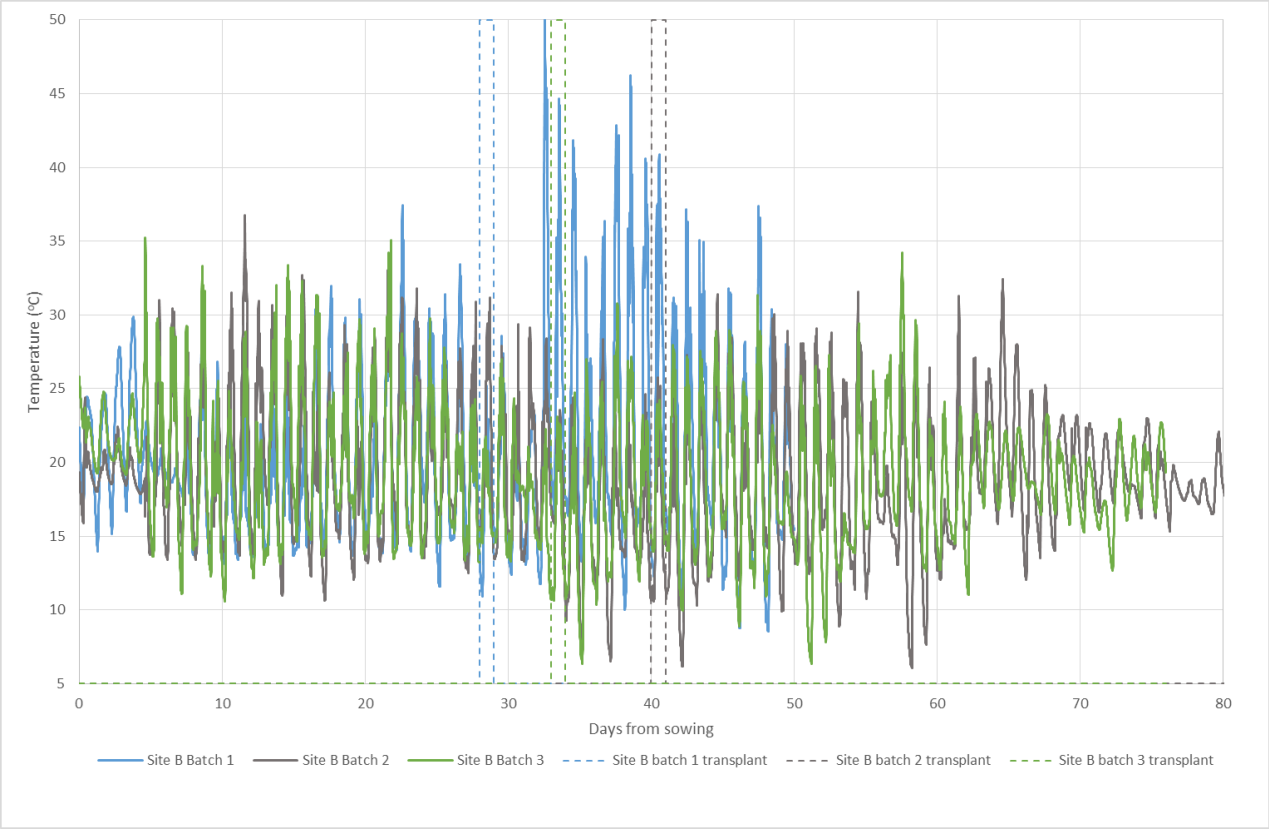

Figure 18. Temperature measured for batches 1 to 3 at site B

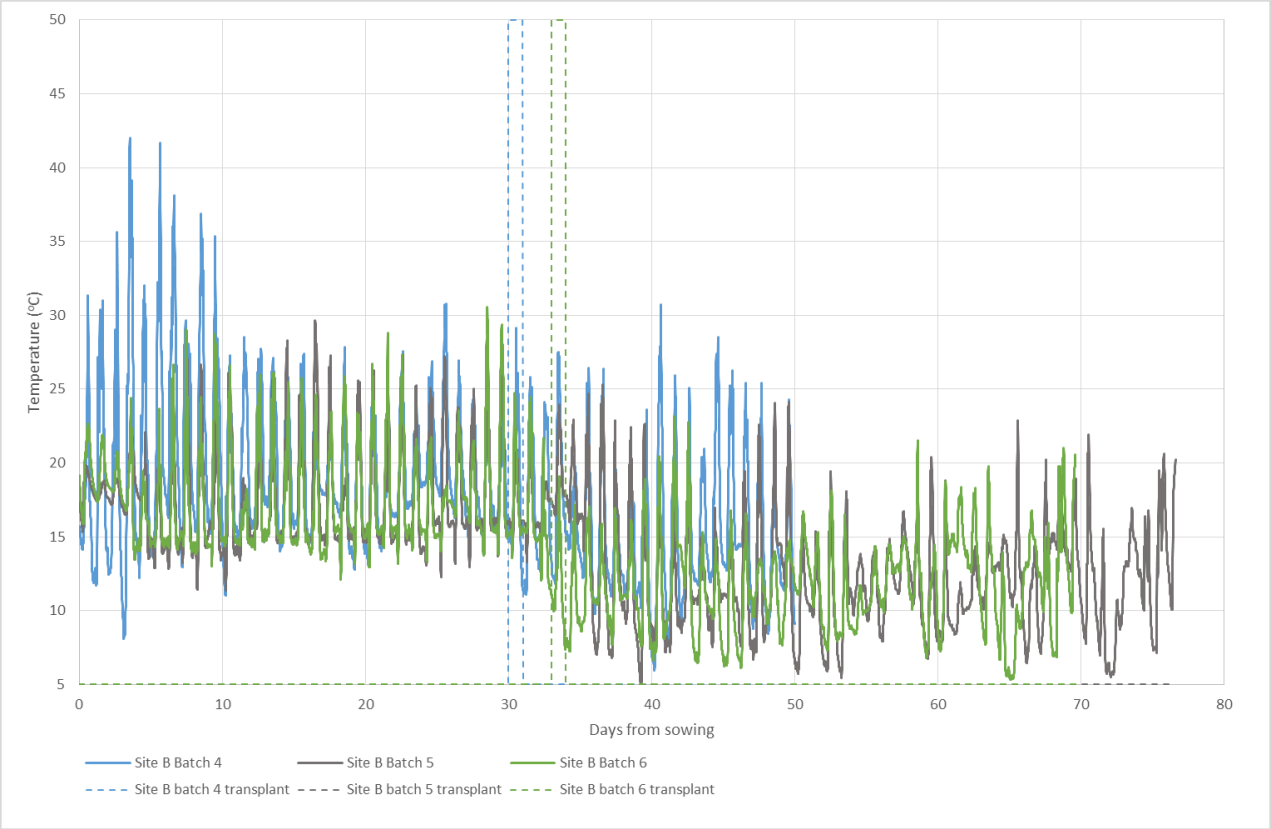

Figure 19. Temperature measured for batches 4 to 6 at site B

The monitoring of soil volumetric water content at site B showed no correlation with the incidence of high VPD or temperature in any of the batches, apart from in batch 4 where there is a significant drop in the volumetric water content around day nine (**Figure 20**). This drop began at a point when VPD and temperature were high and then the VWC plummets to a very low level for a couple of days before rising again. This could be due to the monitored pot not being watered and may not necessarily reflect the conditions in the plug trays in general.

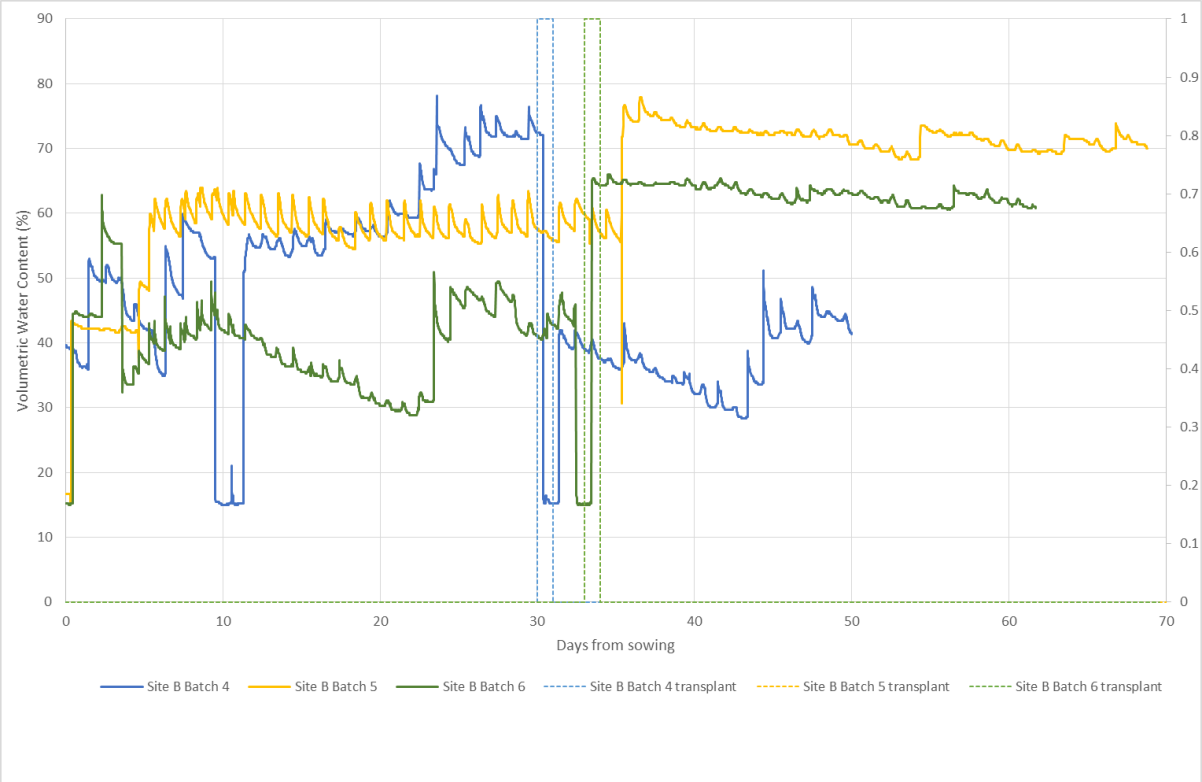

Figure 20. Volumetric water content in batches 4 to 6 at Site B

No symptoms were recorded at sites C and D, despite there being some VPDs greater than 3 kPa and temperatures above 35ºC at both sites.

Objective 2 – association between symptoms of pansy mottle syndrome and VWDaV:
254 Pansy samples were passed to Fera for Taqman® virus testing for *Viola* white distortion-associated virus (VWDaV): 93 distorted, without mottling; 109 with mottling / bleaching; and 52 with no symptoms.

The results of the virus tests were spilt into two groups: those from low populations with a low prevalence of symptoms (1%) and those from a population with a high prevalence of symptoms (75%) (**Table 3**). VWDaV was found to be present in samples from all three symptom categories (distortion only, distortion with bleaching and no symptoms). Samples 1 and 13 (**Table 3**) originated in batches that were monitored under Objective 1 of this project (Site A, Batches 1 and 2, respectively), and plants in both of these samples tested positive for VWDaV: 100% (sample 1) and 60% (sample 13). Statistical analysis was carried out on the data resulting from the virus testing.

Data used in the statistical analysis included information about the source of plants, the number of plants with the two types of symptoms (and no symptoms) selected for testing and the number of samples that had produced a positive result for VWDaV. Samples with incomplete testing information were removed prior to statistical analysis. The results were spilt into two groups: those from low populations with a low prevalence of symptoms (1%) and those from a population with a high prevalence of symptoms (75%) (**Table 3**).

Table 3. Results of the Taqman® virus testing of Pansy samples for *Viola* white distortion-associated virus results. *****These plants were from monitored batches under Objective 1 of this project

Sample	Nursery	No. of plants tested			No. of plants positive for VWDaV			Incidence symptoms
		Distorted, stunted	Mottling, bleaching	None	Distorted, stunted	Mottling, bleaching	None	
1*	A	20	0	20	20	0	20	1%
2	A	1	0	1	1	0	1	1%
3	A	1	0	1	1	0	1	1%
4	A	1	0	1	1	0	1	1%
5	A	1	0	1	0	0	0	1%
6	A	1	0	1	1	0	1	1%
7	A	1	0	1	0	0	0	1%
8	A	2	18	6	1	17	6	1%
10	A	4	4	4	4	4	3	1%
12	A	11	11	11	11	9	3	1%
13*	A	0	16	16	0	12	7	1%
16	A	5	5	5	5	5	0	1%
17	A	5	5	5	0	0	0	1%
18	A	10	10	10	10	10	10	1%
24	B	0	0	4	0	0	4	1%
20	A	6	0	3	0	0	0	1%
21	C	4	5	5	4	0	1	75%
22	C	5	5	5	2	5	5	75%
23	C	5	5	5	5	5	5	75%
Sum 1%		69	69	90	55	57	57	
Sum 75%		14	15	15	11	10	11	

The extent to which symptoms may be caused by the virus was assessed by making the following observations:

$P(S)$: the proportion of plants that were symptomatic.

$P(V|S)$: the proportion of symptomatic plants that contained the virus,

$P(V|{\sim}S)$: the proportion of non-symptomatic plants that contained a virus.

Estimates of three proportions were derived:

$P(V)$: the proportion of plants that contain the virus,

$P(S|V)$: the proportion of plants with the virus that were symptomatic

$P(S|{\sim}V)$: the proportion of plants without a virus that were symptomatic.

Estimates were derived from the law of total probability:

$$P(V) = P(V|S)P(S) + P(V|{\sim}S)\big(1 - P(S)\big)$$

[Equation 1]

and Bayes' Theorem

$$P(N|V) = \frac{P(V|S)P(S)}{P(V)}$$

$$P(N|{\sim}V) = \frac{P({\sim}V|S)P(S)}{1 - P(V)}$$

[Equation 2]

The size of the uncertainty associated with observed proportions was estimated using a Modified Jeffreys interval [2], where given x 'positives' out of n observations the probability p underlying the observed proportion is with confidence 1-α

$$B(\alpha/2\,, x + 0.5, n - x + 0.5) \leq p \leq B(1 - \alpha/2\,, x + 0.5, n - x + 0.5)$$

[Equation 3]

where $B(\alpha, b, c)$ is the α quantile of the $Beta(b, c)$ distribution

Table 4. Estimates of the prevalence of virus and the effect of its removal on the prevalence of symptoms. P = proportion.

% of symptoms observed in crop	% Plants with symptoms and detected virus			% of non-symptomatic plants with virus detected			% of plants with virus detected			% Plants with virus that also had symptoms			% Plants with Symptoms and no virus detected			Effect of removal (%)		
	Lower CI	Central	Upper CI	Lower CI	Central	Upper CI	Lower CI	Central	Upper CI	Lower CI	Central	Upper CI	Lower CI	Central	Upper CI	Lower CI	Central	Upper CI
1%	74.0	**81.1**	87.0	53.1	**63.3**	72.7	53.3	**63.5**	72.8	1.08	**1.27**	1.53	0.332	**0.516**	0.797	20.3	**48.4**	66.8
75%	54.7	**72.4**	86.0	48.4	**73.3**	90.3	57.8	**72.6**	83.7	67.6	**74.8**	82.2	54.5	**75.6**	90.5	-20.7	**-0.84**	27.2

Estimates are given as a central estimate and 95% confidence interval (CI).

The uncertainty associated with derived estimates was estimated by generating independent random (uniform (0,1)) quantiles for each of the observed proportions (Equation 3) and calculating derived values using Equations 1 and 2. 95% confidence intervals were taken from the 2.5th and 97.5th percentiles of 100000 derived values.

Table 4 shows estimates of the prevalence of the virus in symptomatic and asymptomatic plants and the potential reduction in symptomatic plants that may accompany a removal of the virus if it is indeed a causal agent for the symptoms.

In plants taken from low prevalence (1%) populations symptomatic plants are estimated to be at a higher prevalence among plants in which the virus is present (range of **1.08 to 1.53%**) than among those plants in which the virus is absent (range of **0.332 to 0.797%**). If the virus is a causal agent for the symptoms then it is estimated that this accounts for about half (range of **20.3 to 66.8%**) of the 1% of symptomatic plants observed in the population.

No association between the virus and symptoms was found in the higher prevalence (75%) population. The prevalence of plants in which the virus was detected was approximately equal in symptomatic and asymptomatic plants. The virus *may* account for a maximum (97.5% confidence) of **27.2% of the 75%** of symptomatic plants.

Discussion

Objective 1 - environmental monitoring:

The monitoring from 2015 proved to be inconclusive as although environmental conditions similar to those observed in 2013 that were thought to trigger PaMS did occur for most of the batches in which distortion or distortion plus mottling symptoms were observed in 2015, there were two batches where there were no obvious adverse or stressful environmental conditions. In addition, adverse conditions were seen in batches where no symptoms were observed (e.g. batches 1 and 3 at site A, batches 1 and 2 at site C). The lack of consistency would indicate that either the environmental conditions identified in 2013 were not the conditions that trigger PaMS, were specific to the batch that developed symptoms in 2013 or there are other factors involved that we have not been able to identify from the nursery monitoring.

Monitoring of the volumetric water content (VWC) of the soil did not show any clear relationship between the occurrence of PaMS symptoms and conditions of low VWC, high VPD and high temperature. For batch 2 at Site A, there was a co-occurrence of low VWC with high temperature and VPD approximately 2 weeks prior to observation of distortion symptoms, but this combination of low VWC, high VPD and high temperature was not observed for any of the

batches at site B. The results from the monitoring do not unequivocally support the hypothesis that PaMS symptoms are associated with adverse environmental conditions and low water availability (water stress).

Over the three years of monitoring, PaMS has been observed in eight batches out of 40 batches that were monitored. Eight batches is a very small sample size given the huge variation that occurs in the environmental conditions from year to year, between sites within a year and between batches within sites. Combining the data from all batches at all sites in all years is unlikely to provide any further information as the variation in the data would act to mask the effects of any possible correlation between environmental trigger conditions that may be present and the presence of PaMS symptoms. Whilst further monitoring might eventually aid the identification of a set of environmental trigger conditions, a large effort would need to be put into the sampling in order to achieve a sufficiently large set of positive samples to increase the probability of finding a set of environmental trigger conditions.

Objective 2 – association between symptoms of pansy mottle syndrome and VWDaV:
The analysis of pansy samples for presence of VWDaV showed that for those batches with low prevalence (1%) of symptoms, if the virus were not present there would be the potential for an estimated 50% reduction in symptoms. However, for those samples from batches where approximately 75% show symptoms, no association was found between the virus and symptoms.

For a virus, the aim is not to kill the plant but to reach an equilibrium whereby it can exist within the plant. The virus may also be present without symptoms being expressed, and it may be that the virus has an effect on the plant at the molecular or genetic level that only becomes apparent under stress conditions. The conditions that trigger symptom expression may be a combination of environmental parameters and the quantity of virus present. For the samples tested, there was a relatively even amount of infection, regardless of symptomatic status.

There are several points for discussion:

- Samples were taken for virus testing at one time point based on visual symptoms, and it may be that the visually unaffected plants tested may have developed symptoms if left to develop.

- Ilarviruses are thought to be spread through seed and pollen, and mechanical transfer. It would be possible to carry out testing of seed lots to identify if the virus is detectable in infected seed using the Fera TaqMan test.
- As VWDaV is mechanically transmissible e.g. handling and pruning where sap may be transferred by contact with contaminated plants or plant debris, tools, or workers, and this would facilitate spread in a production unit. There is no cure for viruses, but measures that will help to keep them in check include destroying badly affected plant material and good nursery hygiene e.g. disinfecting tools with a disinfectant that is effective against viruses such as Unifect-G, Menno Florades or Jet 5 (O'Neill *et al.,* 2014).
- The Italian scientists who initially discovered VWDaV concluded from the limited number of samples they had tested, that the new virus could not fully account for the symptoms expressed in the plants in their experiments.
- There remains the possibility that there could be another unidentified virus or similar involved in causing these symptoms, and this could account for the results where there were symptoms but no virus detected.

Conclusions

Some plants express the whole range of symptoms, including stunting, distortion, apical blindness and mottling/bleaching and will never develop beyond a small 'rosette' – a very small percentage (estimated <1% of symptomatic plants) are usually affected to this degree. In 2015, growers noticed distortion in Pansies from around three weeks after seed sowing, including plants from Site B, Batch 1 which were included in the virus testing. Of the plants from this batch that remained on the nursery, many grew out of the symptoms. Similarly, it has been noted that plants with mottling and distortion can produce new growth that doesn't show any symptoms given time. It is not suggested that these would be high quality plants that would meet marketing schedules, but is an indication of the transient nature of the event that triggers symptom expression.

It is not possible to reach a definitive conclusion with regard to the effect of environment on expression of PaMS from the findings of this work to date, and the variation in environmental conditions experienced from season to season make it difficult to compare or combine findings across batches, sites and years. There is also the potential that symptom expression is linked to the VWDaV virus, or another virus as yet undetected.

Further work to investigate VWDaV, including how it affects plants at the molecular/genetic level, and confirmation that is seed-borne, including transmission testing, would help to provide answers for growers. Any future work looking at the effect of environment on PaMS should be carried out under controlled environment conditions to reduce the variables, but would be dependent on locating equipment or conditions capable of providing the high stress conditions that have appeared to be implicated in PaMS expression, and should be linked to investigation of the involvement of VWDaV or another as yet undetected virus.

Knowledge and Technology Transfer

An article was published in the July/August 2015 issue of the AHDB Horticulture Grower journal.

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Appendix 1. Grower monitoring template

Project title:	The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS) 2015	
ADAS:	AHDB: PO 016a	
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 Pansy batch / sowing date:		Date: Growth stage (no of leaves):			
Plant no.	**Root hair score (0-3 scale)**	**Comments**	**Plant no.**	**Root hair score (0-3 scale)**	**Comments**
11			4		
2			5		
3			6		

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

Separate Appendices are available as follows:-

Appendix 2. Site data

Appendix 3. Environmental conditions at sites A-D