HORTICULTURE

Grower Summary

PO 016a

The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS)

Final *2016*

Disclaimer

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

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Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

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Project title:	The role of environmental factors in the incidence of Pansy mottle syndrome (PaMS)
Project number:	PO 016a
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Report:	Final report, April 2016
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Date project commenced:	1 May 2015
Date project completed (or expected completion date):	30 April 2016

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**).

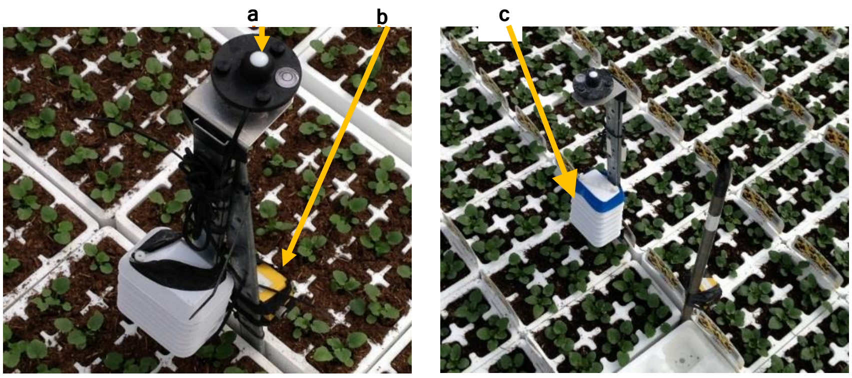

Figure 2. Positioning of data loggers and light sensor within a batch of pansies: a) LightScout Quantum Light 3 Sensor PAR probe; b) Tinytag Plus 2 data logger (temperature and humidity); c) Watchdog 1000 series data logger housed within a radiation shield for protection against solar radiation and water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

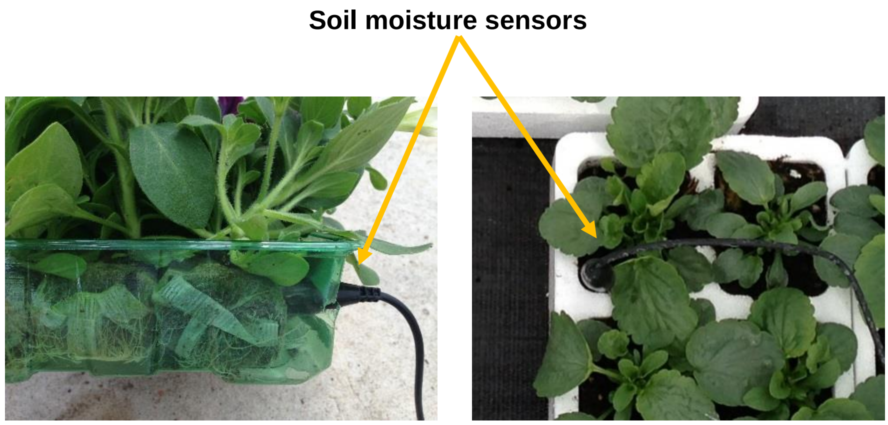

Figure 3. Positioning of SM100 Soil Moisture Sensor within a coir system, inserted horizontally (image left); and in a peat based system inserted vertically (image right) production systems

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.