Project title	Bedding Plants: Investigating the cause and prevention of "Pansy Mottle Syndrome".
Project number:	PC 286
Project leader:	Dr G M McPherson
Report:	Final report, 31 July 2010
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Project Manager **Key staff:**	Cathryn Lambourne Iwona Burdon Deborah Liddell*
Location of project:	Stockbridge Technology Centre Ltd
Project coordinator:	Mr S Coutts (Ornamentals Consultant, retired)
Date project commenced:	July 2008
Date project completed (or expected completion date):	July 2010
Key words:	Pansy, *Viola wittrockiana*, leaf distortion, mottle, virus, growth regulation, blindness, chemical damage, 'stress-induced' response, downy mildew, chimaera.

* left STC October 2009

The results and conclusions in this report are based on an investigation conducted over a two-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 G M McPherson

Science Director

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GROWER SUMMARY

Headline

- Pansy mottle syndrome (PaMS) is an intermittent condition which appears to be the result of a physiological response to stress.

- Comprehensive systematic testing of a wide range of agronomic factors has failed to pinpoint any single factor or combination of factors that may be consistently linked to the condition.

Background and expected deliverables

Symptoms consistent with what is now called ‘pansy mottle syndrome’ were observed on a range of bedding species as far back as the 1960’s. The symptoms have been reported on *Antirrihinum*, marigold, pansy, *Petunia*, stocks, sweet pea and *Verbena*.

The term (PaMS) was coined in recent years to describe a particular set of symptoms seen primarily, though still not exclusively, in pansy plants (similar symptoms have also been recorded in *Petunia* for example). The symptoms include leaf distortion, mottling and bleaching of the leaves, stunting, and in severe cases apical blindness (as shown below).

(courtesy of Will Healy- Ball Colegrave)

Figure 1 GS. Examples of PaMS symptoms

The condition, which previously was only seen intermittently by UK growers now appears to be becoming more prevalent. Early work carried out in 1990 (PC 27) and 1993 (PC 27a) suggested that the problem may be linked to a bacteria, and a later review carried out in 2005 by Stuart Coutts and Neil Bragg (PC 211) supported this view. A comprehensive literature review of the current state of knowledge in the UK was subsequently carried out by Nigel Paul at Lancaster University in 2007 (PC 211). The aim of the current HDC funded project (PC 286) was to examine the various possibilities arising from the review to establish potential causes or contributory factors causing or triggering PaMS in pansy production.

Summary of the project and main conclusions

Approximately 140 UK bedding plant producers were surveyed to gather their experiences and information on PaMS. A response rate of 40% was achieved and the information was used as a basis to plan experiments to evaluate some of the potential 'trigger' factors that might be implicated in the disorder.

Based on data collated from the survey a number of initial experiments were undertaken (Year 1) using some of the PaMS affected plants received from growers and propagators.

Seed collected from severely distorted PaMS affected plants was sown alongside a similar variety of commercial seed but there were no significant differences in numbers of PaMS affected plants resulting from the two sources of seed indicating that PaMS is not carried genetically through seed. Lack of symptom expression in other trials in year 1 prevented identification of other agronomic factors that may be directly linked to the incidence of PaMS.

During the spring and summer of 2009 (Year 2) larger trials were undertaken at STC. Propagation trials focused on growing pansy and *Viola* varieties in different module sizes under a range of different, and overlapping, growing regimes examining light, irrigation and plant growth regulator applications. PaMS symptoms were successfully reproduced particularly with certain specific varieties in the studies. However, these studies failed to identify any of the above factors as specifically triggering PaMS and symptoms were observed in all treatments.

Subsequently, a much larger, fully replicated glasshouse trial was carried out using several varieties of pansy and *Viola* (three of each, using cultivars reported to have been potentially susceptible by growers). Plants were propagated by two commercial propagators (and also

by STC Ltd). The plants were potted-on into 6-packs to allow the crop to be monitored for a longer period. The plants were arranged on 20m^2 benches and all combinations of the following factors were applied to the plants in an attempt to identify the key variables which could influence the incidence of PaMS:

Module tray size	308	576	
Light level	Low (shaded)	Ambient	High (Lit)
Plant growth regulator regime	None	One application	
Irrigation regime	Standard	Low	

On close inspection during transplanting it was observed that a small to moderate percentage of the seedlings propagated at STC were already showing early PaMS symptoms (6% of the total number of plants for Pansy A and *Viola* A), whereas a much lower percentage (1% and 3.5%) of those propagated commercially were affected at this stage. Irrespective of this early appearance of PaMS symptoms the full range of treatments continued to be applied, the crops being assessed and monitored regularly for PaMS symptoms.

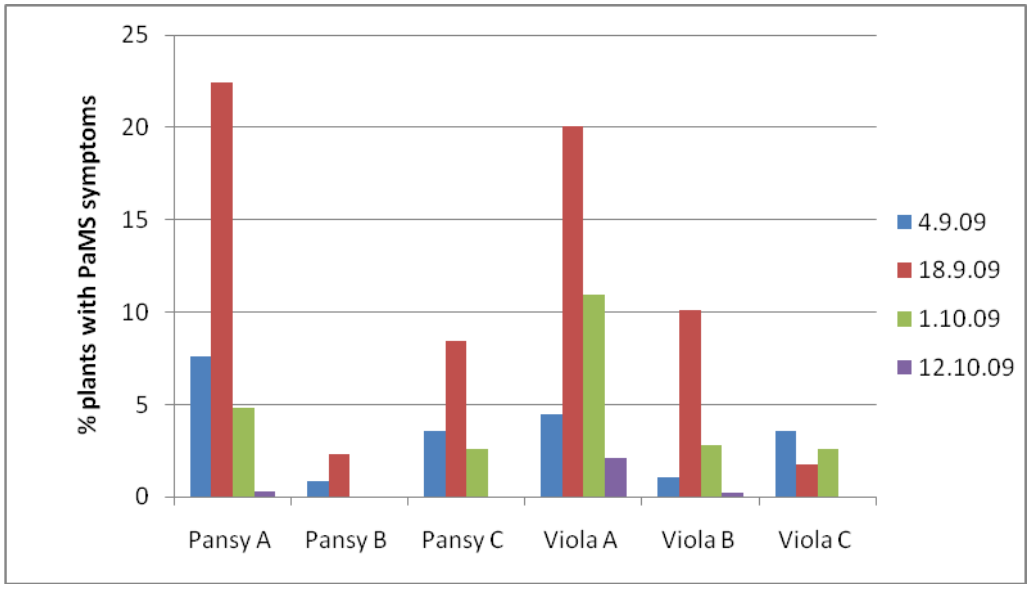


Figure 2 GS. Chart showing the percentage of plants with PaMS symptoms at each assessment date in the glasshouse trial

* 1st assessment on 4.9.09 was made a few days after transplanting from module trays.

The chart shows that the percentage of affected plants increased sharply at the second assessment date, particularly in Pansy A and *Viola* A, and this was in part due to the fact

that the first and second pairs of true leaves had emerged and distortion was observed in these leaves rather than the cotyledons (indicating this was probably a continuation of symptom expression from an earlier cause rather than new symptom development). In nearly all cases the number of affected plants dropped off in later assessments. This was largely due to plants growing-away from the symptoms and producing new healthy foliage. In the vast majority of cases the PaMS symptoms were transient or temporary and most plants grew away from the symptoms over time (but this is not always the case under commercial conditions).

There was no observable pattern to the development of symptoms in the plants that could consistently be attributed to a particular set or combination of imposed factors.

A selection of plants showing symptoms were used to carry out additional investigations such as virus testing and maintaining plants for later seed collection. No virus was detected in any of the affected plants. No differences in tissue or growing media nutrient levels were observed. No systemic downy mildew was detected when the affected plants were tested by molecular analysis using polymerase chain reaction (PCR).

A gas chromatography mass spectroscopy test (GCMS) did show a very slight peak for a chemical product that was detected in the distorted plants, which was not present in the unaffected plants. This was identified as belonging to the natural plant hormone methyl-salicylate (MS). This product can be released by 'stressed' plants and is involved in 'turning-on' the natural plant host defences. A small-scale laboratory study to investigate whether this product could induce PaMS symptoms was subsequently undertaken.

Seed was germinated in sealed containers containing beakers of MS at five different concentrations (0, 50, 200, 500 and 1000ppm). It was found that the higher concentrations impacted severely on germination. Seedlings treated with lower concentrations of MS did not show any PaMS symptoms. This suggests that MS is not responsible for the development of PaMS symptoms, but instead, that its presence merely reflects that affected plants were showing 'stress' although the tests done were not extensive.

Seed collected from affected *Viola* plants was sown alongside another variety of *Viola* to determine whether the symptoms may be carried genetically. No evidence was found to support this hypothesis.

Findings from these trials suggest that there may be another, so far unconsidered, factor or factors causing the development of PaMS. An important observation in these studies was the very early incidence of PaMS in young plants prior to the start of the trial. The difference in the initial level of PaMS noted (dependent upon the site of propagation) could indicate that a particular factor or a range of factors influenced by the different propagation regimes may be implicated in the development of PaMS symptoms. However, it could also simply be a reflection of the different quality control procedures employed by businesses leading to slightly different sub-standard seedling rejection rates within trays of commercial plants prior to dispatch.

Financial benefits

Plant losses due to PaMS (at the propagation stage and finished plant stage) are difficult to quantify due to the variable and intermittent nature of the problem. Published Defra statistics (2002) indicate that around 9.5 million pansies are produced annually with a wholesale value of almost £2.1 million. (These figures undoubtedly underestimate the pansy crop currently grown in the UK). Official production figures for other crop groups which also suffer from similar symptoms (*Petunias* for example) do not exist. The main period when the symptoms are noted is late summer and early autumn, so only a percentage of the pansy and *Viola* crop is affected. Losses may be around 1% on average, equating to around £20,000 based on the Defra figures, but this figure doesn't take into account the costs incurred to 'make up' affected plug trays or finished packs of plants as a result of odd affected plants (to avoid product rejection) and the other plant species affected by this disorder.

Clearly, in years when PaMS symptoms are more severe on specific nurseries or if there is a continuing increase in the frequency and severity of symptoms this will have more of a financial implication for growers. It is hoped that the data and information collected during this study will improve awareness within the industry and help growers implement strategies to reduce the risk and incidence of PaMS.

Action points for growers

- If propagating plants ensure that good quality seed is used.
- The symptoms do not appear to be viral in nature (confirming the findings from HDC project PC 211) and therefore measures to minimise the spread of virus can be ruled out as important requirements for the control of the problem.

- No single factor has been conclusively linked to the incidence of PaMS but it is advised that plants are propagated and grown on under conditions where plant stress is minimised to avoid symptom development. This would include the use of good quality growing media, uniform and appropriate irrigation, using irrigation water of suitable quality, provision of shade as required, ensuring adequate levels of air movement over the crop and avoidance of intensive spray programmes, especially over very young plants.

- If plant stress levels cannot be minimised on the nursery during the propagation phase then consider buying in young plants at certain times of the year when the symptoms are an issue.

- If certain varieties or flower colours are more prone to the problem try substituting them for others, or take extra care with the production of these.

- The project indicated that the plants may eventually grow away from the symptoms, but regular inspections of growing crops (both seedlings and finished plants) will help to ascertain the level of incidence and the need to rogue-out plants to help facilitate better crop management later on.

SCIENCE SECTION

Introduction

Symptoms of pansy mottle syndrome (PaMS) have been observed sporadically in UK pansy and viola crops for many years, and were also observed on a range of other bedding crops including Antirrhinums, Petunia, Stocks, Marigolds, Sweet Pea, Pansy and Verbena until more recent times. Symptoms are now seen less frequently in many of these crops which may be linked to improvements in production and gapping-up techniques. However, in pansy and viola, the symptoms, which include: leaf thickening and distortion, mottling, bleaching, stunting, reduction in flowering and in severe cases, apical blindness (photographs of the symptom are attached in Appendix 1), appear to have increased in frequency over the last 5-10 years (Coutts & Bragg 2005) and this may be linked partly to the move to all year round production which can result in young plants being raised in more stressful conditions over the summer months.

This project was initiated to firstly assess the state of knowledge on PaMS, also to collect images and samples and to clarify definitively, what the symptoms associated with the syndrome were and on what plant species. Lastly we wanted to try and determine what factors might be involved in triggering the PaMS symptoms in plants. To achieve these goals we surveyed and collected information from growers, collected samples of distorted crops and attempted to recreate PaMS symptoms in crops using a range of production scenarios.

We have used a logical stepwise approach to test the different hypotheses for potential causes of PaMS; including virus infection, systemic downy mildew infection, seed and graft transmission, production factors such as temperature, light levels, humidity, crop nutrition and various other stress factors.

In 2005 HDC funded a review which was carried out by Stuart Coutts and Neil Bragg. This was followed by a thorough literature review conducted by Dr Nigel Paul at Lancaster University (PC 211/211a). The review collected anecdotal evidence from 39 growers in the UK. The literature review covered information on the syndrome held in the UK and the US where pansy production had also been seriously affected. The main findings of both pieces of work are summarised below:

- There may be more than one factor which triggers or causes PaMS.
- The syndrome does not appear to spread to other nearby plants i.e. it is not an infective agent.
- There may be a genetic (seed transmission) link, but that this alone was not the cause and it may need to be triggered by some other factor.
- Other species e.g. Antirrhinum can be affected and show the same, or similar, symptoms.
- Severely affected plants rarely recover without some action.
- The symptoms are worse on some colours, and possibly some cultivars.
- Symptom expression, according to many growers, is linked to plant stress.
- The stress can take a number of forms but may be linked to high light, high temperatures possibly combined with fungicide, PGR or other chemical applications.
- Bacterial and virus infections were initially ruled out, but work by Dr John Hammond at USDA suggests that an ilarvirus may be implicated, although again, it is not considered to be the sole cause of the problem.
- Damage caused by tarsonomid mite has been ruled out.
- Work by Dr Douglas Bailey (North Carolina State University) looked at links between a condition causing PaMS symptoms, although not called PaMS and nutritional deficiencies e.g. boron. Dr Paul, in his review, felt that although possibly implicated this could not entirely explain the problem due to the variable expression of symptoms in crops which were managed in the same way across different nurseries.
- It was considered that the symptoms are not directly linked to PGR applications, but that the symptoms could show up more on PGR treated plants.
- Different growing substrates and water supplies have been used on different nurseries and these factors have previously been discounted as possible causes of the syndrome.

Information from the earlier review was used alongside data collected from growers during this project to design trials carried out at STC where we wanted to recreate PaMS symptoms in crops under a range of different conditions.

Materials and methods

Plant Grafting

Following the receipt of several severely affected trays of pansy and viola provided in September 2008 it was decided to use the plants to carry out some grafting experiments to investigate whether the syndrome could be transferred to unaffected material.

Trays (405 modules) or 6 packs of the following varieties of pansy and viola were received, with varying levels of symptom expression.

Series	Cultivar	Incidence of PaMS/tray
Magic Designer	Primrose (2 trays)	40 & 70%
Magic Select	Deep Blue	50%
Magic Designer	Beaconsfield	10-15%
Nature	Frosty Rose	10%
Magic Designer	Yellow with blotch	2%
Sweets	Sweeties	1-2%

As trays of unaffected plants were also provided we opted to carry out grafting on the Beaconsfield and Primrose cvs. Affected and non-affected plants were potted-on into 6 pack trays to allow the plants to get to a size which would permit manipulation of the shoots.

Plants were grafted in both combinations of 'healthy' rootstock with affected scion (shoot material) and also with 'healthy' scions grafted on to the rootstock of affected plants. Tomato grafting clips (1.2mm) were used to hold the graft union in place after earlier attempts using Parafilm proved unsuccessful.

Questionnaire

A detailed questionnaire was developed and circulated to growers via the HDC database. The questionnaire (Appendix 2) showed a series of images of the various symptoms described to ensure that growers could identify the problem correctly. The questions posed attempted to gather information regarding the distribution of the problem on pansy/viola and also determine whether similar symptoms had been seen on other bedding plant crops. Information was also gathered on topics such as timing of symptoms and possible triggers e.g. environment, lighting, application of crop protection products.

The questionnaire was distributed to all growers who attended the BPOA Disease Seminar in February 2009. Extra copies were circulated to bedding plant growers who did not attend via the HDC.

Propagation trial

Data from the grower survey had suggested that some growers only saw symptoms during propagation. It was not clear whether symptoms occurred exclusively in propagation i.e. symptoms exhibited as seeds germinated and prior to potting-on into 6-packs possibly followed by plants growing out of the symptoms, or whether propagators were simply discarding affected seedlings at the potting-on stage during 'gapping-up'. We designed a trial, using possible stress factors suggested from the survey, to investigate whether any of them could trigger PaMS symptoms under carefully controlled conditions at STC.

Cultivar	Two cultivars were selected for study – pansy cultivar A* and viola cultivar D*
Module Size	Two different size modules – (576 and 308) were selected for the study.
Lighting	Three light regimes – low (shaded), ambient, high light (16hrs/day) were selected under which to grow the crops.
PGR	A PGR application (B-Nine) was made to 50% of the trial with the other 50% receiving no PGRs.
Irrigation	Two irrigation regimes were used. Plants were either irrigated infrequently, being allowed to dry out between watering or were watered using a normal regime.

This gave a total of 24 different treatment regimes. One module tray represented a plot. No replication or randomisation was carried out in the trial area (see plate 2).

The seed was sown using a standard commercial seed & modular compost and the trays were placed in separate bays of a glasshouse, but without the additional lighting regimes for the first week post-sowing to ensure normal and even germination.

* Cultivar names not disclosed for confidentiality purposes.

Crop Diary

20.8.09	Seed sown
27.8.09	Different lighting regimes initiated.
10.9.09	Daminozide (B-nine) applied to treatments 7-19.
14.9.09	1st assessment for PaMS affected plants in trial area.
21.9.09	Samples collected for analysis.
28.9.09	2nd assessment for PaMS symptoms in trial area.
7.10.09	Samples of distorted plants dispatched for virus testing.

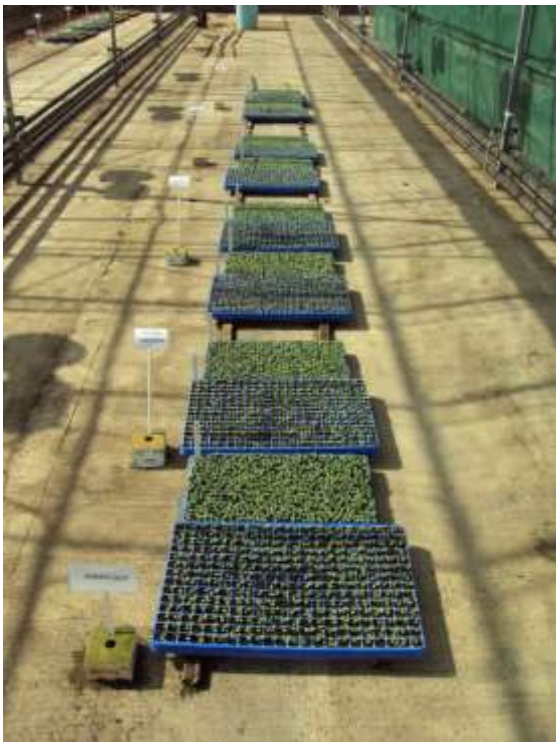


Plate 1: Pansy & viola seedlings in two module size trays under ambient light conditions. Identical modules were also grown concurrently under shading and supplementary lighting conditions.

Assessments

The plants were examined regularly and a detailed assessment conducted as soon as any PaMS symptoms were observed. When symptoms did appear it was noted that affected plants were often seen in patches in module trays with single affected plants seldom being seen. Therefore, to avoid any possible bias during assessments, we opted to assess 100 seedlings in a central quadrat on each tray. The position and type of symptom e.g. distorted, bleached or distorted & bleached was recorded at each assessment date.

During the final assessment the total number of affected plants/tray was recorded.

Table 1: Propagation trial treatment details

Treatment No.	Module size		Lighting Regime			PGR Regime		Irrigation Regime	
	308	576	Ambient	Low	High	None	1 application	Normal	Low
1	✓	X	✓	X	X	✓	X	✓	X
2	X	✓	✓	X	X	✓	X	✓	X
3	✓	X	X	✓	X	✓	X	✓	X
4	X	✓	X	✓	X	✓	X	✓	X
5	✓	X	X	X	✓	✓	X	✓	X
6	X	✓	X	X	✓	✓	X	✓	X
7	✓	X	✓	X	X	X	✓	✓	X
8	X	✓	✓	X	X	X	✓	✓	X
9	✓	X	X	✓	X	X	✓	✓	X
10	X	✓	X	✓	X	X	✓	✓	X
11	✓	X	X	X	✓	X	✓	✓	X
12	X	✓	X	X	✓	X	✓	✓	X
13	✓	X	✓	X	X	X	✓	X	✓
14	X	✓	✓	X	X	X	✓	X	✓
15	✓	X	X	✓	X	X	✓	X	✓
16	X	✓	X	✓	X	X	✓	X	✓
17	✓	X	X	X	✓	X	✓	X	✓
18	X	✓	X	X	✓	X	✓	X	✓
19	✓	X	✓	X	X	✓	X	X	✓
20	X	✓	✓	X	X	✓	X	X	✓
21	✓	X	X	✓		✓	X	X	✓
22	X	✓	X	✓	X	✓	X	X	✓
23	✓	X	X	X	✓	✓	X	X	✓
24	X	✓	X	X	✓	✓	X	X	✓

The PGR treatment – B-nine was applied using a Hozelock hand sprayer at a rate of 4g product/L. Approximately 23ml/tray was applied.

Glasshouse Trial

A large scale glasshouse trial was carried out at STC during the summer/autumn of 2009. In this study we included as many of the possible trigger factors identified in the review work and our grower survey as possible. Two sets of commercially propagated plug plants and one set of STC-raised plug plants were potted-on into 6-packs using a standard growing media at the commencement of the trial with ten 6-packs/treatment. Each of the 3 pansy and viola cultivars were laid out on a separate mobile bench in a 200m^2 glasshouse at STC. The trays were arranged 10 across and 40 along the length thus providing 40 treatment regimes with 10 replicate trays/treatment. The plants were allowed to grow-on for 7 days prior to the start of the chemical applications. A detailed but complex table (Table 5) shows the arrangement of treatments.

Table 2: Treatment factors:

Cultivar	3 pansy and 3 viola varieties (pansy A-C and viola A-C)
Propagator and propagation regime	Seedlings were raised at 2 commercial sites and also at STC (1 pansy and 1 viola cultivar/propagator)
Lighting	In the plants propagated at STC a low light regime was used for 50% of the crop (this was not possible with the commercially produced crops).
Irrigation	Benches were split lengthwise with 5 x 6-packs positioned on capillary matting to maintain moisture and the remainder with no matting – drying partially between watering.
Pesticides	Crops were either left untreated or treated with Octave (prochloraz), Aliette (fosetyl-aluminium) or Amistar (azoxystrobin).
PGR	Plants either received no PGR applications or were treated with B-nine (daminozide), Chlormequat or Bonzi (paclobutrazol).
Adjuvants	The pesticide applications were combined with either: no wetter, Activator 90 or Sprayfast wetter.

Crop Diary

1-3rd Sept 09	All plug plants potted-on into 6-packs and laid out in glasshouse.
4th Sept	1st assessment of distorted plants carried out.
11th Sept	1st application of PGR and fungicide products applied with/without adjuvants.
25th Sept	2nd application of PGR, fungicide and adjuvants carried out applied to half the crop.
18th Sept	2nd in-crop assessment carried out.
1st Oct	3rd in-crop assessment carried out.
12th Oct	4th in-crop assessment carried out.

Table 3: Treatment details

Product	Active ingredient	Application rate (product/L)	Water rate (L/ha)
Octave	prochloraz	2g	300
Aliette*	fosetyl-aluminium	15g	300
Amistar	azoxystrobin	3.33ml	300
B-nine	daminozide	4g	300
Chlormequat	chlormequat	4.4ml	300
Bonzi	paclobutrazol	2.5ml	300
Activator 90	alcohol ethoxylates and natural fatty acids	1ml	300
Sprayfast	terpene polymer and non-ionic wetter	1.25ml	300

* washed-off foliage within 15 mins of application

The products were applied using a Hozelock hand sprayer calibrated to apply 60ml/10 trays.

Plate 2: General view of the glasshouse trial in October 2009

The fungicide and PGR treatments were applied with/without the addition of wetters (see Table 5) to the 6-packs on the 10th September (1 week post potting-on). It had originally been planned to incorporate a further potential stress factor involving differential moisture levels in the plugs at the point of potting-on. However, in practice this was not possible and was omitted from the study. This resulted in there being duplicate batches of plants receiving the same treatment regime e.g. the plants in plots 3 and 4 both received an application of B-nine, with no adjuvant and no fungicide

treatments. The 'spare' treatment was utilised to make a 2nd application of the designated PGR/adjuvant/fungicide treatment to the odd numbered plot in each pairing. This 2nd application was carried out 2 weeks after the 1st application (25.9.09).

Table 4: Propagation details for the plants used in the glasshouse trial

Variety	Propagation location	Propagation regime						
		Module size	**Sowing date (wk)**	**Temp (°C)**	**Lighting**	**Growing media**	**Pesticides or PGRs**	**Irrigation**
Pansy A ..... Viola A	STC	345	33	15-16	½ propagated in low light remainder at ambient	Levington F2+S	None	Hand lance as req'd.
Pansy B ..... Viola B	Northern propagator [NP]	480	32	15-16	Germination chamber for 4 days, then ambient	Jongkind seeding compost with own blend	Subdue Aliette Cercobin Fubol Gold Repulse (all in a 4-wk cycle). B-nine to Pansy only.	O'head gantry, wetter in 1st 7 days of growth.
Pansy C ..... Viola C	Southern propagator [SP]	360	32	16	None	Pindstrup seeding substrate No. 2	B-nine @ 5g/l Cycocel @ 2ml/l	Overhead boom, as req'd.

Table 5 overleaf shows details of the applied fungicides, PGRs and wetters (highlighted boxes represent treatment combinations applied).

Table 5: Glasshouse trial treatment details

Treatment No.	PGRs				Wetters			Fungicides			
	None	B-nine	Chlormequat	Bonzi	None	Activator 90	Sprayfast	None	Octave	Aliette	Amistar
1	✓	x	x	x	✓	x	x	✓	x	x	x
2	✓	x	x	x	✓	x	x	✓	x	x	x
3	x	✓	x	x	✓	x	x	✓	x	x	x
4	x	✓	x	x	✓	x	x	✓	x	x	x
5	x	x	✓	x	✓	x	x	✓	x	x	x
6	x	x	✓	x	✓	x	x	✓	x	x	x
7	x	x	x	✓	✓	x	x	✓	x	x	x
8	x	x	x	✓	✓	x	x	✓	x	x	x
9	x	✓	x	x	x	✓	x	✓	x	x	x
10	x	✓	x	x	x	✓	x	✓	x	x	x
11	x	x	✓	x	x	✓	x	✓	x	x	x
12	x	x	✓	x	x	✓	x	✓	x	x	x
13	x	x	x	✓	x	✓	x	✓	x	x	x
14	x	x	x	✓	x	✓	x	✓	x	x	x
15	x	✓	x	x	x	x	✓	✓	x	x	x
16	x	✓	x	x	x	x	✓	✓	x	x	x
17	x	x	✓	x	x	x	✓	✓	x	x	x
18	x	x	✓	x	x	x	✓	✓	x	x	x
19	x	x	x	✓	x	x	✓	✓	x	x	x
20	x	x	x	✓	x	x	✓	✓	x	x	x
21	✓	x	x	x	✓	x	x	x	✓	x	x
22	✓	x	x	x	✓	x	x	x	✓	x	x
23	✓	x	x	x	x	✓	x	x	✓	x	x
24	✓	x	x	x	x	✓	x	x	✓	x	x
25	✓	x	x	x	x	x	✓	x	✓	x	x
26	✓	x	x	x	x	x	✓	x	✓	x	x
27	✓	x	x	x	✓	x	x	x	x	✓	x
28	✓	x	x	x	✓	x	x	x	x	✓	x
29	✓	x	x	x	x	✓	x	x	x	✓	x
30	✓	x	x	x	x	✓	x	x	x	✓	x
31	✓	x	x	x	x	x	✓	x	x	✓	x
32	✓	x	x	x	x	x	✓	x	x	✓	x
33	✓	x	x	x	✓	x	x	x	x	x	✓
34	✓	x	x	x	✓	x	x	x	x	x	✓
35	✓	x	x	x	x	✓	x	x	x	x	✓
36	✓	x	x	x	x	✓	x	x	x	x	✓
37	✓	x	x	x	x	x	✓	x	x	x	✓
38	✓	x	x	x	x	x	✓	x	x	x	✓
39	x	✓	✓	x	x	✓	x	x	✓	✓	✓
40	x	✓	✓	x	x	✓	x	x	✓	✓	✓

Additional Testing

During the 2009 season samples of PaMS affected plants were used to carry out a series of tests and analyses to help elucidate the cause of the syndrome. These included; virus testing (ELISA, sap transmission and electron microscopy), nutrient analysis of plant tissue and growing media and also a Gas Chromatography Mass Spectroscopy (GCMS) screen of affected and non-affected material.

Further analyses of PaMS affected and non-affected plants was carried out in 2010 using PCR to determine whether the effect may be caused by a systemic infection with downy mildew (*Peronospora violae*) using plants sent into the plant clinic.

Seed collected from distorted plants of Viola A (used in the glasshouse trials) was sown alongside another variety of viola (Viola E) to determine whether the affected plants produced seed which also carried high levels of distortion when grown-on.

Growing media trials

Observations made during the glasshouse trial suggested that plants sown by different propagators could lead to moderately high levels of distortion in plants, notwithstanding any treatments that had been applied. One aspect of this, not previously considered fully, was the substrate, or growing media used. A series of growing media studies were carried out to investigate potential correlations between the choice of growing media and PaMS symptom development.

Therefore a series of trials using a selection of different, commercially available, growing media was used to fill 345 module trays sown with a pansy and viola cultivar. Pansy A seed was sown into duplicate trays containing 4 growing media. The work was subsequently extended with a further 5 media being tested alongside the two which resulted in the highest number of distorted plants in the previous trial (both pansy A and viola E were used in these later trials). Details of the different substrates used and a generic description of the nature of the substrate are shown in Table 6. They are identified only with code numbers to avoid any possible confidentiality issues.

Regular assessments of the seed sown in the module trays were carried out. The number of plants showing possible PaMS symptoms was recorded over the propagation period.

Table 6: Details of the growing media used in the studies.

Growing media identification	Detail of media provided on the packaging
GM1	Seed and modular compost + sand
GM2	Potting substrate based on white peat + 20% frozen black sphagnum peat. Contains a wetting agent.
GM3	Irish sphagnum moss peat. Contains a wetting agent.
GM4	Organic 100% peat-free compost. Made with renewable resources of UK origin with added nutrients.
GM5	Professional multi-purpose, peat-free organic compost. Manufactured entirely from peat-free composted green waste mixed with plant nutrients. Contains a wetting agent.
GM6	Compost for all plants. 50% sustainable peat replacement product added.
GM7	Multi-purpose compost enriched with recycled materials contains patented wetting agent.
GM8	Organic, peat-free.100% chemical free. A blend of peat-free compost and organic plant food.
GM9	Multi-purpose compost produced from high quality sphagnum moss peat, blended with plant nutrients, lime and wetting agent. 40% peat, 60% FG+.

Laboratory study

An interesting GCMS analysis result which identified high levels of the plant hormone methyl-salicylate in the affected plants prompted us to carry out a small-scale study in the laboratory to investigate whether PaMS symptoms could be induced by methyl-salicylate or whether the methyl-salicylate was being produced in response to the foliar damage. Seed of pansy A and viola D were sown onto filter paper over moist sterile vermiculite in deep petri dishes. Seeds were maintained in covered and sealed germination chambers in the presence of beakers containing methyl salicylate at 0 (water control), 50ppm, 200ppm, 500ppm and 1000ppm. Seedlings were left to germinate and grow for approximately 3-4 weeks. The seedlings were then examined under a low power binocular microscope for symptoms of leaf distortion consistent with those of PaMS.

Results

Questionnaire

A detailed questionnaire was circulated to approximately 140 growers either via the BPOA Disease Seminar (February 09) or to non-attendees at a later date.

We had a pleasing 40% response rate. The data collected was extremely useful in providing detail on crop production methodologies which may or may not have been potential trigger factors for PaMS symptoms in crops. The main points identified from the survey are shown below.

Plant Grafting

Several attempts at grafting PaMS affected foliage onto unaffected root-stock plants and unaffected foliage onto PaMS affected young plants failed due to technical difficulties associated with the grafting technique using such small and tender material.

Monitoring the affected plants showed that, in many cases the symptoms became less obvious and the plants 'grew-away' from the symptom suggesting that the factors causing the leaf distortion and mottling were transient and that once the growing conditions became more favourable the plants were able to recover and produce normal growth. This was not always the situation though and some plants occasionally remained distorted – this was often the case where the main growing point had been irreversibly damaged, causing blindness.

Summary of the collated data.

1. 68% of respondents stated that they had seen symptoms conforming to those identified as being linked to PaMS on their nursery. Distribution of the incidence appears to be fairly even across the UK.

2. Approximately half felt that they had seen similar symptoms on crops other than pansy or viola. These included: Nicotiana, Impatiens, Primula, Petunia, Antirrhinum, Geranium and Cyclamen. The wide host range of the symptoms suggest that they are not caused by a primary disease problem, signifying rather that it may be a more generic condition relating to some intervention treatment allied to prevailing physical conditions.

3. Just over 71% of those growers who had seen the problem previously saw it on bought-in plugs, while 29% saw PaMS on their own seed-raised material.
4. Approximately 42% reported that some cultivars were more susceptible to the problem than others. Susceptible cultivars named included the Delta, Matrix and Whiskers series of Pansy. Some growers felt that Viola were more susceptible than Pansy, especially Rocky,

Etain, Tricolor, Beaconsfield, True Blue, Magnifico, Coconut swing, Sorbet (orange & blue) and Penny White. Petunia cv Mirage Red was also reported as being susceptible.

5. Almost 89% of growers were sure that the symptoms had not spread between plants, only 5% felt they may have done, with around 8% being unsure. This information provided further evidence to suggest it is unlikely to be caused by a readily transmissible or mobile agent e.g. pest or pathogen. In contrast, it suggested the problem to be more associated with a non-mobile, possibly an physiological condition, brought about by some factor(s) or 'trigger(s)' on specific nurseries at certain times of year.

6. All plants for growing-on were watered overhead. One respondent felt that plants bought-in from propagators who used sub-irrigation were rarely affected.

7. 98% of those who responded discarded the affected plants, indicating that when it occurred in severe form significant financial losses could occur.

8. The majority of those growers who had raised their own plants provided details of the location, growing media, PGR and pesticide application and also on the use of wetting agents or adjuvants. There was a great deal of variability, particularly in the growing media used. Few pesticides had been applied. Approximately half of the growers had used a range of growth regulators.

9. 55% of those growers who had bought-in plug plants and who had seen PaMS provided similar information. Once again a wide range of growing media had been used from several different suppliers. 29% had not applied any PGR products, whilst 39% had used chlormequat, 32% had used B-nine (daminozide) and 22% had used Bonzi (paclobutrazol). The majority (81%) used a wide range of pesticides during the growing-on period. Just over half did not use any wetting agents, whilst the remainder were evenly spread between Activator 90, Agral, Sprayfast and SW7. It is known that composts are often pre-treated with wetting-agents and growers are not always aware (or in control) of treatments that may have been applied to plants during the plug production phase and this potentially causes a degree of unease in the industry with regard to what exactly they are being exposed to.

10. Seed was bought from a variety of suppliers.

Syngenta	31%	Moles	15%
Ball Colegrave	15%	N. Seeds	8%
Sakata	23%	Jelitto	8%
Flower seeds Direct	8%	Other	38%

(some growers bought from more than 1 supplier).

This suggests that PaMS is not necessarily a seed-house and/or a cultivar or 'series' related issue. However, the information provided was useful in that it ensured that we used cultivars where the problem has been reported previously in our later R&D studies.

11. Where plugs were bought-in these came from a variety of suppliers. This suggests that PaMS is not a specific nursery problem, though it could, of course, be a common factor or factors on each of these nurseries.

12. The timing for symptom expression varied. Some reported seeing the symptoms in young plugs, some at potting-on or within 2-3 weeks of potting-on. Weeks 31-35 (early to late August) were noted, also early autumn for winter crops as being times when PaMS symptoms were more prevalent. The available evidence here would appear to suggest that PaMS is initiated quite early in the production cycle, probably during propagation.

13. Growers provided information regarding which years they had seen plants affected by PaMS. The information was interesting as it appeared to increase in incidence/severity from 2003 onwards. This might potentially coincide with a change in production practice and/or use of new product(s) following launch. One such example would be the introduction and use of azoxystrobin (Amistar) and this approach helped to shape subsequent studies, though it could also potentially coincide with changes in other practices e.g. the use of green waste substrate in growing media.

14. All growers grew-on pansies at low/ambient temperatures, often with ambient light and tried to reduce the humidity as much as possible.

We asked growers to give us their opinion of possible causal factors for PaMS. A summary is reported below.

- Lack of calcium getting to the shoot tip.
- Weak varieties, genetic problem, suggested by colour and variety susceptibility.
- Low irrigation or water shortage at some growth stage.
- Symptoms worse in early sowings e.g. cooler and low light.
- Temperature related, both high and low stated.
- Plant stress.
- Use of PGR's.
- Transplanting dry plugs
- Boron deficiency.
- Herbicide drift damage.
- High light levels.
- Virus infection.
- Chemical damage.

Overall, the information gathered from the questionnaires provided good data on the incidence and severity of PaMS over recent years. The information regarding growing regimes used showed that there was great variability between growers. This may suggest that some factors such as plug supplier, growing media, PGRs, pesticides and the use of wetters may not directly show a pattern, however, a combination of these factors, possibly linked to others which may induce stress may hold the clue to PaMS.

During the BPOA Disease Seminar day (18th Feb 09) Will Healy from Ball Colegrave presented data and information they had collated on the syndrome during recent years. Work had been done in 1999, 2006/7 and a PhD study had been carried out in 2007 by Brian Krug at North Carolina State, University, Raleigh, North Carolina [http://www.lib.ncsu.edu/theses/available/etd-12072007-142004/unrestricted/etd.pdf].

The main findings of this work were:

- No viral, viroid, fungal or bacterial association
- Calcium and Boron uptake was implicated
- Transpiration was a critical factor
- Plant stress exacerbated by other factors e.g. PGRs, fertiliser issues, pesticides could result in PaMS affected plants.

Reproduced from the BPOA Disease Seminar presentation

These results, along with the data generated by our survey provided some very valuable information that helped focus the experimental work more effectively.

Propagation Study

This trial was set up in week 34 (late August) 2009. Germination was generally good across all trays. No leaf distortion was observed on the cotyledons. However, as the true leaves started to emerge symptoms consistent with those of PaMS were observed. The first full assessment was carried out on the 14th September. Further assessments were carried out on the 28th September and the 7th October.

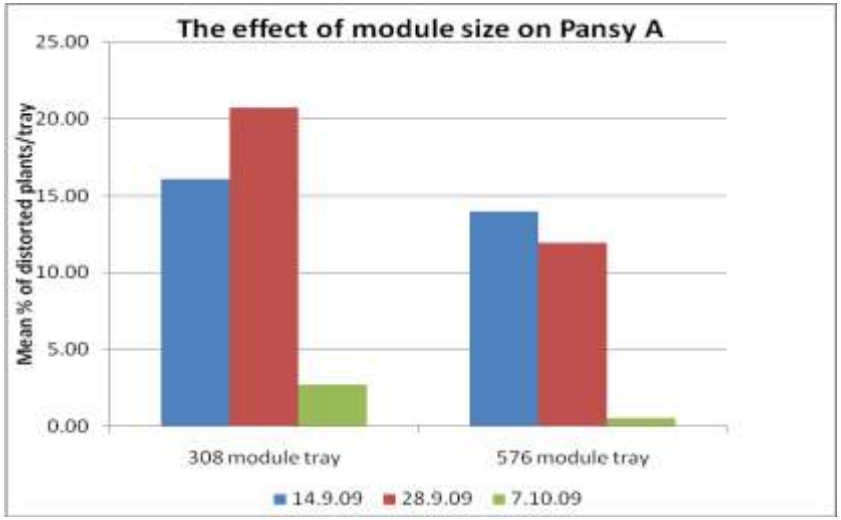
The collected data shows that in general higher numbers of distorted seedlings were seen in pansy cultivar A (Charts 1-4) than in the viola cultivar (Charts 5-8) used in this study (D). Whilst the percentage of distorted viola seedlings was similar to the corresponding pansy plants for each 'treatment' during the 1st assessment, the incidence of PaMS symptoms reduced faster in the viola than the pansy cultivar, suggesting that the viola plants grew away from the effect more quickly or that they were more tolerant to a specific 'trigger factor'. Higher numbers of distorted plants were observed in all the treatment regimes in the earlier assessments. At each assessment date we noted that many of the previously distorted plants had produced 'healthy' sets of leaves and appeared to 'grow-out' of the distortion.

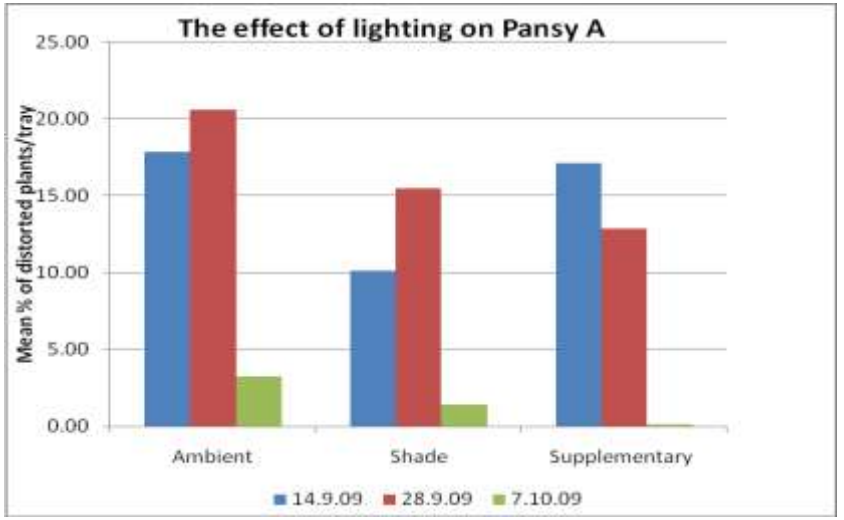
Observations from this trial indicated that:

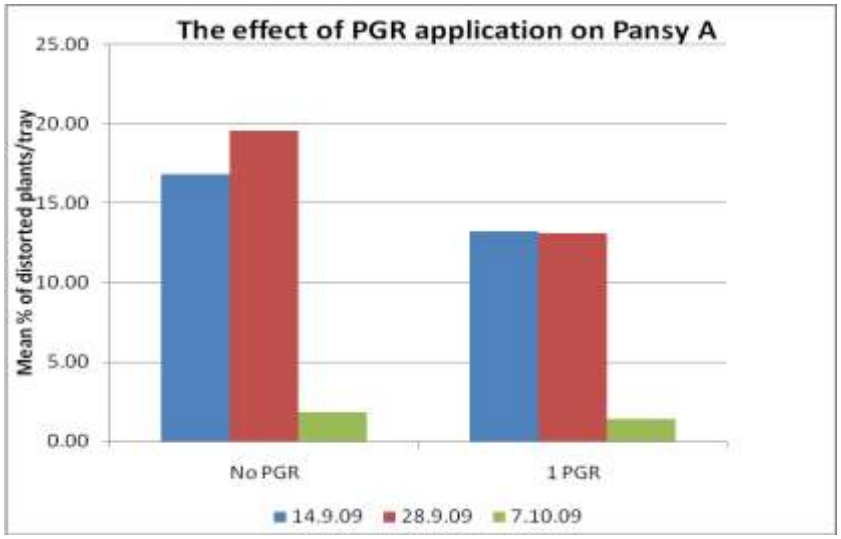
- the slightly larger (308) module trays resulted in higher levels of distorted plants in both cultivars.
- Slightly higher numbers of plants displayed PaMS symptoms under the ambient lighting regime than in either the shaded or supplementary lighting area which both resulted in similar levels of distorted plants.
- The number of distorted plants was higher in the seedlings which did not receive a PGR application. Those receiving PGR were less affected.
- Slightly higher numbers of plants were distorted when a 'normal' irrigation regime was applied compared to irrigation that was somewhat reduced.

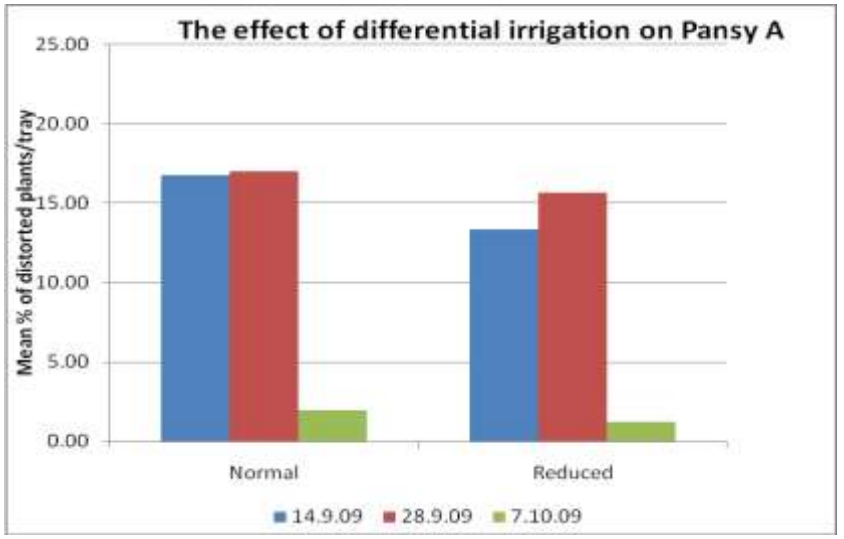
These observations are perhaps contrary what we may have expected from the survey and other comments. We may have expected to see the highest numbers of distorted plants where a small module size was used due to increased risk of 'stress', with reduced irrigation and with a PGR application – also providing, what we assumed, was a more stressful growing scenario. However, the percentage difference in the number of distorted plants within the regimes is quite small (3-5%) and may not be significant. When the various factors are considered in combination e.g. a 308 module grown in shade with a single PGR application etc. the differences are less clear cut or consistent. Therefore, whilst this unreplicated study proved very useful it was not possible to draw firm conclusions from the study.

Charts 1 – 4: Impact of Potential 'trigger' or stress factors on the incidence of Pansy Mottle Syndrome in Pansy A[1].



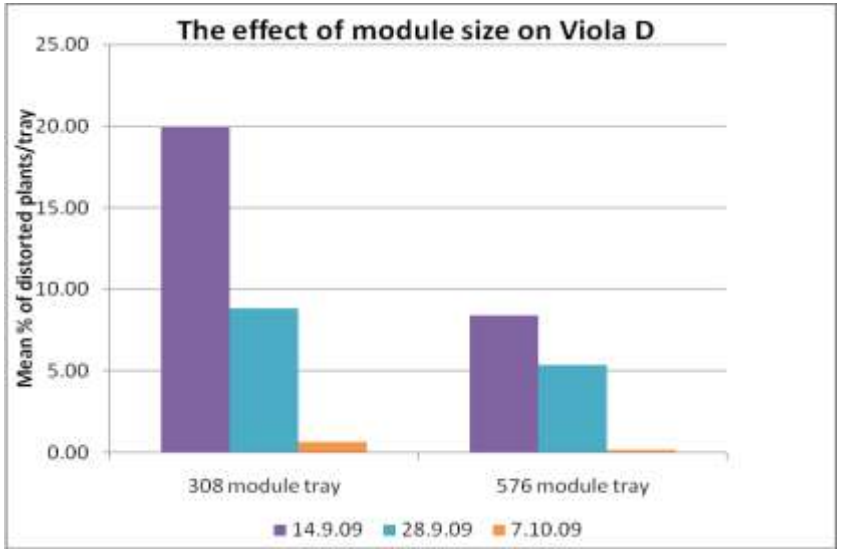


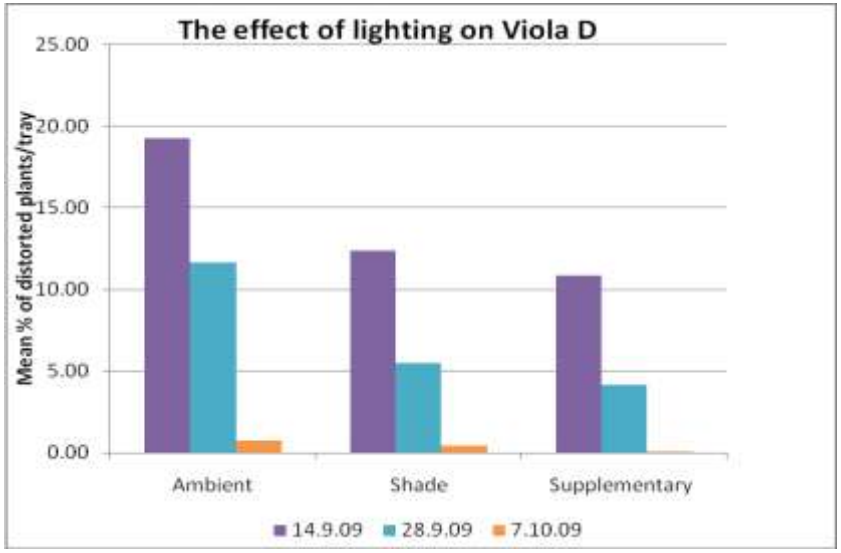


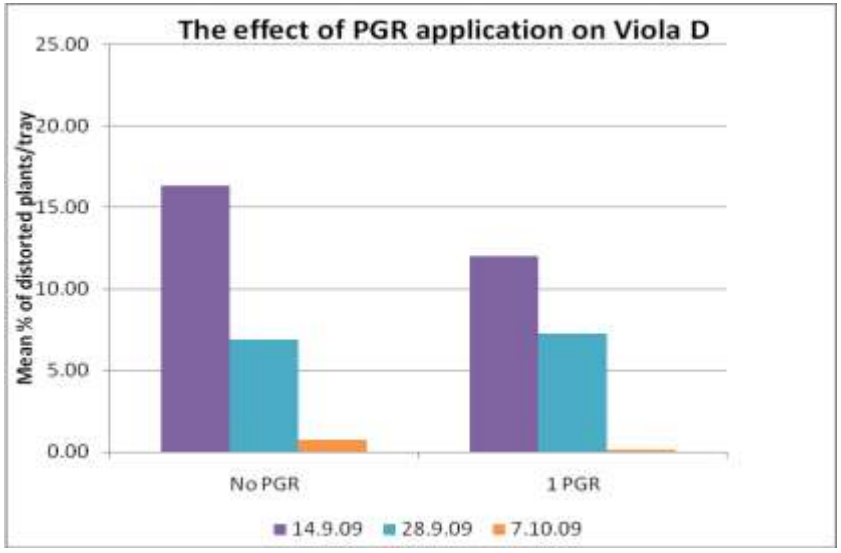


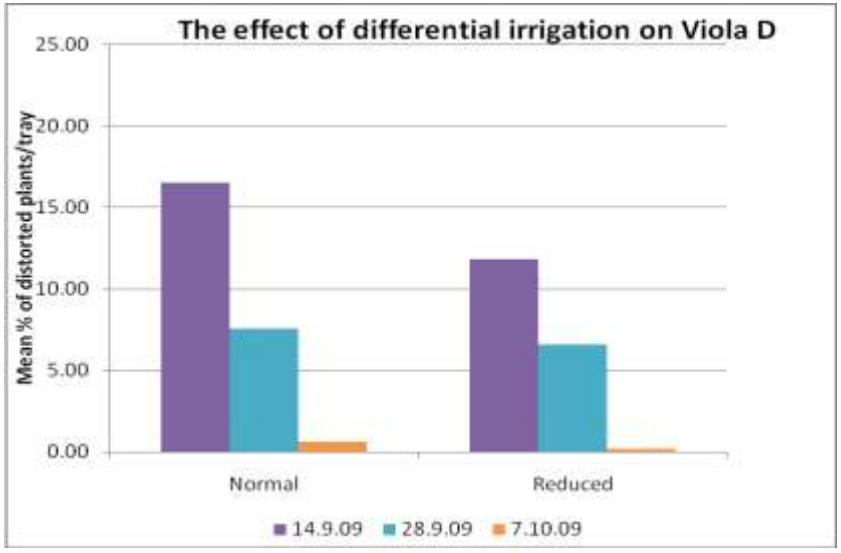
[1] During the assessments carried out on the 14th and the 28th September the number of distorted plants within a 100 seedling central quadrat was counted on each tray. During the final assessment the total number of distorted seedlings/tray was counted and is shown as a percentage of the whole tray.

Charts 5 – 8: Impact of Potential 'trigger' or stress factors on the incidence of Pansy Mottle Syndrome in Viola D. [2]









[2] During the assessments carried out on the 14th and the 28th September the number of distorted plants within a 100 seedling central quadrat was counted on each tray. During the final assessment the total number of distorted seedlings/tray was counted and is shown as a percentage of the whole tray.

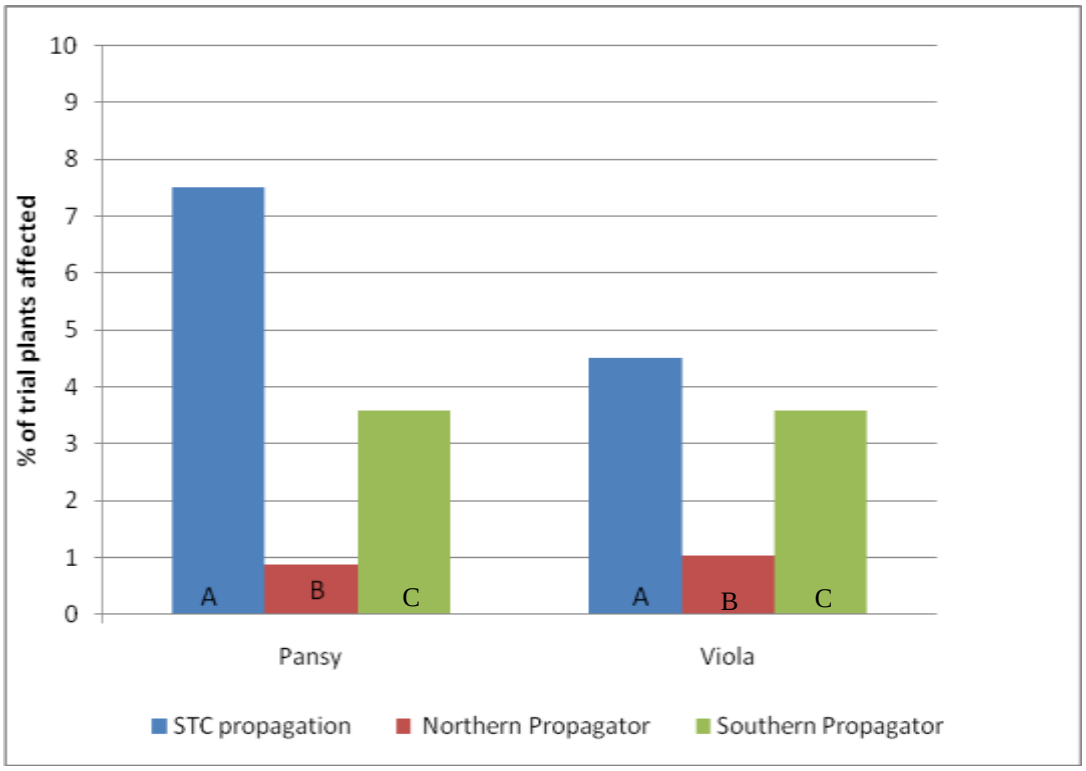
Glasshouse (post-propagation) study

During the potting-on process it was noted that there were quite a number of distorted plants amongst the plants propagated at STC (pansy A and viola A) compared to the plants received from the other propagators. Possible reasons for this could be:

- The cultivars used.
- Differences in the propagation regime (pesticide applications, lighting etc).
- De-selection of distorted plants during 'gapping-up' prior to despatch from commercial propagators.
- The choice of growing media or substrate.

Plants were laid out randomly on each bench; however it was decided to carry out an assessment of the number of distorted plants/variety prior to the application of any chemicals.

Chart 9: Glasshouse study - % plants with PaMS symptoms prior to commencement of treatments (4.9.10)



An interesting correlation can be seen from these results. The number of distorted, blind or blind & distorted plants was high (pansy 7.5 and viola 4.5% of the total number of plants/cultivar) in the STC raised plants. This is higher than that recorded for the commercial propagators (NP - <1% and SP - 3.6%). One of the possible explanations for this result is that differences in the production regime may have caused higher numbers of PaMS affected plugs. Table 4 (Methods & Materials) gives details of the regimes used. It shows that both the commercial propagators (NP and SP) applied pesticides and/or PGR products during the propagation period, whereas STC applied neither. This would suggest that the application of these products is not responsible for

PaMS in young plants. Other cultural/environmental factors were generally similar e.g. temperature, lighting etc.

Irrespective of the early occurrence of PaMS on the young seedlings during propagation the glasshouse study was continued and the chemical applications were carried out with regular monitoring of plants. Further assessments were carried out on the 18.9.09, 1.10.09 and 12.10.09.

Chart 10: Glasshouse Study - the percentage of distorted plants seen in each cultivar during the trial period.

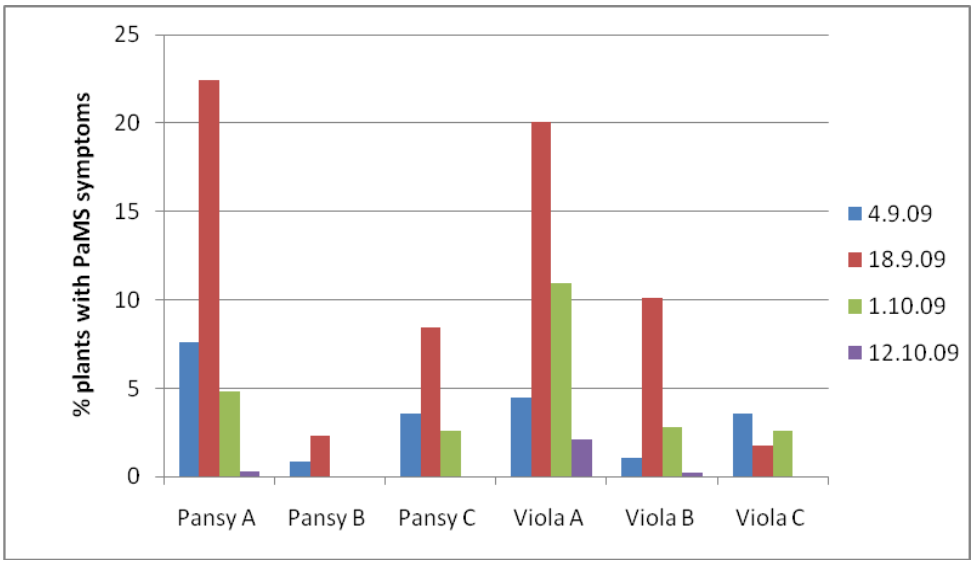


Chart 10 shows the total number of distorted, bleached or bleached and distorted plants observed in the trial on each of the assessment dates. Much higher levels of distortion were seen in the plants propagated at STC (pansy A and Viola A) throughout the trial compared to the other cultivars propagated at commercial nurseries. The chart also clearly shows that in the majority of cases the highest numbers of distorted plants were seen during the 2nd assessment (18.9.09) than during later assessments. This confirms our earlier observations that in many cases the 1st and 2nd pair of true leaves were often quite severely distorted, but that later developing leaves were normal and the plants often grow away from the problem and subsequently developing normally. However, low numbers of distorted plants in pansy A and viola A & B were still evident at the completion of the trial and this also indicates that if the problem is particularly severe it is so damaging that the seedlings are unable to recover.

Plate 3: Images of distorted plants at different stages in the glasshouse trial.

Note normal cotyledons and distorted 1st true leaves

Note normal older leaves on severely distorted plant

Some plants which had been treated with Aliette during the 1st spray application (11.9.09) showed symptoms of spray damage, despite the application of water post-application. We saw very high levels of distortion in these plants, however although the effects were recorded the symptoms were different to PaMS distortion and data from these plots has been omitted from the charts shown. The collected data was analysed statistically using Agricultural Research Manager (ARM) software. The plots which showed a significantly higher level of distortion compared to Treatment 1 and 2, which were effectively untreated control plots, are shown in Table 7 below.

Table 7: Glasshouse Study - Details of trial plots which showed significantly higher levels of distortion at each assessment date

Cultivar	**Treatment No. of plots where significantly higher levels of distortion were recorded**			
	4.9.09 (pre-treatment)	**18.9.09**	**1.10.09**	**12.10.09**
Pansy A (STC)	12(RI)	-	-	-
Pansy B *(NP)	-	-	-	-
Pansy C* (SP	-	-	13, 17(RI)	-
Viola A (STC)	-	-	15, 21, 23, 39, 40	39
Viola B* (NP)	-	23, 25, 35, 36, 37	23, 37	13 (RI)
Viola C* (SP)	-	10, 26, 36	5, 15, 23	-

RI – Plots receiving reduced irrigation

* commercial propagation

The data shown in the table above suggests that there was no obvious pattern to the high levels of distortion seen following application of the treatments e.g. the same set of factors were not consistently causing distortion on the plants, although Trt 23 appears fairly consistently in each of the Viola crops during the assessment on the 1st October. This treatment involved applying Octave with Activator 90 on both spray occasions. No obvious phytotoxicity problems were observed on the plants receiving the Trt 23 regime; however analysis does highlight these plots as having significantly higher levels of distortion than both the untreated controls and other plots receiving treatments. (Full details of the statistical analysis are shown in Appendix 5). The analyses carried out only compare treatments within the same cultivar – not between cultivars. In other studies the azole fungicide prochloraz-Mn (Octave) is known to carry a slight risk of phytotoxicity, usually via root uptake (observations by GM McPherson). It is possible that a low level of crop damage occurred here, though whether it is the same as PaMS is doubtful, especially as the PaMS symptoms were initiated prior to any pesticide application here.

Additional Testing

Samples of distorted plants were collected from various batches of affected plants over the duration of this study. A range of analyses were carried out on the plants to try and determine whether there were any other factors which might be involved (Table 8).

Table 8: Details of additional tests and analyses

Source material	Test details	Result
Affected and un-affected plants from propagation study	Tissue and growing media nutrient analysis.	No significant differences between the samples (Appendix 4) (Pers. Comm. Neil Bragg).*
	GCMS analysis	Graph shown in Appendix 3. 1 unusual peak at RT 15.2. This belongs to methyl salicylate which was approx 5 x greater in the affected sample than the unaffected sample.
	Virus testing ELISA (*Cucumber mosaic virus* and *Cherry leaf roll virus*) sap inoculation and electron microscopy.	ELISA testing negative. Sap inoculation negative (no symptom development). No virus particles seen under EM.
PaMS affected plants received in the STC Plant Clinic	Downy mildew PCR. Molecular DNA analysis.	No downy mildew (*Peronospora violae*) detected in either the affected or 'healthy' plants.

* Full details of analysis are provided in Appendix 4.

With regard to the GCMS analyses, the following comment was provided by the analytical laboratory that carried out the tests.

"Unmethylated 2-hydroxybenzoic acid (detected in the affected plants following GCMS analysis) is a phenolic phytohormone found in plants with roles in plant growth and development, photosynthesis, transpiration, ion uptake and transport. It is also involved in plant defence by systemic acquired resistance (SAR) – a resistance that can be transferred to nearby plants by conversion to the volatile methyl ester (the compound that was detected)."

Report comment by Dr R Macdonald, RPS Mountainheath Laboratories.

This information suggests that in the affected plants the SAR may have been 'switched-on' resulting in the release of methyl salicylate. Interestingly we often observed that in our propagation study individual plants were seldom affected – instead we saw a grouping effect with 1 or 2 very severely affected plants central to the group and slightly less affected plants surrounding them, symptoms declined and disappeared 3-5 plants away from the worse affected plants. These

observations would appear to fit a signal transference scenario, though there are also other potential explanations for such a pattern of symptom distribution. The results of a small-scale laboratory study carried out to investigate the possible effects of methyl-salicylate are reported below (Laboratory study).

In the collected seed trial, higher levels of distortion were seen in the plants grown from the collected seed during early assessments of the plants (Table 9), however the affected plants in both batches gradually started to produce normal foliage in the majority of cases and the total number of plants with very slight distortion at the final assessment date (20.8.10) was very low.

Table 9: Number of plants with distortion symptoms in a comparative growing trial (collected seed Viola A and Viola E).

Cultivar	**No. of distorted plants/cultivar on each assessment date (of a total 345 seed sown)**				
	24.6.10	**30.6.10**	**6.7.10**	**20.7.10**	**20.8.10**
Viola A (collected seed)	23	30	21	16	2
Viola E	5	11	23	27	9

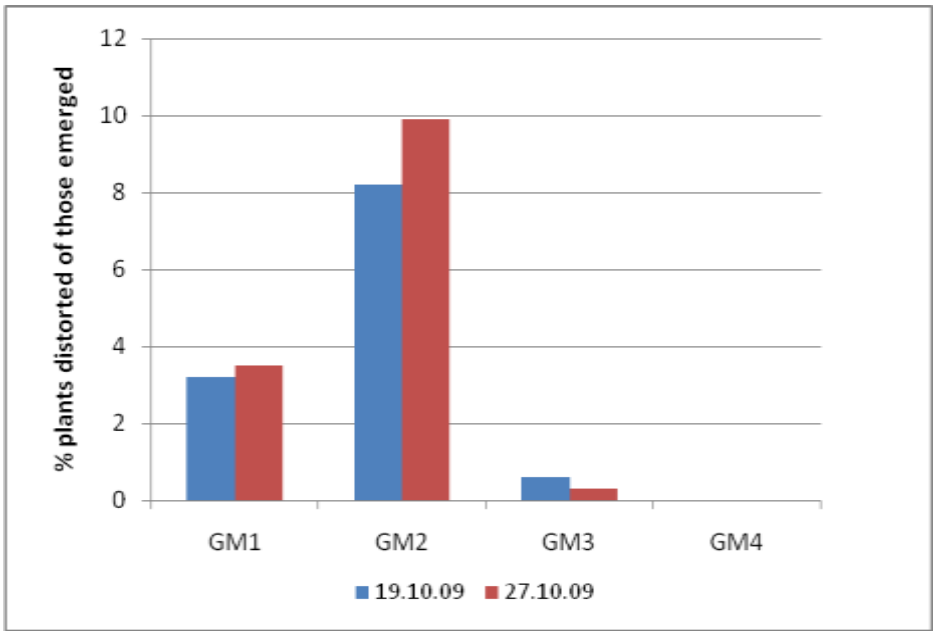
Symptom expression was relatively weak throughout. Those plants with signs of distortion at the final assessment date (20.8.10) showed only mild crinkling or curling of the leaves. All of the plants grown from the collected seed flowered normally. These results tend to suggest that the distortion is not carried genetically in the affected plants though further, more detailed studies would be necessary to confirm this.

None of the other tests carried out on the affected and non-affected plants appear to link the symptoms with clear parameter or cause. However, they have provided information to enable us to rule out some potential causes for PaMS.

Growing media trials

The results from the studies carried out using different substrates are shown in Charts 11-13 below.

Chart 11: The percentage of distorted plants (Pansy A) observed in each growing media on 2 assessment dates.



An initial study (Year 1) used 4 different growing media products. Although the overall percentage of distorted plants was relatively low, slightly higher numbers of seedlings showed signs of distortion when grown in the GM2 substrate, lower levels of distortion were seen in the GM1, with negligible numbers of distorted plants in the remaining 2 substrates (GM3 & GM4). This suggested that there were differential effects on the incidence of PaMS observed when seed was germinated in different substrates. All other propagation factors were the same. The results shown are based on duplicate trays; very similar numbers of distorted plants were seen in each tray.

The later more comprehensive growing media study using both a pansy and viola cultivar resulted in much higher levels of distortion compared to the earlier growing media study the PaMS level in Pansy A seedlings were generally much higher than in the Viola D plants grown in the same media. Once again some of the substrates used appeared more suited to seedling production than others, GM5 and GM8 resulted in quite poor germination and seedling development which may have masked PaMS symptoms.

Chart 12: The percentage of distorted plants (Pansy A) observed in each growing media on 2 assessment dates.

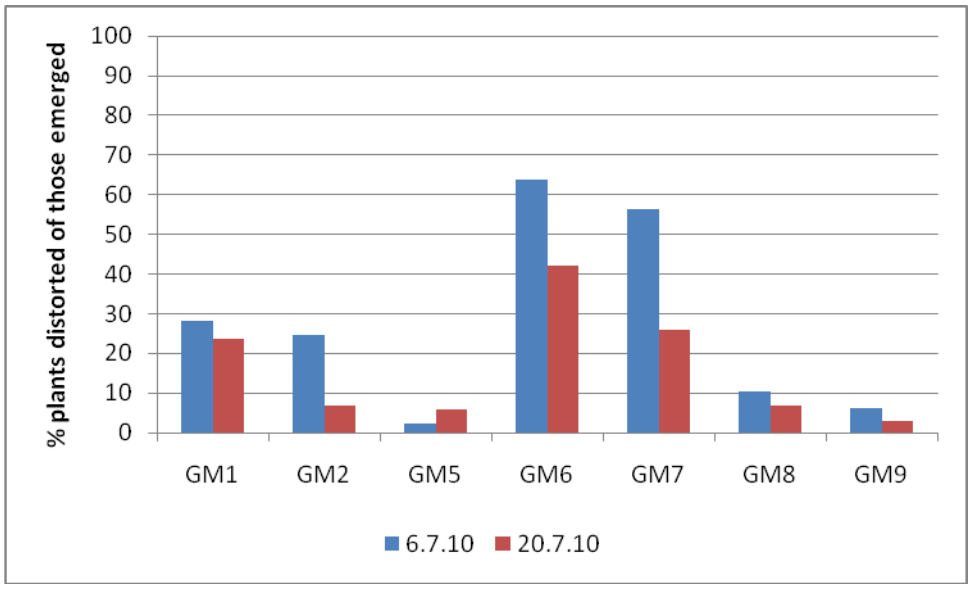
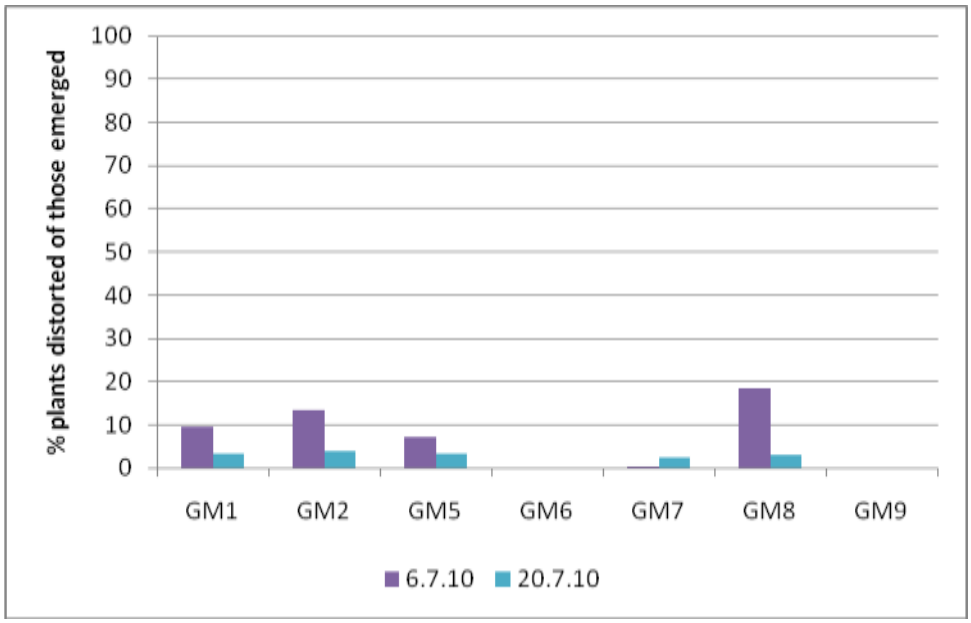


Chart 13: The percentage of distorted plants (Viola D) observed in each growing media on 2 assessment dates.



In this study, the substrates designated GM6 and GM7 gave rise to seedlings with very high levels of distortion in Pansy A. In the GM6 and GM7 trays sown with Viola D the seedlings were all very poor and did not grow normally, finally rotting-off completely making plant counts impossible. In

these substrates it was not clear whether the seedlings were distorted with true PaMS type symptoms prior to dying.

Interestingly the number of distorted plants in Pansy A was higher (2-3 x) in GM1 and GM2 in the year 2 study compared to the earlier growing media study. The reasons for this are not clear at this stage. However, the results are interesting and highlight the need for further work to investigate this potentially important 'trigger' factor in greater detail.

Discussion

The grower questionnaire circulated at the start of this project in 2008 provided us with a wealth of anecdotal evidence from propagators and growers regarding their experiences of PaMS over several years. The information gave us a number of valuable clues regarding possible trigger factors to investigate in our trials. Following a slow-start in 2008, when there seemed to be only a very low incidence of PaMS in the industry; in 2009/2010 we were able to reproduce PaMS symptoms in trials carried out at STC. This was a very important development as it allowed us to investigate various factors to account for the occurrence of PaMS symptoms in pansy & viola.

The propagation and replicated glasshouse (6-pack) trials carried out at STC in 2009 demonstrated that PaMS symptoms were often not displayed in the plants until the 1st and 2nd true leaf pairs of leaves emerged. The fact that we found higher levels of PaMS in the 2 cultivars (pansy and viola A) propagated for the glasshouse trial at STC than in varieties propagated by other commercial propagators is probably quite significant. Possible explanations for this are that the two cultivars of seed used at STC were more susceptible to PaMS than the other commercial cultivars (though it has not been possible to eliminate the possibility that any PaMS affected plants grown by commercial propagators were removed during mechanised 'gapping-up'), or alternatively that a factor, or factors, involved in the STC propagation regime including the substrate used created the 'stress' factor(s) that induced higher levels of PaMS here. Although symptom development did vary following applications of fungicides, adjuvants and PGR products, there was no clear pattern which linked a certain treatment (set of factors) to increased PaMS consistently. It was considered that the increase in numbers of plants with PaMS symptom expression was linked more to the early developmental stage of the plants rather than to the various treatments imposed later.

The propagation study was carried out using seed of Pansy A and Viola D. We saw greater numbers of Pansy A seedlings affected than Viola D. All of the trays in both cultivars had at least a few distorted seedlings – none were entirely symptom-free. We did observe some small

differences in the percentage of distorted plants seen under the different regime imposed. However, we were not able to identify a particular link between symptom development and a particular set of treatment factors that consistently resulted in increased levels of PaMS in this study.

The virus testing carried out on the affected plants was rather limited, due primarily to the limited number of samples of PaMS that occurred commercially during the investigation. Sap transmission and electron microscopy together with specific ELISA testing was used by the virus laboratory selected (Fera), though they were not able to undertake specific ELISA tests for ilavirus as appropriate antisera was not available. However, it was hoped that the more generic sap inoculation and EM tests would serve as a 'catch-all' in this regard. All tests proved negative for the presence of virus. However, further work in this area may still be required before final conclusions regarding the importance of potential virus infection can be drawn.

The tissue and media nutrient analysis provided a useful comparison of affected and apparently 'healthy' plants. Although there were some slight variations between the determinants (see Appendix 4), it was not felt that any differences were severe enough to result in the type of distortion seen (pers comm. Neil Bragg).

We were able to use the *Peronospora violae* PCR test developed as part of the Red Beet project (FV 226 c) to test the distorted seedlings for a systemic infection with downy mildew. The negative result generated has allowed us to eliminate systemic downy mildew infection as a possible cause of PaMS. The fact that the symptoms have also been shown to occur on other hosts also tends to support this result as most downy mildew fungi are host specific and the chances of simultaneous systemic infections on a range of different hosts are very low. In the case of Primula, downy mildew is not known to occur (at least in the UK) and this further supports this result; assuming of course that the symptoms in the different hosts have the same cause.

The GCMS analysis graph was interesting and resulted in the need for an additional small-scale study to investigate the possible implications of the presence of methyl-salicylate. The product is produced by plants in response to stress. It can 'switch-on' plant host defences and send a 'signal' to nearby plants of a potential 'threat'. In our laboratory study we did not find any increase in seedling distortion in the presence of varying concentrations of MS. However, further work may be required to identify greater detail regarding the link and timing of the stress event and MS release. The fact that in our propagation trial we seldom observed single plants which were distorted was of particular interest and perhaps supports the idea of a 'signal' being transmitted to surrounding plants. Alternatively, the adjacent plants may all separately be experiencing the same stress factor resulting in the development of PaMS symptoms.

The growing media trials were instigated following the observation that varying degrees of distorted plants were generated in module trays sourced from different propagators. This would suggest that some difference or differences in the propagation regime at the different sites may be triggering the development of PaMS in some plants. The highest numbers of distorted plants were seen in the STC raised plants. These plants had received no pesticides or growth regulators (PGRs) and they had been watered using mains water – in essence providing what could reasonably be described as a 'best case scenario' for seedlings in terms of minimising possible stress events. The seedlings propagated on our behalf by two commercial propagators had received pesticide applications, but showed much lower levels of distortion on receipt and this may suggest that these potential 'trigger' factors may be considered less important. However, we also considered other possible factors including the different substrates used and the susceptibility of the cultivars being grown.

As a result of these initial observations we subsequently carried out studies using different commercially available growing media. Some of the substrates used resulted in higher levels of distortion using two pansy and viola cultivars. However, it must be noted that not all substrates used in these studies were suited to seedling production and low germination and seedling death in these substrates should not be considered to be due to PaMS symptom development. The observations made during this study, and possible reasons for the distortion seen in seedlings cannot be fully explained at this time, but may warrant further investigation in a separate study.

Conclusions

The project has provided an opportunity to investigate some of the anecdotal evidence relating to Pansy Mottle Syndrome, as well as considering the information already in the public domain. We have been able to investigate a range of potential hypotheses regarding the cause of the severe distortion seen by some growers, and during this study several have either been disproved, or discounted and this helps us to at least start to eliminate some of the causes originally proposed.

A summary of our findings:

- Seedlings of the 3 seed varieties (Pansy A and Viola A & D) propagated at STC consistently developed higher numbers of seedlings affected with PaMS symptoms compared to seedlings propagated by two commercial propagators.

- Symptoms of PaMS were not seen on cotyledons, but were first observed on the 1st and 2nd true leaves. This suggests perhaps that the distortion occurs in response to initial root development (cotyledon formation relying more on the energy reserves in the seed itself).

- The vast majority of plants grew-out of the symptoms, producing healthy leaves – this suggests that whatever 'causes' PaMS it would appear to be transient in nature.

- The application of fungicides, adjuvants and/or pgr's, as tested, do not appear to directly cause PaMS.

- No lighting or irrigation regimes were found that directly and consistently correlated with the development of PaMS in Pansy and Viola seedlings.

- Plug size did appear to have a slight impact on PaMS, with the larger module increasing risk of PaMS. However, this result may relate more to the substrate used rather than the module size *per se*.

- A stress-hormone – methyl-salicylate appears to be associated with PaMS in some way. Though this chemical did not induce PaMS symptom development in specific laboratory studies at STC where a range of concentrations were used, it perhaps signifies that the affected plants are under stress.

- Trials using a range of different growing media provided an indication that differential levels of PaMS incidence and severity can be influenced by using different growing media.

- Virus testing carried out on affected and non-affected plants were all negative, however further testing may be required to completely rule out this possibility.

A look back at some of the factors identified by the grower survey and the review carried out by Nigel Paul may now be useful.

- We have observed PaMS type symptoms in other crops e.g. primula.

- Our findings confirm that the PaMS in found most often in plants early post-germination e.g. at the 1 – 2 true leaf stage.

- We observed higher levels of distortion in one pansy and one viola cultivar than in others used in our trials possibly suggesting a cultivar susceptibility link. Further work is required to investigate this further.

- It is evident that symptoms do not spread from one plant to another, though if a volatile or water soluble agent is responsible this could potentially account for small groups of plants being affected in module trays.

- Our results suggest that pesticides/pgrs/adjuvants used routinely during crop production are not directly implicated in the development of PaMS, but may still add to plant stress. In the studies reported we observed symptom expression prior to the application of any pesticides suggesting that this is not a direct cause of the symptoms.

- The PhD thesis by Brian Krug suggested a link with Boron or Calcium deficiency; however our tissue analyses results do not support this hypothesis.

- Our propagation and glasshouse studies do not support the hypothesis that low irrigation could be a causal factor.

- Tests carried out to-date do not support a link between symptom expression and virus. Reported testing using an ilavirus PCR test in the USA by USDA states:

  "Pansy ilavirus is present in many plants without PMS symptoms, and absent in many plants showing pronounced PMS symptoms. The 'Pansy ilarvirus' therefore is not a unique incitant for PMS but may contribute to the development of PMS which appears to be associated with multiple stresses."

This investigation has succeeded in shedding some light and detail into the determination of the possible cause(s) of PaMS. Tests and experiments carried out by STC and elsewhere support the theory that a stress factor, probably transient in nature, is the most likely cause of symptom expression. However a definitive cause to account for this stress factor is still a little unclear. The experiments conducted as part of this project suggest that a number of previously proposed factors e.g. pesticide applications, lighting, virus, irrigation variations etc. are not necessarily responsible for PaMS development. A possible correlation between increased PaMS and the choice of substrate requires further investigation.

Technology transfer

25th June 2008. Presentation to BPOA – Cathryn Lambourne.
18th February 2009. Presentation to the BPOA Disease seminar – Dr G M McPherson

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Jordan R, Huang Qi, & Hammond J (2007) Annual report on Detection, Identification, and Characterization of new and emerging viral and bacterial diseases of ornamental plants. USDA publication.

Krug BA, (2007), PhD thesis: Physiological and Environmental Factors Affecting Shoot Tissue Boron Concentration of Pansy, Petunia and Gerbera Plugs. North Carolina Institute.

PC 27 Distortion in bedding plants: a survey to determine the incidence and potential losses caused by the disorder 1990

PC 27a Distortion in bedding plants: investigation into the agents causing this disorder 1993

Appendix 1 – Photos of PaMS symptoms

Appendix 2 – Questionnaire

IN STRICT CONFIDENCE

Pansy Mottle Syndrome (PaMS) survey

Section A

1 Contact details

Your name/ nursery					
Address					
Post code					
Telephone		**Mobile**		**Fax**	
E-mail					

Please tick the box if you would be happy to be contacted by STC where further information is required. ☐

Section B

2 Please take a look at the photographs of Pansy on pages 5 to 8 (removed for the purpose of this report appendix) **and indicate if you have seen any with similar symptoms on your nursery (on pansy and other plants)?**

Yes	**No**

If you have answered **NO** to question 2, please return the full form to STC as the information is still relevant and useful.

If you have answered **YES** to question 2, please try to answer all the questions listed below and then return the form as requested.

3 If you answered YES to question 2 please can you indicate in which years you have seen the problem on your nursery?

2008 2007 2006 2005 2004 2003 2002 2001 2000 1999 1998 1997

Please circle the years you think PaMS has been a problem on the nursery

4 Have you also seen similar symptoms on crops other than pansy on the nursery?

Yes	No

If so, please specify which crops …….....

5 Did you notice any particular pattern of distribution of the PaMS affected plants?

Yes	No

If so, can you describe an obvious pattern in the crop ……...

……

6 Were the affected plants raised by you from seed or bought in from a specialist propagator?

Yes	No

Please specify which here …….....

7 If applicable, please give details of the seed supplier for any cultivars/species on

which you saw symptoms

……

8 Were some cultivars affected more than others?

Yes	No

If so, please specify which ones were affected …….....

9 Can you recall at what crop stage the symptoms were first seen?

Yes	No

If so, please specify what week no. or date the problem appeared….....

10 Did the PaMS symptoms spread to affect adjacent plants?

Yes	No

11 Can you provide the following details:-

	Propagation	Growing-on
Propagation location		N/A
Growing medium used		
Plant growth regulator (PGR) # used		
Pesticides applied #		

Wetter and/or adjuvants applied #		

Please include products, rates, no. and timing of applications here if possible

12 Can you provide details of the environmental regime the plants were grown under during propagation and growing-on?

Yes	No

	Propagation	**Growing-on**
Temperature		
Light		
Humidity		

13 Was the watering system applied overhead to the plants?

Yes	No

If so, please specify whether it was by gantry, hose-pipe or other system. If not overhead, please advise what irrigation system was used ……..

……..

14 Were the affected plants discarded?

Yes	No

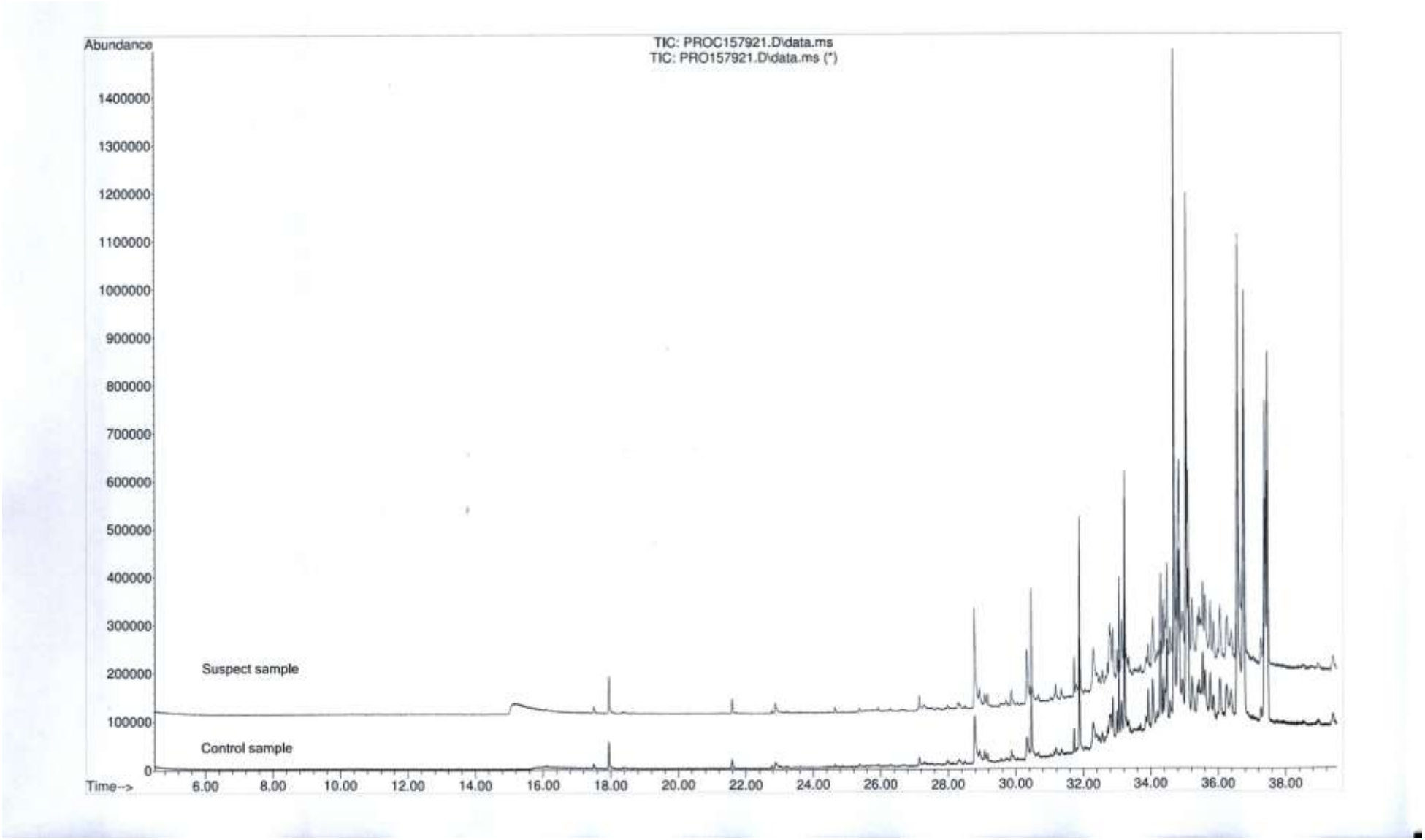
If no, can you comment as to whether the symptoms got worse or disappeared as the crop reached maturity? ……

……..

15 We would value your opinion as to possible cause(s) of PaMS

Please take this opportunity to add your comments below as this could provide helpful in pin-pointing the primary cause of the problem. Also, if you have any photographs of the disorder from your own nursery we would be pleased to receive them. You can either send photo's or digital images on cd via the address below or alternatively email digital images directly to Dr McPherson (email address below)

Appendix 3 GCMS trace showing small peak at Time 15.2 on suspect sample line.



Appendix 4

Tissue Analysis

Determinand	Units	Unaffected	Affected
Nitrogen	%	3.39	3.32
Phosphorus	%	1.32	1.62
Potassium	%	6.96	7.00
Magnesium	%	1.15	1.13
Calcium	%	1.00	1.21
Manganese	mg/kg	337	318
Copper	mg/kg	7.6	9.7
Sodium	%	0.29	0.28
Iron	mg/kg	188.9	260.6
Zinc	mg/kg	69.3	91.3
Molybdenum	mg/kg	0.90	0.51
Boron	mg/kg	26.6	30.5
Sulphur	%	0.29	0.31
N:S ratio		11.7:1	10.8:1

Compost Analysis

Determinand	Units	Unaffected	Affected
pH		6.47	6.42
Conductivity	uS/cm	61	43
Density	kg/m^3	350	350
Dry Matter	%	29.8	26.4
Dry Density	kg/m^3	104.3	92.4
Ammonia-N	mg/l	3.7	3.5
Nitrate-N	mg/l	<0.6	<0.6
Total Soluble N	mg/l	3.7	3.5
Chloride	mg/l	30.8	3.9
Phosphorus	mg/l	16.9	12.7
Potassium	mg/l	17.0	14.5
Magnesium	mg/l	8.2	5.9
Calcium	mg/l	7.4	5.5
Sodium	mg/l	30.0	19.0
Molybdenum	mg/l	0.07	0.07
Manganese	mg/l	<0.06	<0.06
Copper	mg/l	<0.06	<0.06
Iron	mg/l	0.72	0.63
Zinc	mg/l	<0.06	<0.06
Boron	mg/l	<0.06	<0.06
Sulphate	mg/l	38.1	29.0

Appendix 5. Statistical analyses of Glasshouse trial results

TRT	Mean no. distorted plants/treatment on 4.9.09											
	Pansy A		Pansy B		Pansy C		Viola A		Viola B		Viola C	
	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI
1	0.20b	0.20b	0.00a	0.20a	0.00a	0.00a	0.00a	0.20a	0.00a	0.00a	0.80a	0.00a
2	1.00ab	1.00ab	0.00a	0.00a	0.40a	0.00a	0.00a	0.00a	0.20a	0.20a	0.00a	0.40a
3	0.00b	0.40ab	0.00a	0.40a	0.00a	0.00a	0.00a	1.00a	0.20a	0.60a	0.00a	0.80a
4	0.40ab	1.40ab	0.00a	0.40a	0.20a	1.00a	0.20a	0.00a	0.00a	0.00a	0.60a	0.00a
5	0.80ab	0.00b	0.00a	0.00a	0.20a	0.20a	0.40a	1.00a	0.00a	0.20a	0.00a	0.00a
6	1.20ab	0.60ab	0.00a	0.00a	0.00a	0.20a	0.40a	0.60a	0.20a	0.00a	0.20a	0.40a
7	1.00ab	0.00b	0.00a	0.00a	0.20a	0.00a	0.00a	0.20a	0.00a	0.20a	0.20a	0.40a
8	0.40ab	0.80ab	0.00a	0.00a	0.00a	0.80a	0.25a	0.00a	0.20a	0.00a	0.60a	0.20a
9	0.40ab	0.20b	0.00a	0.00a	0.00a	0.00a	0.60a	0.20a	0.00a	0.20a	0.00a	0.00a
10	0.00b	0.80ab	0.00a	0.00a	0.20a	0.00a	0.60a	0.20a	0.00a	0.20a	0.00a	0.80a
11	0.80ab	0.00b	0.00a	0.00a	0.40a	0.20a	0.60a	0.00a	0.00a	0.00a	0.00a	0.40a
12	0.20b	1.80a	0.40a	0.00a	0.20a	0.00a	0.00a	0.50a	0.00a	0.00a	0.20a	0.00a
13	0.00b	0.20b	0.00a	0.00a	0.00a	0.00a	0.40a	0.60a	0.00a	0.00a	0.40a	0.20a
14	0.60ab	0.00b	0.00a	0.00a	0.20a	0.00a	0.20a	0.40a	0.00a	0.20a	0.20a	0.80a
15	0.60ab	0.20b	0.00a	0.40a	0.20a	0.00a	0.20a	0.00a	0.00a	0.00a	0.00a	0.00a
16	0.40ab	0.40ab	0.00a	0.00a	0.00a	0.20a	0.25a	0.20a	0.00a	0.00a	0.20a	0.20a
17	0.40ab	0.20b	0.20a	0.00a	0.20a	0.20a	0.00a	0.60a	0.00a	0.20a	0.20a	0.00a
18	0.40ab	0.20b	0.40a	0.20a	0.40a	0.20a	0.20a	0.60a	0.00a	0.00a	0.00a	0.20a
19	0.40ab	0.20b	0.00a	0.00a	0.20a	0.40a	0.00a	0.40a	0.20a	0.20a	0.40a	0.20a
20	0.00b	0.60ab	0.20a	0.00a	0.40a	0.00a	0.00a	0.25a	0.00a	0.00a	0.20a	0.00a
21	0.60ab	0.20b	0.00a	0.00a	0.20a	0.40a	0.40a	0.40a	0.00a	0.00a	0.20a	0.00a
22	0.00b	0.60ab	0.00a	0.00a	1.00a	0.00a	0.20a	0.20a	0.00a	0.00a	0.20a	0.00a
23	0.20b	0.80ab	0.20a	0.00a	0.20a	0.00a	0.60a	0.20a	0.00a	0.00a	0.40a	0.20a
24	0.20b	0.00b	0.00a	0.00a	0.00a	0.20a	0.20a	0.20a	0.00a	0.00a	0.60a	0.80a
25	0.60ab	1.00ab	0.00a	0.00a	0.00a	0.40a	0.20a	0.60a	0.00a	0.20a	0.00a	0.20a
26	0.40ab	0.60ab	0.00a	0.00a	0.60a	0.20a	0.20a	0.60a	0.00a	0.00a	0.00a	0.00a
27	0.40ab	0.20b	0.00a	0.00a	0.00a	0.40a	0.00a	0.00a	0.00a	0.00a	0.20a	0.00a
28	0.40ab	0.20b	0.00a	0.00a	0.60a	0.00a	0.20a	0.20a	0.00a	0.20a	0.00a	0.00a
29	0.20b	0.60ab	0.00a	0.00a	0.20a	0.60a	0.60a	0.00a	0.20a	0.00a	0.20a	0.00a
30	0.60ab	0.80ab	0.00a	0.00a	0.00a	0.20a	0.20a	0.00a	0.00a	0.00a	0.20a	0.40a
31	0.60ab	0.00b	0.00a	0.00a	0.20a	0.40a	0.20a	0.20a	0.00a	0.20a	0.00a	0.00a
32	0.40ab	0.60ab	0.00a	0.20a	0.20a	0.20a	0.80a	0.00a	0.00a	0.00a	0.40a	0.00a
33	0.40ab	1.20ab	0.20a	0.00a	0.00a	0.40a	0.40a	0.00a	0.00a	0.00a	0.20a	0.40a
34	0.40ab	1.00ab	0.00a	0.20a	0.20a	0.60a	0.40a	0.40a	0.00a	0.00a	0.60a	0.20a
35	0.60ab	0.80ab	0.20a	0.00a	0.40a	0.60a	0.20a	0.00a	0.00a	0.00a	0.00a	0.20a
36	0.20b	0.40ab	0.20a	0.20a	0.20a	0.20a	0.40a	0.25a	0.00a	0.20a	0.40a	0.20a
37	0.40ab	0.20b	0.00a	0.00a	0.20a	1.00a	0.20a	0.20a	0.00a	0.20a	0.20a	0.40a
38	0.60ab	0.00b	0.00a	0.00a	0.00a	0.00a	1.20a	0.20a	0.20a	0.00a	0.60a	0.20a
39	0.60ab	0.20b	0.00a	0.00a	0.20a	0.00a	0.50a	0.00a	0.40a	0.00a	0.00a	0.20a
40	0.80ab	0.20b	0.00a	0.00a	0.20a	0.20a	0.20a	0.00a	0.00a	0.00a	0.00a	0.20a
LSD	0.84		0.27		0.57		0.68		0.31		0.61	
SDev	0.68		0.22		0.46		0.55		0.25		0.50	
CV	147.9		415.1		209.8		196.9		400.5		230.7	

Means followed by the same letter do not significantly differ (P =0.05, Student-Newman-Keuls)

Highlighted cells show values significantly higher than the untreated control.

TRT	Mean no. distorted plants/treatment on 18.9.09											
	Pansy A		Pansy B		Pansy C		Viola A		Viola B		Viola C	
	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI
1	1.0 cde	1.20cde	0.0 b	0.0 b	0.4 abc	0.2 bc	1.2 b-f	0.8 c-f	0.0 f	0.0 f	0.0 b	0.0 b
2	3.0 bcd	1.00cde	0.0 b	0.2 b	1.0 abc	0.0 c	1.2 b-f	0.6 def	0.0 f	0.0 f	0.0 b	0.0 b
3	2.4 b-e	0.80cde	0.0 b	0.4 b	0.0 c	0.4 abc	0.7 c-f	1.2 b-f	1.2 c-f	0.4 def	0.0 b	0.0 b
4	1.4 cde	1.60b-e	0.0 b	0.0 b	0.4 abc	0.4 abc	1.4 b-f	0.6 def	0.0 f	0.0 f	0.2 ab	0.0 b
5	2.0 b-e	1.40cde	0.0 b	0.0 b	0.8 abc	0.4 abc	1.6 a-f	2.0 a-f	1.0 c-f	0.2 ef	0.0 b	0.0 b
6	1.4 cde	0.80cde	0.0 b	0.0 b	0.0 c	1.0 abc	1.6 a-f	0.8 c-f	0.2 ef	0.4 ef	0.0 b	0.2 ab
7	3.4 bc	0.80cde	0.2 b	0.0 b	0.4 abc	1.2 abc	0.8 c-f	0.6 def	0.4 def	0.0 f	0.0 b	0.0 b
8	2.0 b-e	0.60de	0.2 b	0.0 b	0.4 abc	0.2 bc	0.7 c-f	0.4 ef	0.2 ef	0.0 f	0.6 ab	0.0 b
9	1.6 b-e	1.00cde	0.0 b	0.0 b	0.8 abc	0.6 abc	0.8 c-f	1.0 c-f	0.0 f	0.2 ef	0.2 ab	0.0 b
10	1.2 cde	1.20cde	0.0 b	0.0 b	0.8 abc	0.4 abc	1.0 c-f	1.0 c-f	0.0 f	0.2 ef	0.8 a	0.0 b
11	2.8 b-e	1.20cde	0.0 b	0.0 b	0.6 abc	0.0 c	1.0 c-f	0.4 ef	0.4 def	0.0 f	0.2 ab	0.0 b
12	1.8 b-e	1.60b-e	0.0 b	0.0 b	1.0 abc	0.4 abc	1.2 b-f	1.7 a-f	0.2 ef	0.0 f	0.0 b	0.0 b
13	0.6 de	0.80cde	0.0 b	0.0 b	1.2 abc	0.4 abc	1.6 a-f	0.4 ef	0.8 def	2.0 c-f	0.0 b	0.4 ab
14	1.4 cde	0.20e	0.0 b	0.4 b	0.6 abc	0.2 bc	0.4 ef	0.6 def	0.0 f	0.2 ef	0.0 b	0.2 ab
15	0.6 de	0.60de	0.4 b	0.2 b	0.0 c	0.4 abc	1.8 a-f	0.4 ef	0.4 def	0.3 ef	0.0 b	0.0 b
16	1.6 b-e	1.00cde	0.2 b	0.0 b	0.0 c	0.4 abc	1.2 b-f	1.0c-f	1.2 c-f	0.4 def	0.2 ab	0.0 b
17	2.0 b-e	0.40de	0.0 b	0.0 b	0.6 abc	0.6 abc	2.0 a-f	1.6 a-f	1.4 c-f	0.0 f	0.0 b	0.0 b
18	1.4 cde	0.40de	0.0 b	0.0 b	0.8 abc	0.4 abc	0.6 def	1.2 b-f	0.6 def	0.0 f	0.0 b	0.0 b
19	1.2 cde	1.80b-e	0.2 b	0.0 b	0.6 abc	1.2 abc	1.0 c-f	0.8 c-f	0.8 def	0.2 ef	0.2 ab	0.0 b
20	0.4 de	1.20cde	0.0 b	0.0 b	0.4 abc	0.8 abc	1.2 b-f	0.4 ef	0.2 ef	0.0 f	0.0 b	0.0 b
21	1.2 cde	3.00bcd	0.0 b	0.0 b	0.6 abc	0.6 abc	2.8 a-d	1.6 a-f	2.0 c-f	0.0 f	0.0 b	0.0 b
22	0.6 de	0.80cde	0.4 b	0.0 b	1.8 ab	0.4 abc	3.4 ab	1.0 c-f	1.4 c-f	0.0 f	0.0 b	0.0 b
23	2.0 b-e	2.2 b-e	0.0 b	0.2 b	0.6 abc	0.2 bc	3.0 abc	1.6 a-f	2.2 cde	0.2 ef	0.6 ab	0.0 b
24	2.6 b-e	1.8 b-e	0.2 b	0.0 b	0.8 abc	0.6 abc	1.6 a-f	1.8 a-f	1.0 c-f	0.2 ef	0.2 ab	0.0 b
25	1.4 cde	0.8 cde	0.2 b	0.0 b	0.4 abc	0.8 abc	1.6 a-f	1.6 a-f	2.4 cd	0.2 ef	0.0 b	0.0 b
26	2.4 b-e	2.0 b-e	0.0 b	0.0 b	0.6 abc	0.0 c	1.9 a-f	1.4 b-f	0.6 def	0.0 f	0.8 a	0.0 b
27	3.4 bc	4.2 b	0.4 b	1.0 b	0.2 bc	0.8 abc	3.4 ab	1.8 a-f	0.8 def	1.0 c-f	0.2 ab	0.0 b
28	6.0 a	0.6 de	3.0 b	0.2 b	2.0 a	0.6 abc	3.8 a	1.4 b-f	5.4 a	4.2 b	0.0 b	0.0 b
29	1.2 cde	0.4 de	0.2 b	0.0 b	0.0 c	0.8 abc	1.2 b-f	0.9 c-f	0.0 f	0.4 def	0.2 ab	0.0 b
30	1.4 cde	1.0 cde	0.2 b	0.0 b	0.2 bc	0.2 bc	1.2 b-f	0.4 ef	0.8 def	0.0 f	0.0 b	0.0 b
31	1.0 cde	0.8 cde	0.4 b	0.0 b	0.2 bc	1.2 abc	1.6 a-f	1.0 c-f	0.4 def	0.2 ef	0.0 b	0.0 b
32	3.0 bcd	0.4 de	0.0 b	0.2 b	1.2 abc	0.2 bc	1.4 b-f	0.8 c-f	0.0 f	0.0 f	0.0 b	0.0 b
33	1.0 cde	1.6 b-e	0.0 b	0.0 b	1.2 abc	0.8 abc	1.2 b-f	1.0 c-f	0.8 def	0.0 f	0.2 ab	0.0 b
34	0.8 cde	1.8 b-e	0.2 b	0.2 b	0.8 abc	1.2 abc	0.8 c-f	0.8 c-f	1.2 c-f	0.0 f	0.2 ab	0.2 ab
35	1.0 cde	2.2 b-e	0.2 b	0.0 b	0.4 abc	1.0 abc	0.8 c-f	0.6 def	2.2 cde	0.2 ef	0.0 b	0.0 b
36	1.6 b-e	1.0 cde	0.0 b	0.2 b	0.8 abc	0.2 bc	1.8 a-f	0.9 c-f	2.4 cd	0.2 ef	0.8 a	0.2 ab
37	1.4 cde	0.8 cde	0.0 b	0.0 b	1.2 abc	1.4 abc	1.4 b-f	0.6 def	3.0 c	0.2 ef	0.0 b	0.2 ab
38	1.8 b-e	0.8 cde	0.0 b	0.2 b	0.4 abc	0.2 bc	1.6 a-f	0.8 c-f	0.6 def	0.0 f	0.0 b	0.6 ab
39	1.20cde	1.4 cde	0.0 b	0.0 b	0.0 c	0.0 c	2.7 a-e	0.6 def	1.0 c-f	0.0 f	0.4 ab	0.2 ab
40	2.0 b-e	0.8 cde	0.0 b	0.0 b	0.8 abc	0.2 bc	2.2 a-f	0.2 f	1.2 c-f	0.4 def	0.2 ab	0.0 b
LSD	1.44		0.59		0.97		1.26		1.13		0.38	
S.D.	1.17		0.47		0.78		1.02		0.91		0.31	
CV	78.96		364.2		134.59		80.89		145.34		291.78	

Means followed by the same letter do not significantly differ (P =0.05, Student-Newman-Keuls)

Highlighted cells show values significantly higher than the untreated control.

TRT	Mean no. distorted plants/treatment on 1.10.09											
	Pansy A		Pansy B		Pansy C		Viola A		Viola B		Viola C	
	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI
1	2.0 a-e	1.2 b-e	0.0 b	0.0 b	0.0 c	0.0 c	0.8 f-i	0.4 hi	0.0 d	0.0 d	0.0 c	0.0 c
2	2.6 a-e	0.4 de	0.0 b	0.0 b	0.0 c	0.0 c	0.8 f-i	0.0 i	0.4 d	0.0 d	0.0 c	0.0 c
3	1.4 b-e	1.0 cde	0.0 b	0.0 b	0.0 c	0.0 c	0.2 hi	0.6 ghi	0.06d	0.4 d	0.0 c	0.0 c
4	1.0 cde	0.6 de	0.0 b	0.0 b	0.0 c	0.0 c	0.8 f-i	0.0 i	0.0 d	0.0 d	0.0 c	0.0 c
5	1.0 cde	1.0 cde	0.0 b	0.0 b	0.2 c	0.0 c	0.2 hi	0.0 i	0.2 d	0.0 d	1.2 ab	0.0 c
6	1.2 b-e	0.4 de	0.0 b	0.2 a	0.0 c	0.0 c	0.6 ghi	0.2 hi	0.0 d	0.0 d	0.2 bc	0.2 bc
7	2.8 a-d	0.4 de	0.0 b	0.0 b	0.0 c	0.0 c	1.2 f-i	0.0 i	0.2 d	0.0 d	0.2 bc	0.0 c
8	1.6 b-e	0.6 de	0.0 b	0.0 b	0.2 c	0.0 c	0.0 i	0.0 i	0.0 d	0.0 d	0.2 bc	0.0 c
9	1.4 b-e	1.6 b-e	0.0 b	0.0 b	0.0 c	0.0 c	0.6 ghi	0.2 hi	0.0 d	0.0 d	0.2 bc	0.0 c
10	0.6 de	2.0a-e	0.0 b	0.0 b	0.0 c	0.0 c	0.6 ghi	0.0 i	0.0 d	0.0 d	0.2 bc	0.0 c
11	2.0 a-e	1.4 b-e	0.0 b	0.0 b	0.4 c	0.0 c	0.2 hi	0.0 i	0.0 d	0.0 d	1.0 abc	0.0 c
12	1.2 b-e	2.2 a-e	0.0 b	0.0 b	0.2 c	0.0 c	0.4 hi	0.7 ghi	0.0 d	0.0 d	0.0 c	0.0 c
13	0.4 de	1.0 cde	0.0 b	0.0 b	1.6 b	0.0 c	0.4 hi	0.0 i	0.0 d	0.0 d	0.6 abc	0.0 c
14	0.8 cde	1.4 b-e	0.0 b	0.0 b	0.0 c	0.0 c	0.6 ghi	0.2 hi	0.0 d	0.0 d	0.2 bc	0.0 c
15	1.6 b-e	0.6 de	0.0 b	0.0 b	0.0 c	0.0 c	4.6 ab	0.0 i	0.0 d	0.0 d	1.4 a	0.0 c
16	1.8 a-e	1.6 b-e	0.0 b	0.0 b	0.0 c	0.2 c	0.2 hi	0.2 hi	0.4 d	0.4 d	0.0 c	0.0 c
17	1.2 a-e	1.6 b-e	0.0 b	0.0 b	0.4 c	4.6 a	1.2 fi	0.2 hi	0.2 d	0.0 d	0.0 c	0.0 c
18	0.8 cde	0.2 e	0.0 b	0.0 b	0.0 c	0.0 c	0.0 i	0.4 hi	0.0 d	0.0 d	0.0 c	0.0 c
19	0.2 e	1.4 b-e	0.0 b	0.0 b	0.0 c	0.4 c	0.8 fi	0.4 hi	0.0 d	0.0 d	0.4 bc	0.0 c
20	1.2 b-e	1.0 cde	0.0 b	0.0 b	0.4 c	0.0 c	0.6 ghi	0.2 hi	0.0 d	0.0 d	0.2 bc	0.0 c
21	2.0 a-e	2.6 a-e	0.0 b	0.0 b	0.4 c	0.0 c	3.6 a-d	2.2 c-h	0.4 d	0.0 d	0.6 abc	0.0 c
22	0.8 cde	2.4 a-e	0.0 b	0.0 b	0.6 c	0.0 c	2.2 c-h	1.0 f-i	0.8 d	0.0 d	0.2 bc	0.0 c
23	2.8 a-d	3.6 ab	0.0 b	0.0 b	0.2 c	0.2 c	4.8 a	1.6 e-i	1.8 bc	0.4 d	1.4 a	0.0 c
24	2.8 a-d	2.0 a-e	0.0 b	0.0 b	0.2 c	0.0 c	5.0 d-i	1.6 e-i	0.4 d	0.0 d	0.8 abc	0.0 c
25	2.2 a-e	1.0 cde	0.0 b	0.0 b	0.4 c	0.0 c	2.6 c-g	1.2 f-i	1.2 cd	0.0 d	0.0 c	0.0 c
26	3.2 abc	2.6 a-e	0.0 b	0.0 b	0.0 c	0.2 c	2.0 d-i	0.6 ghi	0.0 d	0.2 d	0.2 bc	0.0 c
27	2.4 a-e	2.0 a-e	0.0 b	0.0 b	0.2 c	0.0 c	2.8 c-f	0.2 hi	0.0 d	0.0 d	0.2 bc	0.0 c
28	4.2 a	1.0 cde	0.0 b	0.0 b	0.2 c	0.0 c	3.8 abc	0.6 ghi	0.0 d	3.8 a	0.0 c	0.0 c
29	1.2 b-e	0.6 de	0.0 b	0.0 b	0.0 c	0.0 c	0.4 hi	0.5 hi	0.0 d	0.0 d	0.6 abc	0.0 c
30	0.8 cde	0.6 de	0.0 b	0.0 b	0.0 c	0.2 c	0.4 hi	0.0 i	0.0 d	0.0 d	0.0 c	0.0 c
31	0.8 cde	0.4 de	0.0 b	0.0 b	0.0 c	0.0 c	0.8 f-i	0.4 hi	0.0 d	0.2 d	0.0 c	0.0 c
32	5.4 a-e	0.8 cde	0.0 b	0.0 b	0.2 c	0.0 c	0.2 hi	0.0 i	0.0 d	0.0 d	0.0 c	0.0 c
33	2.2 a-e	0.8 cde	0.0 b	0.0 b	0.0 c	0.0 c	0.6 ghi	0.8 f-i	0.0 d	0.0 d	0.0 c	0.0 c
34	1.4 b-e	1.6 b-e	0.0 b	0.0 b	0.2 c	0.0 c	0.6 ghi	0.0 i	0.2 d	0.0 d	0.0 c	0.0 c
35	2.2 a-e	1.8 a-e	0.0 b	0.0 b	0.6 c	0.0 c	0.2 hi	1.4 hi	0.6 d	0.0 d	0.0 c	0.0 c
36	1.4 b-e	1.4 b-e	0.0 b	0.0 b	0.0 c	0.0 c	1.8 d-i	0.7 ghi	0.4 d	0.0 d	0.6 abc	0.0 c
37	3.2 abc	0.2 e	0.0 b	0.0 b	0.4 c	0.0 c	1.0 f-i	0.8 f-i	2.2 b	0.0 d	1.0 abc	0.0 c
38	2.2 a-e	0.2 e	0.0 b	0.0 b	0.2 c	0.0 c	1.2 f-i	0.4 hi	0.8 d	0.0 d	0.0 c	0.0 c
39	1.8 a-e	0.8 cde	0.0 b	0.0 b	0.2 c	0.0 c	4.9 a	1.8 d-i	0.6 d	0.0 d	0.8 abc	0.0 c
40	1.4 b-e	0.6 de	0.0 b	0.0 b	0.0 c	0.0 c	3.2 b-e	0.4 hi	0.2 d	0.2 d	0.0 c	0.0 c
LSD	1.37		0.06		0.59		1.10		0.68		0.54	
SD	1.10		0.05		0.47		0.89		0.55		0.44	
CV	75.46		1554.12		287.91		97.820		256.7		278.3	

Means followed by the same letter do not significantly differ (P =0.05, Student-Newman-Keuls)

Highlighted cells show values significantly higher than the untreated control.

TRT	Mean no. distorted plants/treatment on 4.9.09											
	Pansy A		Pansy B		Pansy C		Viola A		Viola B		Viola C	
	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI	NI	RI
1	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.4 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
2	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
3	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.2 b	0.0 b	0.0 a	0.0 a
4	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
5	0.2 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
6	0.6 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
7	1.0 a	0.2 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
8	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
9	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
10	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
11	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
12	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.2 bc	0.0 b	0.0 b	0.0 a	0.0 a
13	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.0 c	0.0 b	0.6 a	0.0 a	0.0 a
14	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.2 bc	0.0 b	0.0 b	0.0 a	0.0 a
15	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.4 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
16	0.0 a	0.2 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
17	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
18	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.2 bc	0.0 b	0.0 b	0.0 a	0.0 a
19	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.2 bc	0.0 b	0.0 b	0.0 a	0.0 a
20	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.5 bc	0.0 b	0.0 b	0.0 a	0.0 a
21	0.0 a	0.2 a	0.0 a	0.0 a	0.0 a	0.0 a	0.4 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
22	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
23	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.8 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
24	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
25	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.6 bc	0.2 bc	0.0 b	0.0 b	0.0 a	0.0 a
26	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.6 bc	0.0 b	0.0 b	0.0 a	0.0 a
27	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
28	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	1.0 b	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
29	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
30	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
31	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
32	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
33	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
34	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
35	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.2 bc	0.0 b	0.0 b	0.0 a	0.0 a
36	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
37	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
38	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
39	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.8 a	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
40	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.8 bc	0.0 c	0.0 b	0.0 b	0.0 a	0.0 a
LSD	0.33		0.00		0.00		0.51		0.14		0.00	
SD	0.26		0.00		0.00		0.41		0.11		0.00	
CV	881.72		0.00		0.00		289.95		1120.16		0.00	

Means followed by the same letter do not significantly differ (P =0.05, Student-Newman-Keuls)

Highlighted cells show values significantly higher than the untreated control.

Section 2

A suggested hypothesis for the link between substrate and PaMS symptom development.

One of the potential factors in the development of PaMS affected plants may be the substrate. Following differential development of PaMS in plants raised at STC and those raised by 2 commercial propagators for our glasshouse house study we felt that it was worth considering the possibility that the growing substrate was a point of difference between the raised plants and worth further consideration. During both 2009 and 2010 we carried out a series of experiments and tests using a range of different growing substrates. These studies highlighted a differential response regarding PaMS with some substrates inducing a greater incidence of PaMS than others. This has led us to hypothesise that one or more 'factors' or substances within the growing substrate have the potential to trigger PaMS; the fact that seedlings in general have a tendency to 'grow out' of the symptom supports the view that whatever the cause it is likely to be transient. Possible contributory factors may be the addition of novel wetting agents, 'glues' or other treatments to growing media. Perhaps of greater significance is that in our growing media studies we also used growing media containing peat-replacement products e.g. bark, green-waste. The possible implications of the inclusion of these types of materials, particularly the green-waste, are unknown at present. However, the risk of possible contamination of substrates with residual pesticides and, in particular, with persistent hormone herbicide products cannot be ignored at this stage.

It is possible that contamination with such products sufficient to affect seedlings immediately post-germination (as the initial roots tap into the substrate) and at levels below the limit of determination in conventional laboratory bioassays e.g. phenoxy-acid herbicides[3] may be present. This could potentially account for the sporadic and at times, erratic, appearance of PaMS in crops. It could also help explain why crops can sometimes grow out of the problem and why it affects quite a wide range of species rather than just pansy and viola. Some of these compounds operate in vapour phase and this could potentially account for the distribution or grouping of affected seedlings in module trays.

Further work is required to investigate this hypothesis though, in the meantime, growers and especially propagators need to be wary of using any substrates that may contain (or be

[3] Lawn treatments contain phenoxy-acid herbicides and in the UK at least such material is collected as a component of green waste. Studies elsewhere have demonstrated that the same chemicals can persist through the composting process – the risk from such chemicals is therefore currently not known but theoretically possible.

contaminated) by green waste or similar substrates. In this regard, it worth noting that some of the persistent herbicides are highly water soluble and as stacks of green waste and peat are held outdoors there is also a slight risk of contamination via run-off water.

Although the incidence and severity of symptoms seen by the bedding industry seems to vary from year to year, the problem of PaMS has certainly not gone away. Propagators and growers should therefore ensure that they keep accurate records of substrates used and, where possible, retain a representative sample of the substrate stored under cool (1-3°C) conditions until such time that the crop has been grown successfully. Naturally they should also ensure that bedding plant crops are subjected to as few stresses as possible.

