Project title:	**Investigation into the widespread death of *Clematis armandii* subjects.**
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Contents

Grower Summary

Headline

There is no evidence to suggest that the premature death of C*lematis armandii* is due to a virus.

Background and Expected Deliverables

Several nurseries throughout the UK have experienced a similar problem with *Clematis armandii*; of leaf crinkling followed by sudden death.

Many pest and disease investigations have been carried out over the past four years by growers of *Clematis armandii* but none have been able to identify a conclusive, common cause of failure.

Summary of the Project and Main Conclusions

Samples were collected and tested using ELISA test kits, sap inoculation into indicator plants and electron microscopy on Clematis leaf material.

If plants were found to be infected with a virus, then a strategy to control this problem would be to find stock free of virus for future propagation or to produce virus free stock.

No viral infections were found in the plant samples. Therefore, this is not a likely causal organism of the wilt symptoms experienced by growers. What was observed was that *Clematis armandii* have a much softer internal stem construction than other Clematis varieties. This allows cracking and damage to the internal stem structures, during many standard husbandry operations. This damage ruptures the xylem and phloem, disrupting water supply at that point. Disease also develops at the rupture site.

Action points for Growers

- Be careful when handling *Clematis armandii* so as not to disturb or damage the crown as fungal infections that cause Clematis wilt readily infect this species at this entry point.

- Tie the stem close to the crown and apply extra ties that will reduce the risk of stem twisting. Keep the tying operation more frequent and do not allow the foliage to become tangled.

- Spray a wide spectrum fungicide such as Bravo (chlorothalonil) after trimming, training or husbandry activities, to provide prophylactic cover to reduce incidence of Clematis wilt.

Science section

Introduction

Growers have reported that infection of *Clematis armandii* with 'Clematis wilt' in recent years has consistently been higher than in other Clematis species.

Picture 1
Leaf crinkling on *Clematis armandii*

Growers have sent samples for fungal pathogen testing but there have been no consistent findings. Literature searches were carried out in the CABI, HDC and Web of Science databases to determine if there were any previous records of virus infection in *Clematis* spp. No viruses have been recorded, to date, in Clematis. The biology and cultural recommendations of HNS 52 have enabled clematis growers to produce crops with fewer wilt incidences but the problem still emerges on some species with more regularity. HNS 52 determined that clematis wilt was associated with *Phoma clematidina* and could be kept under control by combinations of fungicide applications and cultural techniques. Other research, by the Tree and Shrub Nursery Experiment Station in Boskoop, Netherlands, Proefstation voor de Boomwekerij te Boskoop (PBB) showed that the disorder can also be associated with *Coniothyrium clematidis-rectae* and *Ascochyta clematidina*.

Due to the virus-like symptoms that were observed in specimens of *Clematis armandii* investigations were performed to see if there was any evidence of either known or unknown viruses in the samples.

Materials and Method

Plant samples were taken from two different clematis growers in Lincolnshire and Somerset. Two plants from each site were collected in the summer of 2002 and the whole of the plant submitted to Central Science Laboratory (CSL), York, for investigation. There, the samples were tested for tomato spotted wilt virus (TSWV), impatiens necrotic spot virus (INSV), tobacco streak virus (TSV) and cucumber mosaic virus (CMV) by ELISA. Leaf samples were also tested by electron microscopy and, plant tissue from young leaves with symptoms was sap inoculated onto indicator plants.

Results and Discussion

Symptoms of 'clematis wilt' were first apparent in 2lt and 3lt pots at 18 months to two years after propagation. Often plants in 2lt and 3lt pots showed quite a large amount of upper leaf crinkle, from which point the plant would progressively deteriorate around the crown which would lead to complete plant death.

Picture 2
Leaf crinkling on *C. armandii*

Foliage symptoms varied, but in most cases a yellow mottle developed over most of the younger leaves. The mottling symptoms were similar to those typical of root damage caused by over watering or root loss. However, in most cases the root system remained

intact, clean and healthy. Cutting back plants to the crown sometimes resulted in re-growth which, when grown on for a few weeks, appeared normal.

In many cases the symptoms were typical of 'Clematis wilt', but were preceded by a distinctive crinkle to these large-leaved species, which is not typical 'Clematis wilt'.

Phoma clemitidina was recovered from the stem base of one plant but no wilt symptoms were present in the first set of tests. Two other plants submitted at the same time had no *Phoma clematidina* present but CSL reported that pathogens which cause a weakening of the plant, rather than death, were found (e.g. *Pestalotiopsis* spp). Some physical damage was apparent at the stem base which may have occurred in transit to the laboratory and had subsequently been colonised by such fungi.

The results of ELISA and sap inoculation testing for TSWV, INSV, TSV and CMV were negative. No viruses were detected by electron microscopy and no viral symptoms developed on indicator plants.

Conclusions

The virus testing done provided no evidence that the causal organism of clematis wilt was a virus.

Previous literature has shown that clematis wilt was associated with *Phoma clematidina* (HNS 52) and can also be associated with *Coniothyrium clematidis-rectae* and *Ascochyta clematidina* (Proefstation voor de Boomkwekerij te Boskoop and Van de Graaf, P). No leaf crinkle symptoms are associated with these fungal infections.

The leaf crinkle would appear to be the result of physiological response from the plant. *Clematis* spp. often react to extreme temperature differences in the spring between night and day and applications of Flexidor (isoxaben). No applications of Flexidor had been made on any of the test plants or on nearby plants.

It would seem from knowledge and experience of *C. armandii* that the wilting effect is a response of the plant to a fungal infection at or near the crown or rupturing of the vascular system.

Clematis armandii stems are much more fragile than other clematis species, so these husbandry practices could easily result in aerial portion wilt. Plants subject to physical movement even by air movement can be injured. This can be demonstrated quite easily by lightly bending a stem until a "crack" is heard. A physical observation will show that no outward appearance of damage is visible. If you leave this cracked stem on the plant it will show wilt symptoms in a few days near the cracked point.

There are many operations on the plant that can render it vulnerable to disease and injury:

- Frost can cause stem splitting, leaving an open wound vulnerable to fungal infection until callusing has sealed it.
- During the potting process the unsupported stems of the plant are often quite floppy and flexible, which results in bending and twisting movements at the crown.
- The husbandry work of twisting, training and tying of the plant can result in damaged or severed stems. This is particularly evident when training is carried out infrequently and the foliage of plants is all inter-twined together. Frequent training should be carried out on this plant subject to avoid foliage tangle and minimal crown disturbance.
- The root system is static in the container but the aerial parts of the plant are quite mobile, even if secured to a framework or canes. Any husbandry work carried out on the aerial portions of the plant subjects the crown of the plant to twisting and bending.

In conclusion, leaf crinkle may be a result of internal damage or other physical agents, but not virus infection. The increased incidence of clematis wilt on C. *armandii* would appear to be the result of internal stem cracking during crop handling.

Future Work

Until now the control of clematis wilt has concentrated on the likely pathogens that cause the disorder. The fact that different species of the genera have varying degrees of physical damage susceptibility suggests that a closer look at the physiological processes taking place within the plant may broaden the understanding of the disorder and lead to improved control.

References

Van de Graaf, P. (1999). **Clematis: aspects of the biology and control of clematis wilt.** Horticultural Development Council final report for HNS 52

Van de Graaf, P., Joseph, M. E., Chartier-Hollis, J. M. and O'Neill, T. M. (2002) **Prepenetration stages in infection of clematis by *Phoma clematidina*.** Plant Pathology 51:331-337

Van de Graaf, P., O'Neill, T. M., Chartier-Hollis, J. M., Joseph, M. E. (2002) **Susceptibility of clematis varieties and species to stem infection by *Phoma clematidina* as an indicator for resistance to wilt.** European Journal of Plant Pathology vol. 107 (6): p.607-614

Netherlands, Proefstation voor de Boomkwekerij te Boskoop (1975)
Yearbook for 1975 of the Tree and Shrub Nursery Experiment Station at Boskoop.

Van Steekelenburg, N. A. M. (1972) **Wilt disease of clematis.** Groen vol. 28