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Susceptibility of clematis varieties and species to stem infection by *Phoma clematidina* as an indicator for resistance to wilt

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Abstract

The susceptibility to stem rot and wilt caused by *Phoma clematidina* was tested for a range of clematis varieties and species widely grown in Europe, and compared with their susceptibility to wilt in practice as judged by amateur and commercial growers in two postal surveys. Most small flowering clematis species did not die back when unwounded stems were inoculated with spores of *P. clematidina*, and were also reported in the surveys as being very resistant to wilt. In contrast, large flowering hybrids were found to be highly susceptible both to stem infection by *P. clematidina* and to wilt in practice according to growers. A significant positive linear relationship existed between the susceptibility scores for disease in the stem inoculation test and those for wilt from the surveys. Fungal isolations were made from the stems of naturally wilted clematis plants from British nurseries and private gardens. Unlike the many other fungal species isolated, *P. clematidina* occurred very frequently, especially in stems of large flowering varieties. Together with the results from the grower surveys and stem inoculation trial, this indicates that this fungus is a regular cause of clematis wilt. The stem inoculation test with *P. clematidina* described in this study would be useful for European breeders and growers to determine the susceptibility of new clematis varieties to wilt before they are marketed.

Introduction

Soon after the start of the large scale cultivation of clematis (*Ranunculaceae*) in the 19th Century, reports began to appear in American and European journals about a widespread, destructive disease, which caused huge losses and subsequently discouraged the breeding of new varieties of this ornamental climbing plant. The symptoms of the disorder, which became known as clematis wilt, were usually described as stem rot, often at soil level, and the wilting of above ground parts of the plant (Arthur, 1885; Klebahn, 1891; Foussat, 1896; Jackman, 1900). Clematis wilt occurs wherever susceptible plants are grown and has been reported throughout Europe (Foussat, 1896; Bücher-Niederwalluf, 1933; Ebben and Last, 1966; Schumann and Bemann, 1972; Riekstins, 1989; van Kuik and Brachter, 1997; Margot, 1998). There is a wide range of pests, pathogens and physiological factors, which can cause wilting symptoms in clematis (Howells, 1993; van de Graaf, 1997), and close examination of diseased plants is essential to make a proper diagnosis. The disease was first associated with a fungus, a *Phoma* species, by Arthur (1885), but it was the American botanist Gloyer (1915), who established Koch's postulates for *Ascochyta clematidina* as a cause of leaf spot, stem rot and wilt in clematis. The fungus was later re-classified as *Phoma clematidina* (Thüm.) Boerema (Boerema and Dorenbosch, 1979). Clematis wilt can be the result of rot following direct penetration of stems or roots by

P. clematidina (Ebben and Last, 1966; van de Graaf, 1999). In addition, the fungus may enter the clematis stem via the petiole after colonising the leaf by the formation of dark coloured lesions (Gloyer, 1915; Smith, 1987).

The experience of most growers is that the large flowering clematis hybrids are especially prone to wilt, while most small flowering clematis species and their varieties generally remain healthy (Foussat, 1896; Morel, 1903). Within the large flowering group, plants that flower early in the season appear to succumb more readily to wilt than late flowering varieties (Howells, 1994). Many growers in Europe have been reluctant to accept *P. clematidina* as a common cause of clematis wilt, even though Gloyer's (1915) results have been confirmed by Ebben and Last (1966) and Smith (1987). Pathogenicity studies by these authors have suggested that the varietal differences in wilt susceptibility could be related to variations in susceptibility to infection by *P. clematidina*. However, these investigations were mainly based on leaf spot symptoms, not on stem rot or wilt, while the number of clematis varieties tested was very limited.

The present studies supplement previous work by comparing the susceptibility of a range of clematis varieties and species to stem rot and wilt caused by *P. clematidina* with their susceptibility to wilt in practice. The aim was to develop a rapid test which accurately predicts wilt susceptibility, which would be useful to clematis breeders. The hypothesis is that the reaction to stem infection by *P. clematidina* in a stem inoculation test differs between clematis varieties and species, and that these differences reflect the differences in susceptibility of the same varieties and species to clematis wilt in practice. This would indicate that the fungus is a regular cause of clematis wilt. To test this further, the occurrence of *P. clematidina* in the stems of naturally wilted plants of different varieties and species was investigated. If the hypothesis is true, inoculation of clematis stems with *P. clematidina* would be a useful technique for breeders and growers throughout Europe to test the susceptibility of their clematis varieties and species to wilt.

Materials and methods

Grower surveys

Two postal surveys on clematis wilt, one for amateur and one for commercial growers, were designed. Both surveys contained a list of the most commonly grown large and small flowered clematis varieties and species (see Table 1), which growers were asked to judge for susceptibility to wilt, on the basis of their own practical experience, on a scale of one (very resistant) to five (very susceptible). The questionnaire for amateur growers was distributed by the British Clematis Society (BCS) to 790 private members in the UK in December 1996, while the questionnaire for commercial growers was sent to 207 British clematis nurseries in January 1997.

Fungal isolations

Wilted clematis plants of different varieties were collected from British clematis nurseries and private gardens in the period 1996–1999. In total, 95 wilted clematis plants belonging to 62 different varieties and species were examined. Pieces of diseased stems, especially from parts showing discoloration or lesions, were surface sterilised in 70% ethanol for 10 min. The plant parts were then washed in sterile distilled water (SDW) for the same duration, air dried on filter paper, and plated out on V8-agar (Campbell's V8®-juice in distilled water 1 : 4 v/v, pH adjusted to 6.0 with 1.0 M sodium hydroxide, 20 g Oxoid® agar no. 3 added per litre, and autoclaved at 6.8 kg for 15 min at 115 °C) with added streptomycin (130 $\mu g\,ml^{-1}$) and penicillin (300 $\mu g\,ml^{-1}$) (VSP-agar). Plates were sealed with laboratory film and incubated in the dark at 20 °C.

After several weeks, fungi were transferred individually to fresh V8-agar plates and identified. *P. clematidina* was distinguished from other *Phoma* species by observing characteristics such as growth rate (25–40 mm at 22 °C), conidial shape and size (3.5–9 × 2–3.5 μm), the formation of pycnidia (110–120 μm diam) exuding a salmon coloured spore mass (cirrhus), and, most importantly, the presence and shape of chlamydospores (unicellular diameter 8–10 μm or irregular multicellular 3–50 × 12–25 μm) (see Figure 1(a)) (Boerema, 1993).

Stem inoculation

Unwounded, non-woody stems of healthy clematis plants of 11 different varieties and species, which were also listed in the grower surveys (see Table 2), were inoculated with a mix of conidia in SDW of six different isolates of *P. clematidina*, previously recovered

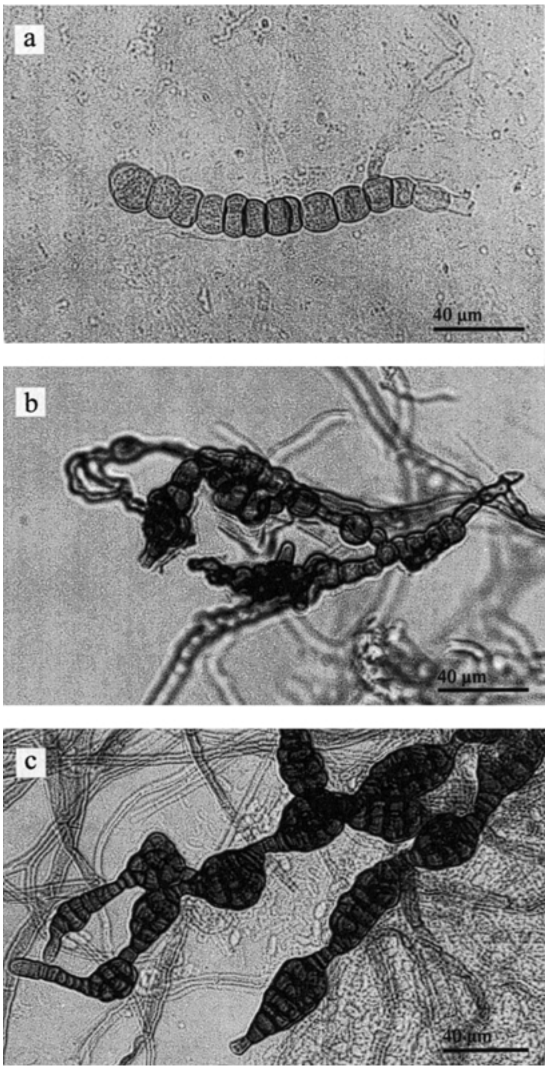

Figure 1. Morphology of chlamydospores formed by three *Phoma* species isolated from wilted clematis stems: (a) *Phoma clematidina*; (b) *Phoma pomorum* var. *calorpreferens*; (c) *Phoma glomerata*.

from wilted clematis plants. A piece of filter paper was moistened with 0.2 ml of the spore solution (5×10^6 spores ml^{-1}), placed against an internodal section of the stem, wrapped in moist tissue paper, and sealed with laboratory film. Controls were inoculated with filter paper moistened with SDW. Only one stem per plant was used and there were ten replicates per treatment. Plants were checked daily for wilting symptoms for 60 days. Wilted stems were examined for stem rot symptoms, and some were plated out on VSP-agar to check for the presence of *P. clematidina*.

All plants used were supplied by Guernsey Clematis Nursery Ltd., six to eight months old at the start of the experiment, and grown in a mix of peat and bark (3 : 1, v/v) in 2 l plastic pots; these were kept in a ventilated polytunnel in randomised blocks, shaded by green netting, and watered daily at the stem base by hand.

Results were statistically tested using one-way ANOVA and Duncan's Multiple Range tests (time before wilting), χ^2-tests (percentage wilted plants) or *t*-tests (linear regression) ($\alpha = 0.05$).

Results

Grower surveys

The response to the clematis surveys was 22% and 47% for amateur and commercial growers respectively. According to the respondents, clematis wilt occurred in 50% of the nurseries and 81% of the private gardens involved in the period 1991–1996. Amateur and commercial growers largely agreed on the susceptibility of the different clematis varieties and species listed in the questionnaire (see Table 1). Overall, the small flowering species were judged to be much more resistant (average score 1.2) than the large flowering varieties (average score 2.1). Within the group of large flowering varieties, there was also a clear difference in average score between the group of early flowering varieties (average score 2.2) and the group of late flowering varieties (average score 1.8). However, as Figure 2 shows, the variation in susceptibility within the early flowering group was large and there was some overlap with the late flowering group. In both surveys, cv. Vyvyan Pennell was judged to be by far the most wilt susceptible of all varieties, while cvs Ville de Lyon and Hagley Hybrid were thought to be the most resistant large flowering hybrids listed.

Fungal isolations

Of the 95 wilted clematis plants examined, 23 plants, belonging to 20 different varieties and species, yielded *P. clematidina* (24%). The percentage recovery was highest for the group of late large flowering varieties (55%) and much lower for the early large flowering (21%) and small flowering (19%) groups. In total, *P. clematidina* was isolated from 12 out of 70 wilted

Table 1. Clematis varieties (nos 1–17) and species (nos 18–24) listed in the grower surveys and their average wilt susceptibility score (on a scale of 1 = very resistant to 5 = very susceptible) according to commercial and amateur growers

No.*	Clematis variety or species	Commercial grower survey		Amateur grower survey	
		Average score	Number of respondents	Average score	Number of respondents
1	*C*. 'Vyvyan Pennell'	2.5	37	3.0	71
2	*C*. 'Mrs N Thompson'	2.4	26	2.5	47
3	*C*. 'Duchess of Edinburgh'	2.3	34	2.5	57
4	*C*. 'Henryi'	2.3	26	2.4	59
5	*C*. 'William Kennett'	2.2	30	2.5	47
6	*C*. 'Marie Boisselot'/ 'Madame le Coultre'	2.2	37	2.3	65
7	*C*. 'Lady Betty Balfour'	2.2	32	2.2	39
8	*C*. 'Barbara Jackman'	2.3	30	1.7	45
9	*C*. 'Jackmanii'	2.3	42	1.7	99
10	*C*. 'Bees Jubilee'	2.2	38	1.9	34
11	*C*. 'Elsa Späth'/'Xerxes'	2.0	36	2.1	52
12	*C*. 'Ernest Markham'	2.0	45	2.0	75
13	*C*. 'Miss Bateman'	1.9	34	2.0	69
14	*C*. 'Nelly Moser'	2.1	46	1.7	73
15	*C*. 'Comtesse de Bouchaud'	2.0	41	1.7	89
16	*C*. 'Ville de Lyon'	1.9	45	1.6	87
17	*C*. 'Hagley Hybrid'	1.8	43	1.5	107
18	*C. integrifolia*	1.5	19	1.1	71
19	*C. orientalis*	1.4	34	1.2	72
20	*C. viticella*	1.4	42	1.2	131
21	*C. alpina*	1.3	43	1.1	105
22	*C. macropetala*	1.3	46	1.1	110
23	*C. tangutica*	1.1	44	1.3	85
24	*C. montana*	1.2	60	1.1	123

*The numbers in this table refer to Figure 2.

plants from nurseries (17%) and 11 out of 25 wilted plants from private gardens (44%).

Apart from *P. clematidina*, several other *Phoma* species were found in wilted clematis stems: *P. exigua* var. *exigua*, *P. glomerata*, *P. macrostoma* var. *macrostoma*, *P. nebulosa*, *P. pomorum* var. *calorpreferens*, and *P. viburnicola*. However, only *P. exigua* var. *exigua* was isolated from more than one plant. In culture, colonies of *P. glomerata* and *P. pomorum* var. *calorpreferens* looked morphologically very similar to those of *P. clematidina*, but the species could be easily distinguished by the shape of their chlamydospores (see Figure 1). Of all fungal species isolated from the stems of the wilted plants, *P. clematidina* was the species occurring most frequently. Only the widespread *Botrytis cinerea* was more common. *P. exigua* var. *exigua* and *B. cinerea* were tested on their pathogenicity towards clematis stems in the same manner as *P. clematidina*, but did not cause any stem rot or wilt.

Stem inoculation

In the stem inoculation trial, there was a significant difference between clematis varieties and species in both the percentage of wilted plants after 60 days and the average time interval before wilting (see Table 2). The first signs of wilt, from the point of inoculation upwards, were observed after 12 days in cvs Miss Bateman and Lady Betty Balfour. Many more plants of different varieties and species followed, and after 60 days, half of all inoculated plants had wilted. As Table 2 shows, only three of the eleven varieties and species tested did not show any signs of wilt. These were all small flowering species, namely *C. alpina*, *C. montana* and *C. orientalis*. There was a significant effect of clematis group on both the percentage of wilted plants after 60 days and the average time interval before wilting. However, differences between varieties within each group were often greater

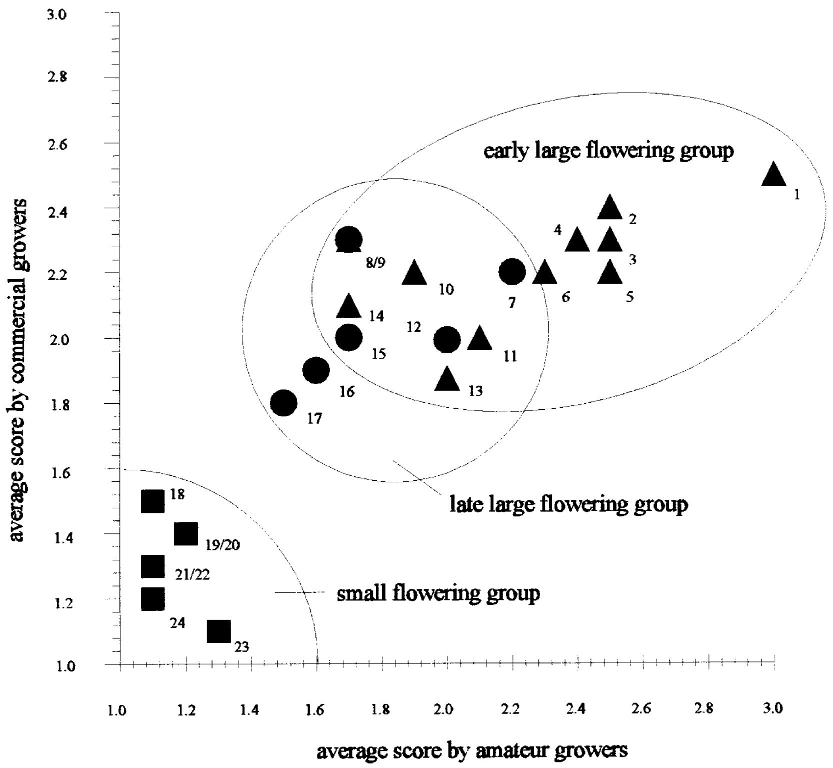

Figure 2. Average wilt susceptibility scores (on a scale of 1 = very resistant to 5 = very susceptible) for three different clematis flowering groups according to commercial and amateur grower surveys. Each number represents a different clematis variety or species listed in Table 1.

than those between the three flowering groups (see Table 2).

Disease progression was especially quick in cv. Ernest Markham and in *C. viticella*. The most resistant large flowering varieties tested were both late flowering, namely cvs Ville de Lyon and Comtesse de Bouchaud. Most plants that wilted (65%) developed symptoms between 20 and 40 days after inoculation.

Wilted stems which were cut open showed a distinctive, black discoloration of the vascular tissues. Usually, the infection had spread over the whole length of the stem covered by the moist tissue paper (±8 cm) and sometimes even beyond, up- and downwards from the point of inoculation. The bark had often started to separate from the inner tissues and white mycelium could be seen in the spaces thus created. Pycnidia were regularly observed on the outside of both the vascular tissues and the bark. *P. clematidina* was recovered from most pieces of rotted stem plated out on agar. None of the control plants inoculated with water without spores showed stem rot and wilt symptoms.

To investigate whether the susceptibility to wilt in practice was correlated to susceptibility to stem infection by *P. clematidina*, the varietal susceptibility to stem infection by the fungus was expressed as a susceptibility score S:

$$S = \frac{\text{(fraction of wilted plants after 60 days)} \times 100}{\text{(the average number of days before wilting after 60 days)}} \times 10$$

The higher the score, the more susceptible the variety or species. For varieties or species that did not wilt the score was taken as zero.

In Figure 3, S is set out against the average susceptibility score from the commercial grower survey as discussed above. Most results indicated a positive linear relationship between the two scores. Only two varieties/species did not fit a linear relationship: cvs Ernest Markham and *C. viticella*. These two clematis types were less prone to wilt in practice than

Table 2. Effect of clematis variety or species on percentage wilted plants and average time interval before wilting after inoculation of unwounded internodal stems with conidia of *Phoma clematidina*, 60 days after inoculation ($n = 10$)

Clematis group and variety/species	Average time before wilting (days) ± S.E.*	Percentage wilted plants*
Early large flowering group		
C. 'Henryi'	36.9 ± 3.5 a	70 abc
C. 'Miss Bateman'	33.6 ± 8.3 a	50 ac
C. 'Vyvyan Pennell'	36.4 ± 3.4 a	90 ab
Group average	35.9 ± 2.6	70
Late large flowering group		
C. 'Comtesse de Bouchaud'	41.6 ± 1.6 b	50 ac
C. 'Ernest Markham'	26.9 ± 3.9 c	100 b
C. 'Lady Betty Balfour'	26.0 ± 3.0 c	70 abc
C. 'Ville de Lyon'	21.5 ± 0.5 d	20 cd
Group average	29.3 ± 2.3	60
Small flowering group		
C. alpina	>60	0 d
C. montana	>60	0 d
C. orientalis	>60	0 d
C. viticella	26.2 ± 2.4 c	90 ab

*Results in the same column not sharing a common letter are significantly different ($\alpha = 0.05$).

could be expected on basis of their susceptibility to *P. clematidina* in the stem inoculation test. When the results from these two varieties/species were ignored, a significant linear relationship was found between the susceptibility of clematis varieties and species to *P. clematidina* in the trial and their susceptibility to wilt in practice according to the grower survey (see Figure 3). The regression line for the scores in the amateur grower survey ($y = 1.13 + 0.057x$; $R^2 = 86.0\%$), which was also tested, did not differ greatly from the line for the commercial grower survey.

Discussion

The results of the amateur and commercial grower surveys showed that clematis wilt is a widespread problem in both nurseries and private gardens. The difference in wilt incidence reported by amateur (81%) and commercial (50%) growers reflects an important aspect of this disease in that it often occurs after a customer has bought a healthy looking plant from a nursery and planted it in the garden. Clematis wilt regularly occurs when plants are subjected to a change in growing conditions (Bücher-Niederwalluf, 1933; Blok, 1965). The transfer from the protected environment of the nursery, where the plants are watered and fed regularly, protected with pesticides and shielded from the elements, to the more hostile garden environment is a shock which may stress plants and predispose them to pathogen attack or induce the spread of infections already present.

The idea that small flowering species of clematis are more resistant to wilt than the large flowering clematis varieties was confirmed by both surveys. The fungal isolations showed that infection by *P. clematidina* is more common in large flowering clematis varieties than in small flowering species. The recovery rate was higher for the late large flowering group than for the early flowering group, which suggests that the early varieties may also wilt due to other causes. These are most likely to be mechanical damage as a result of rough handling or wind, since most early large flowering varieties have very thin, brittle stems supporting a large mass of leaves and flowers. Observations indicate that small flowering clematis species are especially susceptible to root infection by *Phytophthora* which may explain part of the cases of wilt found in this group (Wolff, 1996; van de Graaf, 1999).

At 24%, the overall recovery rate for *P. clematidina* from naturally wilted plants was relatively low. This might have been due partly to the slow growing character of the fungus *in vitro* which meant that it was quickly overgrown by other species, and partly because *P. clematidina* does not spread systemically through the plant so that in some cases sections of the wilted stem were sampled which were not the actual site of infection. Nevertheless, the incidence of *P. clematidina* was much higher than any of the other fungi found, indicating that this species does play an important role in clematis wilt. In the stem inoculation trial, Koch's postulates were established for stem rot and wilt by *P. clematidina* in a range of clematis varieties and species with 49% of the inoculated plants wilting. Similar stem inoculation tests with *B. cinerea* and several other fungi isolated from diseased clematis have not resulted in infection or wilt (van de Graaf, 1999).

The results of the stem inoculation trial showed that differences in susceptibility to stem infection by *P. clematidina* could explain variations in susceptibility to wilt in practice. For nine out of eleven varieties and species tested, a positive correlation was found between the results of the stem inoculation trial and those of the grower survey, supporting the initial hypothesis. Thus, the inoculation of unwounded internodal stem sections with spores of a mix of *P. clematidina* isolates

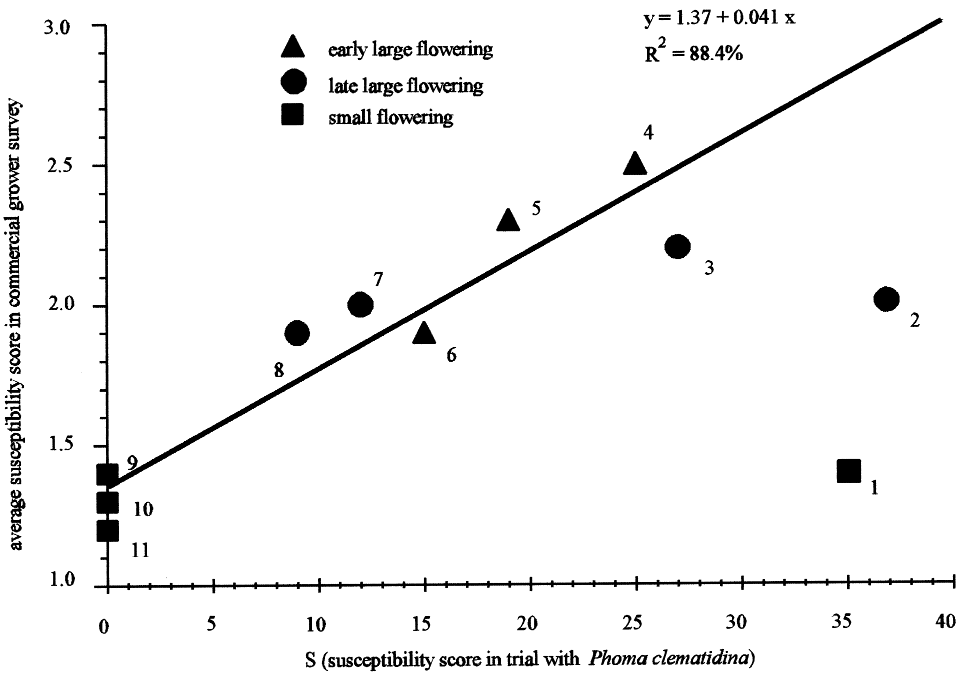

Figure 3. Relationship between *S* (susceptibility score in the stem inoculation trial with *Phoma clematidina* after 60 days) and average wilt susceptibility score in the commercial grower survey (on a scale of 1 = very resistant to 5 = very susceptible) for clematis varieties (nos 1–8) and species (nos 9–11) belonging to three different flowering groups. The regression line is based on varieties/species 3–11. Clematis varieties and species tested in the stem inoculation trail: 1 = *C. viticella*; 2 = 'Ernest Markham'; 3 = 'Lady Betty Balfour'; 4 = 'Vyvyan Pennell'; 5 = 'Henryi'; 6 = 'Miss Bateman'; 7 = Comtesse de Bouchaud'; 8 = 'Ville de Lyon'; 9 = *C. orientalis*; 10 = *C. alpina*; 11 = *C. motana*.

is a simple and effective test to predict the susceptibility of newly bred clematis varieties to wilt in practice, which could be used on clematis nurseries throughout Europe. Only for *C. viticella* and closely related varieties, which includes cv. Ernest Markham, this method would be less reliable. It appears that the general vigour of *C. viticella* and related varieties normally compensates for their susceptibility to *P. clematidina* and that they are able to outgrow infection by the fungus if this occurs naturally. Results of trials by Ebben and Last (1966), who also found that internodal stems of *C. viticella* were susceptible to *P. clematidina*, and Smith (1987), who reported that leaves of cv. Ernest Markham were relatively susceptible to the fungus, support this explanation.

Although the conclusions of the grower surveys and fungal isolations were based on British results, they are applicable to the situation in other clematis growing areas in Europe as well. There is a worldwide trade in clematis plants and *P. clematidina* is geographically widespread (Boerema, 1993). Chemical control of clematis wilt has proved to be difficult, partly because of fungicide resistance in some strains of *P. clematidina* (van Kuik and Brachter, 1997; van de Graaf, 1999). Disease resistance is therefore an important factor in the production of clematis and growers should take the susceptibility of new clematis varieties to wilt into account before deciding on large scale propagation and distribution. The use of only the resistant small flowered clematis species in breeding programmes is economically unattractive, since, despite the problems with clematis wilt, the large flowered varieties are still very popular with the public. Thus, screening of new varieties and species by clematis breeders for susceptibility to *P. clematidina* using the simple stem inoculation test described in this study would be an important step towards the reduction of the incidence of clematis wilt in Europe.

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