Neth J. Pl. Path. 86 (1980) 55-68

Recent observations on leafy gall in Liliaceae and some other families

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Accepted 17 April 1979

Abstract

Corynebacterium fascians, which causes leafy gall, has been shown to be responsible for the unusual symptoms recently found in lilies. The symptoms are described and the different bacterial isolates compared by biochemical, serological and pathogenicity experiments. No evidence could be found for the existence of specialized strains even though considerable variation in virulence could be demonstrated. Although the role of variation in susceptibility of the different lily cultivars should not be underestimated, it would appear that high inoculum levels of *C. fascians* in the soil may be largely responsible for these outbreaks.

Also included in this study are the results of biochemical, serological and pathogenicity experiments of *C. fascians* which has been isolated from *Kalanchoe*, *Euphorbia*, *Brodiaea*, *Hebe* and *Verbascum*.

Additional keywords: Corynebacterium fascians, C. michiganense, C. oortii, C. sepedonicum, Brodiaea laxa, Chrysanthemum morifolium, Euphorbia pulcherrima, Gladiolus, Hebe andersonii, Kalanchoe blossfeldiana, Lathyrus odoratus, Lilium regale, L. speciosum, Pelargonium zonale, Verbascum nigrum, bacterial taxonomy.

Introduction

Although leafy gall (bacterial fasciation) was reported in sweet peas (*Lathyrus odoratus*) by Brown (1927), the cause was not established until 1936 when *Corynebacterium fascians* (Tilford, 1936) Dowson 1942 was found to be responsible for the disease and not *Agrobacterium radiobacter* subsp. *tumefaciens* (E. F. Smith & Townsend, 1907) Keane et al., 1970. Since that time numerous publications have indicated a wide host range for *C. fascians* (Baker, 1950; Sorauer, 1956; Faivre-Amiot, 1967). However, instances of leafy gall in monocotyledonous plants are rare. With the exception of *Asparagus sprengeri* (Lacey, 1936, 1939; Brown and Weiss, 1937) and *Lilium regale* (Lacey, 1939), no further mention has been made of this disease occurring in the Liliaceae.

Recently, during the examination of lilies grown in the Netherlands unusual symptoms were found which appeared to be suggestive of leafy gall caused by *C. fascians*. A preliminary investigation had shown that the symptoms could not be attributed to any disturbance caused by chemical treatments of the soil or plant material; therefore lily bulbs were examined for the presence of *C. fascians*. The main purpose of this paper is

Fig. 1. Scales and bulblets of 'Enchantment' with leafy gall (healthy plant on the left).

Fig. 1. Planten van 'Enchantment' met stengelbollen aangetast door de woekerziekte (links: gezonde plant).

to describe the symptoms produced in the lily, to report the identification of the bacterium isolated and the results obtained from inoculation experiments. In the course of the diagnostic work of the Plant Protection Service (PD) during the past two years other host plants have been recorded for *C. fascians* and these results have also been incorporated into this paper. Although no serious damage of this kind has ever been reported, measures were also taken to prevent the spread of the disease, and research towards its control is now in progress at the Bulb Research Centre in Lisse.

Symptoms in lilies (field observations)

The symptoms of this lily disease, now known in Dutch as 'Woekerziekte', are chiefly found in the bulblets. The scales are deformed, sometimes pointed or rounded and are present in larger numbers than is normally the case (Fig. 1). Beneath these clusters a thickened ridge of yellowish gall-like tissue can often be found. This wild growth can become so serious that the bulb appears as a white cauliflower-like clump (Fig. 2). Sometimes the scales develop into long cream-coloured sprouts often with longitudinal ridges along the upper surfaces which grow out into leaves with thickened bases (Fig. 3). Occasionally infected tissue can be found to have a cream-like glassy appearance. Diseased bulblets usually have a reduced root growth, however, bulblets which produce leaves above the ground often form well developed root systems. Diseased bulblets may be found over the whole length of the underground stem but occur most frequently just under the ground surface. During field observations abnormal growth was not reported in bulblets formed in the leaf axils of the aerial parts of the stem.

Fig. 2. Wild growth of diseased bulblet 'Ranger'.

Fig. 2. Wildgroei van een stengelbol 'Ranger'.

Fig. 3. Scales of bulblets 'Rubrum' which have developed into long cream-coloured sprouts.

Fig. 3. Spruitvormige uitgroei op schubben van stengelbollen 'Rubrum' (sabeltanden).

When diseased bulblets are planted during the following season, sprouting does not always occur although even lightly infected material will sometimes produce weak or short plants.

Symptoms in the mother bulbs are usually light. The outer scales can be deformed and quite often the outer surfaces are ridged giving the bulb a somewhat loose appearance. Wart-like patches may also be present. Often the lower part of the bulb becomes abnormally thickened. In heavily infected plants there is often considerable foliage reduction and root growth stagnation (Fig. 4). Even in lightly infected plants the root development is sometimes retarded. Healthy mother bulbs which are planted in contaminated soil and which produce diseased bulblets show poorer growth than those which produce healthy bulblets even though no symptoms can be found on the mother bulbs themselves. About July-August a yellow discolouration appears in the foliage of lightly infected plants by which disease may be first recognized.

When diseased bulbs are planted in the greenhouse for flower production, abnormal thickening of the stem frequently occurs. Although the thickening may already be present in the bulb, often the first few centimetres of the stem above the bulb will have a normal diameter which is immediately followed by a thickened zone (Fig. 5). This can reach about half way up to the above-ground foliage. There is a noticeable stagnation

Fig. 4. Root growth stagnation of diseased plant 'Enchantment'.

Fig. 5. Thickened stems of diseased plant 'Enchantment' grown in greenhouse.

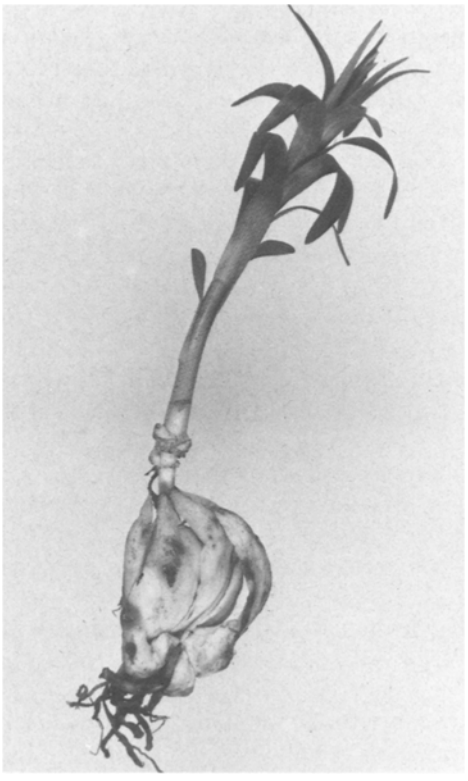

Fig. 4. Reductie van het wortelstelsel van een aangetaste plant 'Enchantment'.

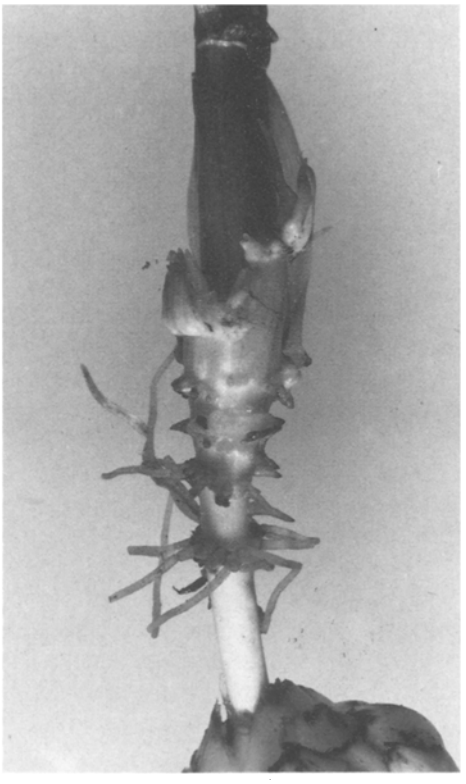

Fig. 5. Verdikte stengel van een in de kas gekweekte zieke plant 'Enchantment'.

of the stem root development, while the lower leaves become broader and heavier than normal. This abnormal development results in diminished flower production.

Materials and methods

Deformed bulbs of the following *Lilium* cultivars grown in sandy soil (pH 5.6–6.0) were collected: Mid Century Hybrid 'Enchantment', *L. speciosum* 'Brabander', 'Journey's End', 'Rubrum' selection no. 10 and 'Uchida' as well as the not yet fully defined hybrids 'Connecticut King', 'Firecracker' and 'Ranger'. Other plant material examined, which showed typical symptoms of leafy gall, included: *Kalanchoe blossfeldiana*, *Euphorbia pulcherrima*, *Brodiaea laxa* 'Koningin Fabiola', *Hebe andersonii* and *Verbascum nigrum*.

After thoroughly washing the bulbs, isolations of bacteria were made from the outer tissue layers on YPG agar plates (0.5% yeast, 1% peptone, 0.5% glucose and 1.5% agar) and maintained on Difco nutrient agar containing 0.1% glucose. Isolations in

Lisse were made on D2 medium (Kado and Heskett, 1970). Comparisons were made with the following *C. fascians* PD isolates: 1054A (*Gladiolus*), 1098 (*Pelargonium*) and S174 (Lisse – orginally an English isolate from *Lathyrus odoratus*).

Colony colour was noted and the following tests were applied: Gram stain, oxidative and fermentative (O & F) reactions of Hugh and Leifson (1953), catalase activity, gelatin liquifaction, nitrate reduction, starch hydrolysis, H_2S production, growth at 37°C, growth in 5% NaCl, salicin utilization (see Ramamurthi, 1959) and urease production (Lelliott, 1966). Serology was effected by means of indirect immunofluorescent microscopy. The antiserum was prepared from culture S174 (IPO 226) by the Research Institute for Plant Protection according to the method of Vruggink and Maas Geesteranus (1975) and checked against *Corynebacterium michiganense*, *C. oortii* and *C. sepedonicum*.

Inoculation experiments were conducted in 1977 and 1978. The preliminary experiments of 1977 were carried out at the Bulb Research Centre in Lisse using lily bulbs of *Lilium* 'Enchantment', which had been planted in steamed soil and had been allowed to grow to a height of approximately 25 cm.

The bulblets were then inoculated as follows:

a) 5 ml bacterial suspension (c. 10^6 cells/ml) were poured along the stem.

b) Bulblets were exposed, without damaging, and soaked with a bacterial suspension from a cotton-wool swab after which the soil was replaced.

c) The bulblets were pricked and covered with cotton-wool containing a bacterial suspension. The soil was replaced.

Five plants per method were used with each inoculum. Inoculations were made on 20 October 1977 with *C. fascians* isolates S174, S224(I9) and S225 (I15). A control group was also included using tap water.

These experiments were repeated on 16 November 1977 with plants which had been allowed to grow one month longer before inoculation. All plants were examined at the end of December 1977.

During 1978, inoculation experiments were conducted at the Plant Protection Service in Wageningen using 10 of the 25 *C. fascians* isolates obtained from the various *Lilium* cultivars. Healthy *Lilium* 'Enchantment' bulbs were used in this series. With each isolate 25 bulbs were dipped in a bacterial suspension (10^7 cells/ml in physiological saline) and pricked. The sterile soil surrounding the bulbs was also infected with the same bacterial isolate. A series of 25 bulbs treated with physiological saline was used as negative control. Plants were examined for symptoms at flowering, almost three months after inoculation. Similar experiments were conducted with sweet peas (*Lathyrus odoratus*) and chrysanthemums (*Chrysanthemum morifolium* 'Dramatic'). Isolates from other plants previously mentioned were also used in inoculation experiments as well as isolates from *Gladiolus* sp., *Pelargonium zonale* and *Lathyrus odoratus*.

Results

During this investigation 20 isolates of *C. fascians* were obtained from different lily cultivars examined at the Plant Protection Service (PD) and five isolates were made from lilies by the Bulb Research Centre, Lisse (LBO), see Table 1. The largest percentage of isolates were obtained from the cultivar Enchantment, which was the

Table 1. Results of physiological tests performed with 33 isolates of *C. fascians* from different host plants.

Isolates from	Gram stain	Colour on YPG	O&F	Catalase	H_2S	Gelatin	Urease	Nitrate	Growth at 37°C	Starch (soluble)	NaCl 5%	Salicin	Serology
Lilium[1] cultivars	+	14 yellow 11 orange	+ –	+	+	–	+	+	+	– (1 ±)[3]	+	–	+
Cult. S174[2]	+	orange	+ –	+	+	–	+	+	+	–	+	–	+
Brodiaea (857)	+	orange	+ –	+	+	–	+	+	+	–	+	–	+
Gladiolus (1054-A)	+	yellow	+ –	+	+	–	+	+	+	–	+	–	+
Pelargonium (1098)	+	orange	+ –	+	+	–	+	+	+	±[3]	+	–	+
Verbascum (1499)	+	orange	+ –	+	+	–	+	+	+	±[3]	+	–	+
Euphorbia (1203)	+	orange	+ –	+	+	–	+	+	+	–	+	–	+
Hebe (1170)	+	orange	+ –	+	+	–	+	+	+	–	+	–	+
Kalanchoe (88)	+	yellow	+ –	+	+	–	+	+	+	–	+	–	+

[1] PD isolates: 142 ('Connecticut King'), 727, 1036, 1037, 1040, 1041, 1042, 1043, 1044, 1046, 1047, 1048, 1050, 1051, 1052 ('Enchantment'), 139, 140 ('Firecracker') and 1038, 1039 ('Uchida').
LBO isolates: I9 (S225), I14, I15 (S224), I17 and I2442 ('Ranger').

[2] Used for preparation of antiserum.

[3] ± weak reaction.

Tabel 1. Resultaten van fysiologische toetsen van 33 isolaten van C. fascians afkomstig van verschillende waardplanten.

Fig. 6. Leafy gall symptoms in *Hebe andersonii*, caused by *C. fascians*.

Fig. 6. Woekeringen (abnormale spruitvorming) bij Hebe andersonii, veroorzaakt door C. fascians.

most frequently examined cultivar although *C. fascians* was also isolated from cultivars 'Connecticut King', 'Firecracker', 'Ranger' and 'Uchida'. During the past two years of routine diagnostic investigation, *C. fascians* has also been isolated from *Brodiaea laxa*, *Euphorbia pulcherrima*, *Hebe andersonii* (Fig. 6), *Kalanchoe blossfeldiana* (Fig. 7) and *Verbascum nigrum* (Fig. 8).

Table 2. Numbers of bulblets cv. Enchantment showing symptoms after inoculation with three isolates of *C. fascians*.

Bacterial isolate	Bulblets with symptoms, compared with total number of bulblets produced					
Inoculated	20 October 1977			16 November 1977		
Inoculation method	a	b	c	a	b	c
S 174	0/14	0/15	0/9	2/10	0/22	0/6
S 224 (I9)	23/27	2/25	15/21	6/21	9/28	10/19
S 225 (I15)	0/19	4/21	0/9	6/33	2/16	7/10
Control	0/7	0/21	0/5	2/21	0/33	0/16

Tabel 2. Aantallen stengeljongen van de cv. Enchantment met symptomen na inoculatie met drie isolaten van C. fascians.

Fig. 7. Leafy gall symptoms in *Kalanchoe blossfeldiana*, caused by *C. fascians*.

Fig. 7. Abnormale spruitvorming bij Kalanchoe blossfeldiana, veroorzaakt door C. fascians.

Fig. 8. Leafy gall of the cauliflower type in *Verbascum nigrum*, caused by *C. fascians*.

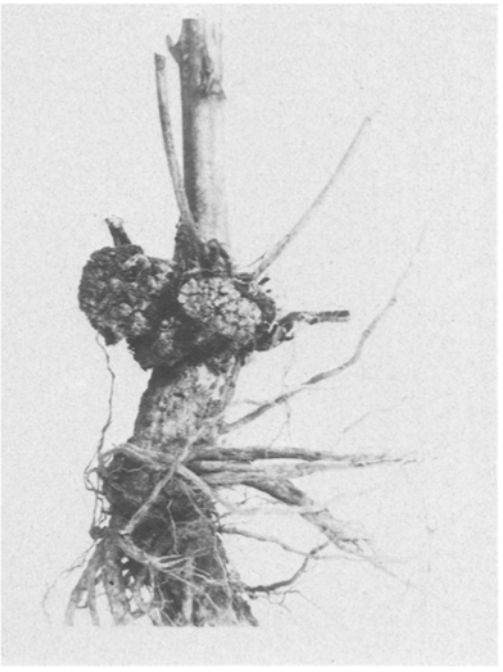

Fig. 8. Bloemkoolachtige woekeringen bij Verbascum nigrum, veroorzaakt door C. fascians.

Colony colour, the Gram reaction and the results of the biochemical tests together with an outline of the serological tests have been collected in Table 1. Inoculation experiments made in 1977 appear in Table 2, while the experiments of 1978, with 10 isolates from lily and 8 from other hosts are presented in Table 3. In the 1978 inoculation experiments no symptoms were found in the bacteria-free controls, but two bulblets of the 1977 series were found to show symptoms.

The pathogenicity tests show that isolates from lily were all pathogenic to lily, but indicate a considerable variation in the severity of symptoms as well as in the percentage of bulbs affected (12–100%). Isolates from other hosts reacted in lily in a similar way. Some isolates e.g. *Verbascum nigrum* were just as pathogenic as the most virulent isolate from lily. Pathogenicity tests on *Chrysanthemum* were doubtful in our hands and often differed from symptom formation in lily. *Lathyrus* was found in most cases to be a good test plant for *C. fascians*. Only one isolate (*Pelargonium zonale* no. 1098), which was pathogenic to *Lilium* and *Pelargonium* did not produce clear symptoms on *Lathyrus*.

All isolates reacted clearly with the antiserum prepared from isolate S174 using indirect immunofluorescence microscopy. In cross-reaction tests no reaction was

Table 3. Comparison of isolates for colony colour, serological and pathogenicity reactions.

Original host plant	Isolate no.	Colour[1] on YPG	Serology titre	Pathogenicity experiments					
				Lilium[2,3] 'Enchantment'	*Chrysanthemum*	*Lathyrus*	*Pelargonium*	*Hebe*	*Kalanchoe*
Lilium									
'Enchantment'	1037	or	1280	++ 100	±	++			
'Enchantment'	1042	or	2560	+ 84	−	−			
'Enchantment'	1047	y	1280	+ 79	++	++			
'Enchantment'	1050	y	640	± 12	−	−			
'Uchida'	1038	y	1280	++ 88	±	+			
'Uchida'	1039	or	1280	++ 92	+	+			
'Firecracker'	139	y	1280	++ 80		+			
'Firecracker'	140	y	640	± 12		+			
'Connecticut King'	142	y	640	+++ 84		+			
'Ranger'	I2442	or	640	++ 84		+			
Lathyrus odoratus	S174	or	1280	++ 90	±	++			
Brodiaea laxa	857	or	640	++ 76	+	++			
Gladiolus	1054-A	y	5120	± 26	−	−	+		
Pelargonium zonale	1098	or	2560	+ 52	±	±	++		
Verbascum nigrum	1499	or	1280	+++100		+	++		
Euphorbia pulcherrima	1203	or	1280		−	−	++		
Hebe andersonii	1170	or	640	++ 83	++	+		++	
Kalanchoe blossfeldiana	88	y	640		−	+			+

[1] or: orange, y: yellow.
[2] Degree of symptom expression: ± = weak reaction; + = positive reaction; ++ = strongly positive reaction; +++ = severe symptoms.
[3] Percentage infected plants.

Table 3. Vergelijking van de isolaten wat betreft koloniekleur, serologische reacties en pathogeniteit.

found between the antiserum and *Corynebacterium michiganense* although weak reactions of less than 1:20 were noted with *C. oortii* and *C. sepedonicum.*

Discussion and conclusion

Corynebacterium fascians, isolated from lilies and other plants, was identified by means of the different tests listed in Tables 1 and 3. These bacteria which are all Gram-positive and morphologically similar, appear to make up a biochemically stable species which is readily distinguishable from most other plant-pathogenic corynebacteria. *C. fascians* typically produces H_2S (Lazar, 1968a) compared with very weak or negative results for other species of *Corynebacterium*, although Schuster (1975) reported positive results for 11 isolates of *C. nebraskense*. Only *C. fascians* and *C. ilicis* produce urease (Lelliott, 1966) and in a medium lacking peptone only *C. fascians* reduces nitrate to nitrite (Ramamurthi, 1959). Salicin utilization, which was considered to be negative for the whole Group 1 of Lelliott, was previously reported by Ramamurthi as being positive for *C. insidiosum*, *C. michiganense* and *C. sepedonicum*. Vidaver and Mandel (1974) reported positive salicin utilization for *C. michiganense* but Schuster's results were negative. Ramamurthi found that only *C. fascians* failed to utilize salicin. In this investigation all isolates were able to grow at 37°C which is not in agreement with Lelliott's findings in which the species of his Group 1 had failed to grow above 35°C. Oxidative metabolism only, catalase production and a negative to weak breakdown of gelatin are common features of the genus. Although Ramamurthi was unable to find starch hydrolysis in any of the corynebacteria he examined, three of the 33 isolates tested in this investigation were found to hydrolize starch, but only weakly. Schuster also found that 7 virulent and 3 avirulent strains of *C. nebraskense* hydrolysed starch. However, more recently Dye and Kemp (1967) reported starch hydrolysis in *C. oortii*, *C. betae* and *C. poinsettiae* as well as variable results in five other *Corynebacterium* species but not in *C. fascians*. All isolates produced a positive reaction with the antiserum and using immunofluorescent microscopy generally gave titres varying between 1:640 and 1:1260. With the *Gladiolus* isolate a titre of 1:5120 was obtained. These variations, however, may be explained in the case of *C. fascians* which is very rich in antibody forming polysaccharide. Lazer (1968b) also reported variation in intensity of his reactions when using double gel diffusion. The serological division of Lelliott's Group 1 according to Lazer (1968b) is also supported by the failure of the *C. fascians* antiserum to react with *C. michiganense* and the very weak reaction with *C. sepedonicum* (see Buchanan et al., 1974).
In the 1977 inoculation experiments (Table 2), isolate S224 caused the strongest symptoms which at first sight may suggest the existence of specialized forms of *C. fascians*. However, there appears to be considerable variability in these results as is also indicated in the more comprehensive experiments of 1978.

In the 1978 inoculation experiments using bulbs of *Lilium* 'Enchantment' (see Table 3), all lily isolates produced symptoms of varying degrees of severity. However, not all lilies used for each isolate produced symptoms. The infection rate varied between 12 and 100%. There are two possible explanations for these differences. Although these results, which also include the pathogenicity tests with other host plants, do not indicate the presence of specialized strains, there would appear to be considerable

variation in the pathogenicity of the isolates as well as differences in the susceptibility of the various lily cultivars. Using the high inoculum levels (10^6–10^7 cells/ml) and considering the method of inoculation, it may be expected that the bacteria would be able to at least inhabit the surface and outer layers of the bulbs.

It would seem that the lily has always been susceptible to infection by *C. fascians*. The fact that now this disease has become serious in the cultivation of lilies at least, may well be related to the absence of crop rotation on a number of nurseries whereby the bacterial populations have reached such abnormally high levels (see also Roberts and Blaney, 1957).

As far as the isolates from other plants are concerned, there is no doubt of their pathogenicity. The pathogenicity neither appears to be related to titre nor does the colony colour of the different isolates, related to variability in pathogenicity in some bacterial species, seem to bear any relationship in the case of *C. fascians*.

Although *C. fascians* may not be as widespread as *Agrobacterium radiobacter* subsp. *tumefaciens* or have such a broad spectrum of host plants, its distribution is large and it would appear to have the ability to infect a greater number of plant species than we had realized. This is reflected by the various new host plants reported in this paper as well as other recent, not yet proven, observations in *Crocus*, *Fritillaria*, *Hyacinthus* and *Muscari* (Dr. G. Weststeijn, Lisse, personal communication). This may become even more noticeable through the increased use of mechanization especially in commercial bulb growing where a considerable amount of plant material may remain in the soil after harvesting.

Acknowledgements

The authors wish to extend their thanks to Mr C. F. M. Dekkers for his technical assistance.

Samenvatting

Recente waarnemingen van aantastingen door Corynebacterium fascians in Liliaceae en enkele andere families

Reeds een aantal jaren was in lelies een woekering in stengelbollen bekend, die echter sporadisch voorkwam en economisch van weinig betekenis was. Tijdens de zomer van 1977 echter trad de ziekte op één enkel perceel zo ernstig op dat zeer aanzienlijke oogstderving het gevolg was. Behalve de stengelbol kan ook de hoofdbol misvormd zijn. Bovendien kunnen misvormde stengelbollen lange spruiten vormen, zgn. sabeltandvorming. Soms is het wortelstelsel gereduceerd; in de kas zijn verdikte stengels waargenomen. De ziekte staat nu bekend onder de naam woekerziekte.

Door de PD te Wageningen en het LBO te Lisse werd de bacterie *Corynebacterium fascians* (Tilford) Dows. uit het zieke materiaal geïsoleerd. In gezamenlijk onderzoek werd nagegaan of de bacterie de veroorzaker is van bovengenoemde symptomen en of hier sprake is van een op lelie gespecialiseerde stam.

Biochemische en serologische vergelijkingen tussen isolaten van *C. fascians* uit lelie, *Kalanchoe*, *Euphorbia*, *Brodiaeea*, *Hebe* en *Verbascum* laten zien dat er zeer weinig verschillen bestaan. Alle isolaten reageren op een standaard antiserum met titers variërend tussen 1:640–1:5120.

Een oriënterende inoculatieproef werd uitgevoerd op het LBO in 1977. Daarna werden op de PD 10 van de 25 isolaten uit lelie en 8 uit andere waardplanten getoetst op lelie, chrysant en lathyrus. Uit de inoculatieproeven blijkt, dat de bacterie de veroorzaker is van de waargenomen symptomen. Tussen de isolaten blijkt een groot verschil in pathogeniteit te bestaan (12–100% aantasting), zonder dat dit aan herkomst gebonden is. Het voorkomen van op lelie of andere waardplanten gespecialiseerde stammen is daarom niet aan te nemen.

In hoeverre de hevige aantasting een gevolg is van een opbouw van de bacteriepopulatie door een te nauwe vruchtwisseling en mechanische rooimethoden moet worden nagegaan.

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