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FINAL REPORT
PROJECT 27A
DISTORTION IN BEDDING PLANTS

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DISTORTION IN BEDDING PLANTS

APPLICATION

This project has shown that the bacterium which causes leafy gall disease, Rhodococcus fascians, is not responsible for the Distortion problem which affects a wide range of ornamental plants, most notably petunia. However, since true leafy gall can occasionally affect bedding plants and other ornamentals, growers should familiarise themselves with the differences between leafy gall and Distortion listed on Page 2 of this report.

SUMMARY

The Interim Report for this project described a number of pieces of work which were carried out to determine i) the extent of the problem of Distortion in bedding plants, and ii) whether the bacterium Rhodococcus (formerly Corynebacterium) fascians is the causal agent of Distortion. This work included a survey of growers, a literature review, and attempts to reproduce the Distortion symptom by manipulating growing conditions or inducing nutrient deficiencies.

The major part of the project (carried out at CSL Harpenden) involved the testing of samples of bedding plants showing Distortion for the presence of the Rhodococcus bacterium. During 1991, 86 samples of bedding plants were tested. A total of fourty-two isolates were obtained which were confirmed as Rhodococcus species, of which twenty-four had high enough Similarity Indices (S.I.) to be considered as Rhodococcus fascians. However, only one of these reproduced symptoms when it was inoculated into a range of bedding plant species. The conclusion was that Rhodococcus fascians was not responsible for the symptom of Distortion in bedding plants, and that any future work should concentrate on possible physiological causes.

At the time of publication of the Interim Report, the results of work on a further 22 samples of bedding plants were still outstanding. This is now complete, and the results are very similar to those of the previous work. Nine isolates were confirmed as Rhodococcus species but none of these produced distortion symptoms when inoculated into bedding plants.

This final report describes the work carried out on these 22 samples, and presents conclusions and recommendations for the project as a whole.

INTRODUCTION

In this section, brief details only are presented of the work described in the Interim Report, which should be consulted for more detailed descriptions. However, it is worth repeating the descriptions of similarities and differences between the symptoms of Distortion (which has been a significant problem on nurseries in recent years) and those of true leafy gall, caused by Rhodococcus fascians.

Symptoms Common to Distortion and Leafy Gall

Swollen buds and shoots, distorted (twisted) stems, thickened misshapen leaves. Formation of gall-like structures.

Symptoms of Leafy Gall Not Exhibited in True Distortion

Fasciation (lateral bud proliferation), proliferation of shoot growth at or below soil level, galls usually at soil level. Plants do not grow out of symptoms.

Symptoms of Distortion Not Exhibited in Leafy Gall

1. Always occurs between cotyledon and 4 - leaf stage
2. Seed-raised plants only are affected
3. Thickened and twisted leaves on otherwise normal stems
4. Reduction of growing point to a knob-like structure
5. Spread from plant to plant does not occur
6. Plants often recover to produce normal growth

The major part of the work in Project 27A involved testing samples of bedding plants showing Distortion for the presence of Rhodococcus fascians, with the aim of determining whether R. fascians had a role in the problem. Samples were sent from nurseries throughout England to bacteriologists in the Plant Health Group at CSL Harpenden. A total of eighty-six samples of plants were received in 1991, the majority of which (36) were petunia.

A few (2-10) samples were received of zonal pelargonium, impatiens, antirrhinum, salvia, verbena, nicotiana, French marigold, pansy and viola. Single samples were received of cineraria, celosia, aster, campanula, primula and lettuce.

Very few samples showed symptoms typical of leafy gall. Most plants showed the symptoms of Distortion described above. In many cases distorted plants grew out of the condition as they developed.

A total of forty-two isolates of Rhodococcus were obtained. These came from twenty of the samples. Twenty-four of these isolates were thought to be Rhodococcus fascians (there are difficulties in identifying Rhodococcus to species level, which will be described later).

Thirty-nine of the Rhodococcus isolates were inoculated into petunia, antirrhinum, pelargonium, French marigold, salvia and impatiens to see whether they would reproduce the Distortion symptoms. Only one isolate, from primula, produced lateral bud proliferation typical of leafy gall. The primula plants from which the isolate was obtained also showed symptoms of leafy gall rather than Distortion. None of the isolates obtained from plants showing Distortion was capable of reproducing the symptoms in test plants, ie the isolates were non-pathogenic.

Also under Project 27A, work was carried out at ADAS Wolverhampton to attempt to reproduce the symptoms of Distortion by simple manipulation of temperature and light regimes during the early stages of plant growth. No symptoms were seen in petunia and zonal pelargoniums, but low temperatures induced a mild leaf curling in impatiens.

Finally, results were presented in the Interim Report (by kind permission of Mr S Coutts, Four Oaks Nurseries) of earlier experiments in which nutrient deficiencies were investigated as possible causes of Distortion. Pansies and zonal pelargoniums sown in composts deficient in either boron, molybdenum or zinc did not develop symptoms of Distortion.

The following pages detail the work done at CSL Harpenden on a further twenty-two samples of bedding plants which was not complete at the time of publication of the Interim Report.

Materials and Methods

All sampling procedures, methodologies and techniques are as described in the Interim Report.

Samples

Twenty-two separate samples of plants were received between September 1991 and June 1992. Samples were sub-divided depending on their size. Sixty-two separate sub-samples were investigated. Table 1 lists the samples received with their details. A summary of numbers of samples received by plant species is given below.

	No of samples
Primula	2
Pansy	5
Primrose	1
Pelargonium	2
Antirrhinum	3
Petunia	6
Alyssum	2
Phlox	1
TOTAL:	22

Symptoms

As in the previous samples, the most common species received was petunia and once again most showed a thickening or clubbing of the growing point. Pansies showed a proliferation of the growing point and some cupping. Two separate alyssum samples had plants with 'bunchy' centres and some stunting. Apart from one sample of primula and one of petunia no specimens showed the typical fasciation of leafy gall.

Table 1
Details of samples received - 5/9/91 - 25/5/92

No	Ref. No	No of Sub-samples	Date	Host	Region	Symptoms	No of *Rhodococcus* sp. on isolation plate
1	90	2	5/9/91	Primula	Kent	Typical fasciation	nil
2	91	2	5/9/91	Primula	Kent	Proliferation and thickening of growing point	nil
3	92	2	1/10/91	Pansy	Dorset	Proliferation and thickening of growing point	nil
4	93	2	10/10/91	Pansy	Dorset	Proliferation, cupping, elongated leaves	nil
5	94	3	25/10/91	Pansy	Norfolk	Proliferation	nil
6	95	2	1/11/91	Primrose	Bristol	Distortion	nil
7	96	4	13/1/92	Pansy	via Wolverhampton	Thickening/cupping	nil
8	97	3	4/3/92	Pelargonium	via Cheshunt	Thickening of growing point	nil
9	98	3	11/3/92	Antirrhinum	via Wolverhampton	Cupping and stunting	nil
10	99	3	24/3/92	Antirrhinum	Cheshire	Proliferation/cupping	1
11	100	3	27/3/92	Pelargonium	Essex	Thickening of growing point	nil

No	Ref. No	No of Sub-samples	Date	Host	Region	Symptoms	No of *Rhodococcus* sp. on isolation plate
12	101	3	27/3/92	Pansy	Cheshire	Thickening/cupping	nil
13	102	3	15/4/92	Petunia	Lancs	Thickening of growing point/distortion	2
14	103	3	23/4/92	Antirrhinum	Beds	Thickening of growing point/cupping	nil
15	104	3	23/4/92	Petunia	via Huntingdon	Thickening of growing point/clubbing	1
16	105	3	23/4/92	Alyssum	via Huntingdon	'Bunchy centres'	nil
17	106	3	1/5/92	Petunia	via Cheshunt	Uneven growth and clubbing	20 +
18	107	3	7/5/92	Petunia	Cheshire	Thickening and clubbing of the growing point	nil
19	108	3	7/5/92	Alyssum	Cheshire	50% v. stunted centres and proliferation	1
20	109	3	7/5/92	Phlox	Cheshire	Proliferation, stunting and death of growing points	nil
21	110	3	7/5/92	Petunia	Cheshire	Thickening of growing point and stunting	nil
22	111	3	27/5/92	Petunia	Cheshire	Classic distortion very like leafy gall	nil

Isolations

Only five of the samples received yielded likely Rhodococcus species, and in all but one case only one or two colonies were found on isolation plates. The exception was in one sub-sample of petunia from a nursery in Hertfordshire where more than twenty colonies were found on the isolation plate. In total, nine separate strains were found and held at -80°C until identification by fatty acid profiling and host-testing could proceed. Seven strains were from petunia, one from antirrhinum and the other from alyssum.

Identification by fatty acid profiling

A fatty acid profile was prepared for each of the nine strains. Problems were encountered in the previous work with fatty acid profiling because unlike other bacteria, Rhodococcus spp. do not seem to produce consistently reproducible fatty acid profiles due to the complexity of their cell walls. Because of this inconsistency, profiles were prepared and run on three separate occasions.

All nine strains were identified as Rhodococcus species, and all but one had similarity indices (SI) high enough to be considered typical of R. fascians. This strain was from petunia.

Host Tests

The nine suspect R. fascians strains were inoculated into petunia, French marigold, pelargonium, impatiens, antirrhinum, alyssum, primula and sweet pea.

Compared to the previous work (see Interim Report) the range of plants was increased to include alyssum, primula and sweet pea. Primula was included because the only confirmed virulent R. fascians strain from the previous eighty-six samples came from primula (sample number A4830/3). Salvia was excluded as no symptoms were seen in 1990-91 even with authentic R. fascians strains taken from the National Collection of Plant Pathogenic Bacteria (NCPPB).

Six R. fascians strains from the NCPPB, together with the positive primula strain (A4830/3) from 1990/91 were used as positive controls.

Plants

All plants were grown from seed in a 50:50 John Innes/peat compost for approximately 30-40 days before inoculation. Cultivars used were:-

Petunia	- Expression mixed
Pelargonium	- Century deep salmon
Primula	- Husky mixed
French marigold	- Aurora gold
Sweet pea	- Galaxy mixed
Antirrhinum	- Coronette
Alyssum	- Snowdrift
Impatiens	- Accent F1 mixed

Inoculation Method

As described in the Interim Report. All plants were inoculated on the same day. Six replicate plants were used for treatment except for primula where only three plants for treatment were available.

Glasshouse Conditions

The plants were grown on in a single large glasshouse under the same conditions as described in the Interim Report, ie at approximately 22°C with an extended day length of 16 hours. The plants were monitored over the test period and assessed for lateral bud proliferation/distortion after nine weeks.

Results

Symptom Development

None of the nine test isolates showed any leafy gall or other symptoms.

NCPPB Culture (Table 2)

All of the NCPPB strains of R. fascians used produced leafy gall in some of the replicates of at least three of the host plant species used. Strain A4830/3 gave some positive results in French marigold, pelargonium and impatiens and showed a weaker response in petunia, alyssum and primula. French marigold appeared to be the most susceptible species used. None of the negative control plants developed symptoms.

Table 2

NCPPB strains and Primula isolate from 1991 survey - No. of plants out of 6 showing proliferation symptoms

NCPPB Culture No	Original host	Petunia	French Marigold	Pelargonium	Impatiens	Antirrhinum	Alyssum	Primula*	Sweet Pea
3267	Pelargonium sp.	3	6	1	0	0	3	0	0
469	Fragaria x ananassa	3	6	1	1	0	4	0	2
1733	Beloperone guttata	1	6	3	3	0	1	0	0
3067 T	Unknown	3	6	6	5	0	5	0	0
1488	Lathyrus odoratus	4	6	2	5	0	6	1	6
1675	Dendranthema x grandiflorum	0	1	1	0	0	2	0	0
188	Dendranthema x grandiflorum	3	6	1	5	0	4	0	0
A4830/3	Primula	0	4	2	1	0	(3)	(2)	0

T = Type culture
* = Three replicates only
( ) = weak positive reaction

Conclusions

During the course of this project one hundred and eight samples (three hundred and twenty-four sub-samples) of bedding plants have been tested for the presence of Rhodococcus fascians.

Plants submitted with distortion-like symptoms included:-

Petunia
Pelargonium
Impatiens
Antirrhinum
Salvia
Verbena
Nicotiana
French marigold
Pansy
Cineraria
Celosia
Primula
Primrose
Alyssum
Phlox

Distortion is most common in petunias, and these have provided the bulk of the specimens.

From the symptomatic plants forty-eight suspect strains of Rhodococcus fascians were isolated. They occurred mostly in single or sparse colonies on isolation plates. In only six cases were relatively large numbers of colonies recorded.

Of the forty-eight strains tested only one produced typical leafy gall symptoms. This same strain produced similar galls in many host plant species.

The NCPPB cultures used as positive controls have proved that virulent strains of R fascians are able to produce typical symptoms under identical glasshouse conditions in many of the host plant species used.

It is now apparent that the Distortion symptom in bedding plants is not caused by Rhodococcus fascians. The true cause is not known. However, in some species, for example petunia and pansy, the symptoms of bedding plant Distortion can be confused with those of R. fascians - induced proliferation or leafy gall. Leafy gall induced by R. fascians is found occasionally in bedding plants, but during the course of this work only one sample received out of a total of three hundred and twenty-four sub-samples contained virulent R. fascians. Non-pathogenic Rhodococcus species are fairly common in soils, composts and on plant surfaces. Fatty acid profiling proved to be very useful in determining the genus but due to certain chromatographic problems caused by some

unknown compounds in Rhodococcus spp, identification at species level was not generally of an acceptable standard. This problem is considered to be unique for Rhodococcus species.

Due to the above problems with fatty acid profiling it is considered that the best means of confirming R. fascians is by colony morphology, Gram-stain and a host test in seedlings of the species from which the original isolation had been made. If a single plant were to be selected, then the use of African marigold (Tagetes erecta) is recommended, for example cv Aurora Gold. The use of sweet pea as indicated in ADAS Microbiological Techniques, Section 2, Technique PP1, H8 is not recommended, as this species is not susceptible to many strains of R. fascians.

In conclusion therefore, the true cause of bedding plant Distortion, a common and sometimes serious problem, is not known. For many years it was thought that Rhodococcus fascians was involved. In the light of some of the problems described in identifying Rhodococcus to species level, it is likely that diagnostic errors may have been made in previous years, with non-pathogenic Rhodococcus species being identified as R. fascians. Thus the 'myth' of R. fascians as the cause of Distortion was perpetuated, and compounded by the occasional confusion of true leafy gall disease with Distortion.

In addition to the differences in symptoms described in the Introduction, one further distinguishing factor between leafy gall and Distortion is that whereas the unknown causal agent of distortion does not seem to show any host specificity, true proliferation or leafy gall is not likely to be found on a range of bedding plants since individual strains of R. fascians rarely have host ranges as wide as those seen with Distortion. In practice however, even Distortion is often only found on one or two species on an individual nursery, whilst the other bedding plant species grow normally.

Recommendations

1. This work has shown that the bacterium Rhodococcus fascians is not responsible for Distortion in bedding plants. The continued submission of bedding plant Distortion samples for bacterial analysis is therefore unnecessary. Growers should be made fully aware of the differences between Distortion and true leafy gall.

2. Any future work should concentrate on plant physiological processes as possible causes of Distortion. The work should be done by experienced plant physiologists at an institution with suitable facilities for complex manipulation of environmental conditions.

Contract between ADAS & CSL (hereinafter called the "Contractors") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT **Contract No: PC 27a**

DISTORTION IN BEDDING PLANTS: INVESTIGATION INTO THE AGENTS CAUSING THIS DISORDER (PC27A)

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Distortion of growth soon after pricking out resulting in malformed leaves, loss of growing point and plant death became a prominent problem on several nurseries in Surrey in the early 1970's. Petunias and Antirrhinums were the main subjects affected but the condition has since been recorded elsewhere on salvia, verbena, alyssum, zonal pelargonium, impatiens, phlox, pansy, marigold and dianthus. Incidence and reported severity have varied erratically since the condition was first recognised but individual growers have suffered significant losses. Establishing the cause of this disorder should indicate means of prevention and lead to improved quality.

The major problem with this disease is determination of the causal agent. Two similar symptoms are commonly seen:

1. Lateral bud proliferation of seedlings consistently yields significant populations of Rhodococcus fascians which are usually pathogenic to the host from which they are isolated. Occasionally R.fascians cannot be isolated and in some cases the R.fascians strains isolated are avirulent.

2. Seeding distortion is perhaps more common, and when it occurs it may affect many unrelated bedding plant species on the holding. Isolation rarely yields large populations of R.fascians. R.fascians and other Rhodococcus spp are known to occur epiphytically and saprophytically in the plant's environment. Whereas most of the occasional R.fascians isolates obtained from distorted plants are not pathogenic to the host from which isolated, some have been shown to be pathogenic.

   This implies that whereas the vast majority of cases of lateral bud proliferation are caused by R.fascians only a few of the cases of distortion are. This situation is further confused by the fact that as proliferation develops the pathogen may die out (a situation parallelled in Agrobacterium induced crown galls) and the fairly recent observation that some trays of infected

plants have shown both symptoms; proliferation apparently succeeding distortion.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

The HDC funded survey (PC27) carried out by ADAS in 1990 showed the growers estimated losses in the region 0.6% of total sales. Assuming a total crop value of £50m, losses of £300,000 occur annually from this disorder. This initial study costing £20,000 over a 3 year period is a cost effective start to solving a long standing unsolved problem.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

1. To review literature and case studies and to summarise their findings.

2. To establish the significance of Rhodococcus species in the Distortion Syndrome.

3. To examine the effect of boron deficiency on symptom development.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

There is no scientific literature on this disorder. The only information available is in the form of popular articles and case studies from NAAS and ADAS records.

6. DESCRIPTION OF THE WORK

1. The main objective is to make a collection of up to 500 R.fascians strains from a range of outbreaks of both symptom types and to assess their relative populations in the diseased tissues. A questionnaire will be designed to maximise efficiency of collecting data regarding each case. Each isolate will be characterised by fatty acid profiling and other key tests. Its host range will be determined using a panel of six bedding plant species including the host from which isolated. In addition, seed samples where available will be tested for the presence of R.fascians.

2. Previous published and unpublished work by ADAS and world literature will be reviewed.

3. Up to 10 selected samples will be checked for presence of virus by EM and sap transfer.

4. Boron deficiency as a potential cause of symptoms will be investigated, using petunias or zonal pelargoniums as the test subject. Choice of cultivar to be agreed with B Crosby. Plants will be tested in standard compost, in composts with and without boron and

compost with and without phosphate. 5 plants/plot; 3 replicates.

7. COMMENCEMENT DATE AND DURATION

1.4.91 for 3 years. The literature review will be completed and report presented by October 1991. The work on boron deficiency will be completed and reported by December 1991. Work on Rhodococcus spp. will continue throughout the 3 year period and reports will be presented as appropriate.

The main effort and therefore costs involved in this study are directed towards establishing the role of the bacterium Rhodococcus in the disorder. If at any stage the anticipated number of isolates (approximately 150 per year) are not obtained or new information becomes available the project leader and co-ordinator should re-examine the work programme and adjust funding accordingly.

8. STAFF RESPONSIBILITIES

Project Leader:	G W Griffin, Plant Pathologist ADAS Wolverhampton
Other Staff:	Dr D Stead) Mrs J Sellwood) CSL Harpenden
	N Bragg Soil Scientist, Wolverhampton

Contract between ADAS & CSL (hereinafter called the "Contractors") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT

Contract No: PC/27a (Shortened contract)

DISTORTION IN BEDDING PLANTS: INVESTIGATION INTO THE AGENTS CAUSING THIS DISORDER (PC27A)

2. BACKGROUND AND COMMERCIAL OBJECTIVE

As for PC/27a.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

As for PC/27a.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

As for PC/27a.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

As for PC/27a.

6. DESCRIPTION OF THE WORK

As for PC/27a.

7. COMMENCEMENT DATE AND DURATION

1.4.91 for 2 years. The literature review will be completed and report presented by October 1991. The work on boron deficiency will be completed and reported by December 1991. Work on Rhodococcus spp. will continue throughout the 2 year period and reports will be presented as appropriate.