Final Report:	5 October 1993
Project Number:	PV/FV135
Project Title:	Lettuce: A non-toxic crop protection system for lettuce (and other vegetable crops)
Project Leader:	Adrian C Newton & Gary D Lyon
Project report authors:	Tony Reglinski, Gary D Lyon and Adrian C Newton
Location:	Scottish Crop Research Institute Invergowrie, Dundee DD2 5DA
Project co-ordinator:	Mr Adrian Berrevoets
Date project commenced:	1 December 1992
Date project completed:	31 May 1993
Key words:	lettuce, *Botrytis cinerea, Rhizoctonia solani*, yeast, resistance elicitors, crop protection

RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application

The objective of this project was to determine whether non-toxic extracts from yeast cell walls could be used to protect lettuce against grey mould and stem rot caused by *Botrytis cinerea*, and bottom rot caused by *Rhizoctonia solani*. The yeast extracts reduced grey mould by up to 90% on detached leaves in laboratory tests and reduced grey mould and bottom rot by approximately 70% in the glasshouse with no observable detrimental effects on lettuce. This alternative control measure may provide a means for reducing fungicide inputs on lettuce grown in the field or under glass.

Summary

Objective

Major crop losses are experienced from diseases caused by fungal pathogens in lettuce. *Botrytis cinerea* is by far the most common pathogen in UK lettuce crops causing grey mould, damping-off and stem rot. However, severe attacks of bottom rot disease caused by *Rhizoctonia solani* have occurred in recent years and this disease is considered to be important in protected crops. These pathogens are commonly controlled using methods such as soil sterilization or fungicide application, however, the development of multiple fungicide resistance and the concern over the application of toxic fungicides to foliage which is for human consumption has prompted a search for safer alternative control measures. The activation of plant resistance mechanisms by application of compounds called elicitors has been suggested as an alternative approach for disease control. We have demonstrated that non-toxic elicitors extracted from yeast cell walls are capable of controlling barley powdery mildew in the field. The objective of this study was to assess the ability of these yeast extracts to protect lettuce from infection with *B. cinerea* and *R. solani*.

Results

Water soluble elicitors extracted from yeast cell-walls reduced *Botrytis cinerea* infection by up to 90% on detached leaves of cv. Little Gem in the laboratory. In the glasshouse *B. cinerea* and *R. solani* infection were reduced by approximately 70% on cvs Little Gem and Patricia, and by approximately 50% on cv. Berlo. Extracts applied with the non-ionic wetter Agral controlled disease more effectively in the glasshouse than those with the penetrating, acidifying surfactant LI-700, thus demonstrating the importance of formulation. The extracts do not exhibit antifungal activity and there was no phototoxic or otherwise detrimental effects observed following application of any of the formulations on any of the lettuce cultivars.

Conclusions

This project has demonstrated the principle of using non-toxic elicitors from yeast cell-walls to be a viable crop protection strategy. It is important to note that these results were obtained under environmental conditions selected to encourage disease in order to provide a stringent test for the yeast extracts. Future research into the use of new formulations containing yeast extracts together with experimentation under more realistic cultural conditions would enable a more accurate assessment of the viability of this technique in terms of the rate, timing, and frequency of application necessary for crop protection. The ultimate aim of this work is to see a new product on the market offering an alternative to conventional fungicides for the grower.

Anticipated benefits for the grower

1) Yeast extracts should be non-toxic and readily biodegradable so providing greater safety for both the sprayer operator and the consumer.
2) Costs should be comparable with fungicide; however, it is likely that several diseases will be controlled by yeast extract leading to possible savings.
3) The extracts are water soluble and can be applied using conventional sprayers.

4) The integrated use of yeast extracts in a spray regime with reduced rate fungicide may provide a viable means of reducing inputs in sustainable horticultural production systems.

5) Yeast extracts will be compatible with other non-chemical controls used on protected crops in controlled systems.

1. INTRODUCTION

A number of fungal pathogens are known to cause rotting of the basal leaves of lettuce in the UK thus rendering plants unmarketable because size, weight and quality limits required by customers are not achieved. The most important of these pathogens are *Botrytis cinerea, Rhizoctonia solani, Sclerotinia minor* and *Sclerotinia sclerotiorum*. *B. cinerea* is by far the most common pathogen in UK lettuce crops causing grey mould, damping–off and stem rot. However, severe attacks of bottom rot disease caused by *R. solani* have occurred in recent years and this disease is considered to be important in protected crops (Griffin and Cox, 1982; Wareing *et al.*, 1986).

There is no lettuce cultivar resistant to *B. cinerea* or *R. solani* and disease is commonly controlled using methods such as soil sterilization or fungicide application. The development of multiple fungicide resistance in *B. cinerea* (Elad *et al.*, 1992) and the concern over the application of toxic fungicides to foliage which is for human consumption has highlighted the need for alternative approaches to disease control which may reduce the use of fungicides. Biological control of bottom rot of lettuce (*R. solani*) using *Trichoderma* spp. has been reported, although results were poorer than those obtained using chemical treatment (Coley–Smith *et al.*, 1991). Few commercial preparations containing *Trichoderma* are available for controlling plant diseases, possibly because non–biological methods of control at present are more effective or reliable. Free radical scavengers have been used to control *B. cinerea* and *S. sclerotiorum* on various crops and their practical use on crops under field conditions has been proposed (Elad, 1992).

The chemical activation of plant resistance mechanisms by application of elicitors has

previously been suggested as an alternative approach for disease control (Cartwright *et al.*, 1977; Schonbeck and Dehne, 1986; Lyon *et al.*, 1990). The stimulation of resistance mechanisms in plants by pre-treatment with various elicitors has been widely reported (Kuc, 1987; Lamb *et al.*, 1989). We have been developing elicitor-active extracts from yeast (Saccharomyces cerevisiae) cell walls for use as a crop protectant and have demonstrated their ability to control barley powdery mildew (*Erysiphe graminis* f.sp. *hordei*) infection and to maintain yield (Newton *et al.*, 1993; Reglinski *et al.*, 1993). In this report we present an assessment of the ability of the yeast extracts to protect lettuce from infection with *B. cinerea* and *R. solani*.

2. MATERIALS AND METHODS

2.1 Selection of Yeast Cell Wall Extracts

Insoluble fractions of dried yeast (*Saccharomyces cerevisiae*) were extracted to give phytoalexin elicitor-active fractions. Details of extract preparation methods are confidential for commercial reasons. During the course of the work alterations to extract preparation were made and new extracts were tested on soybeans before testing on barley leaves. Different formulations of yeast extract used are pre-fixed with the letter Y followed by a number eg Y4. Unless otherwise stated, all treatments used on lettuce were applied in 0.02% (v/v) Agral (the concentrate contains 900 g alkyl phenol ethylene oxide condensate per litre, ICI Agrochemicals).

The elicitor activity of yeast extracts was measured using the soybean cotyledon bioassay (Forrest and Lyon, 1990) in which 90 µl of each test solution was applied to soybean

cotyledon wounds. After incubation in the dark for 20 h at 25°C, phytoalexin accumulation in the wound droplets was determined by measuring absorbance at 285 nm (Uvikon 930 spectrophotometer, Kintron Instruments Ltd).

2.2 Isolation of fungi

Infected lettuce were donated by Mr Adrian Berrevoets of West Cranleigh Nurseries, Cranleigh, Surrey. Pieces of leaf material, including the edges of lesions, were surface sterilized for 2 min in 2% (w/v) sodium hypochlorite, rinsed briefly in sterile distilled water and then dissected into smaller sections. These sections were placed onto either potato dextrose agar (PDA) or 0.5% distilled water agar (DWA), each containing 100 $\mu g\ ml^{-1}$ of the antibiotics aureomycin and streptomycin sulphate. Plates were incubated at 20°C for 1 week and the growing tip of emerging fungi plated onto fresh plates for identification. Cultures were maintained regularly to ensure a supply of fresh inoculum. *B. cinerea* was plated on "Medium X" (Last and Hamley, 1956) to encourage sporulation and spore suspensions were prepared from 8–10 day old cultures. Autoclaved barley seed was inoculated with *R. solani* and incubated in sterile Petri dishes at 20°C for 5 weeks prior to use for inoculation purposes (LeClerg, 1941).

2.3 Detached leaf assays

Lettuce cultivars Berlo, Norden, Novita and Patricia were donated by Mr Adrian Berrevoets and cv. Little Gem was donated by Mr Mark Sutton (Scottish Agricultural College, Horticulture Adviser). Lettuce was sown in peat blocks measuring 35 x 35 x 35 mm (Gowrie Growers, Longforgan, by Dundee) and grown in a growth cabinet (Fisons G600) at 15°C with

16 h light (128 μmol.$m^{-2}s^{-1}$)/8 h dark and 75% relative humidity. Seedlings were used when eight leaves were unfolded. Detached leaves were maintained either as intact leaves on moistened filter paper or foam, or as leaf discs, each measuring 15 mm in diameter, on 0.5% distilled water agar (DWA) containing 120 ppm benzimidazole. The leaves were placed in boxes measuring 20 x 80 x 125 mm (Stewart Plastics) and each box contained either 4 intact leaves or 24 leaf discs placed with the adaxial (top) surface uppermost. Leaves were sprayed with 50 μg ml^{-1} of either Y3, Y4 or Y20 almost to run-off, incubated at 15°C for 24 h in the light (140 μmol.$m^{-2}s^{-1}$), blotted and air-dried to remove surface moisture. Inoculation with *B. cinerea* was carried out by placing 10 μl of a spore suspension containing 5 x 10^5 spores ml^{-1} onto the centre of each leaf disc. Boxes containing the inoculated material were placed in polythene bags to maintain high humidity and incubated at 15°C. The leaf discs were inspected at regular intervals and disease was estimated as the percentage of the leaf area infected by *B. cinerea*. All detached leaf tests were carried out three times.

2.4 Glasshouse experiments

Lettuce was sown in peat blocks and grown as described above for one week before transfer to propagators containing John Innes No.2 compost in the glasshouse. Glasshouse temperatures were maintained at 16°C minimum day temperature, venting at 21°C, with a 12°C minimum night temperature, and high humidity was maintained by mist irrigation. Four propagators each containing 12 plants (4 of each cvs Berlo, Little Gem and Patricia) were sprayed with either Y3 (50 μg ml^{-1}) or Y4 (50 μg ml^{-1}) 24 hours prior to inoculation. Inoculation was carried out by placing two barley seed infected with *R. solani* at the base of the growing plant adjacent to the stem and then spraying each plant with 1 ml of a *B. cinerea* spore suspension containing 5 x 10^4 spores ml^{-1}. The plants received two further treatments

at 10 day intervals following inoculation. The symptoms of *B. cinerea* infection cannot be distinguished unequivocally from those of *R. solani* infection and so an overall disease assessment was estimated at regular intervals as percentage of leaves showing disease symptoms.

2.5 Formulation of extracts

The following treatments were applied to leaf discs and intact plants of the cvs Berlo and Little Gem in order to assess the importance of formulation on the efficacy of yeast–derived elicitors: 1) No treatment; 2) Agral (0.02%); 3) LI–700 (Newman Agrochemicals Ltd.)(0.2%); 4) Y4 (50 μg ml^{-1}) in Agral (0.02%)($Y4^{A}$); 5) Y4 (50 μg ml^{-1}) in LI–700 (0.2%) ($Y4^{L}$). Three boxes each containing 24 leaf discs (12 of each cvs Berlo and Little Gem), and three propagators each containing 12 plants (6 of cvs Berlo and Little Gem) were set up for each treatment. Treatment was applied to leaf discs 24 hours prior to inoculation with *B. cinerea* as described above. In the glasshouse, plants were treated 24 hours prior to inoculation with both *R. solani* and *B. cinerea*, as described above, then treated again 7 days and 14 days after inoculation. Disease was assessed 10 days and 21 days after inoculation for the leaf discs and the intact plants respectively.

2.6 Effect of Yeast Extract on fungal growth

To determine the effect of Y4 on the growth of *B. cinerea* and *R. solani*, mycelial plugs (4 mm in diameter) taken from the growing margins of 4–day old colonies on PDA were placed in the centre of DWA plates supplemented with 10, 100 and 1000 μg ml^{-1} Y4. Colony diameter was measured daily and the colony area was calculated. The results expressed are

the mean of 4 replicates.

3. RESULTS

3.1 Elicitor Activity

Yeast cell wall extracts Y4 and Y20 each induced greater phytoalexin accumulation in soybean cotyledons than Y3 (Figure 1).

3.2 Leaf disc assay

Preliminary tests demonstrated that uninoculated lettuce leaf discs remained green for longer when maintained on 0.5% DWA containing 120 ppm benzimidazole than on either 0.5% DWA, moist filter paper or foam. The growth of *B. cinerea* on lettuce leaf discs maintained on 0.5% DWA containing 120 ppm benzimidazole was inhibited by approximately 8% when compared with those on 0.5% DWA. However, the benefit in terms of prolonged leaf viability outweighed this problem. In addition, this method enabled a large number of replicates to be treated at any one time, which is an important factor in a short term pilot project, and therefore was adopted as the standard technique for subsequent detached leaf assays.

Treatment of lettuce leaf discs with Y3, Y4 and Y20, 24 h prior to inoculation with *B. cinerea*, reduced subsequent infection on each cultivar tested but did not provide complete disease control (Figure 2). On untreated controls the most severe infection occurred on cv. Little Gem (58% leaf area infected) followed by Patricia (42%), Novita (28%), Berlo (18%)

and Norden (17%). Y3, Y4 and Y20 each reduced disease levels to between 5–14% on each cultivar except for cv. Little Gem where Y3 only reduced infection to 29%. Best disease control was obtained with Y4 on cv. Little Gem which reduced infection to 6% representing a 90% reduction in disease level compared to the untreated control. Overall, Y3, Y4 and Y20 reduced infection on all cultivars by 58%, 72% and 69% respectively. *B. cinerea* was successfully re–isolated from infected leaf discs sampled at random from each of the cultivars.

3.2 Glasshouse plants

Spray application of Y3 and Y4 onto cvs Berlo (Figure 3), Little Gem (Figure 4) and Patricia (Figure 5) in the glasshouse reduced the level of subsequent infection by *B. cinerea* and *R. solani* although they did not provide complete protection. Y4 was the most effective treatment and reduced disease by approximately 70% in cvs Little Gem and Patricia, and by approximately 50% in cv. Berlo. The 2nd and 3rd applications of Y3 and Y4, carried out 10 and 20 days after inoculation, each coincide with reductions in disease progress between 7–14 days and 21–28 days in each cultivar. Y4 was more effective than Y3 on both cvs Little Gem and Patricia but not on cv. Berlo where both extracts provided similar levels of disease control, although it should be noted that disease levels were generally lower in cv Berlo. *B. cinerea* and *R. solani* were each re–isolated from infected lettuce.

3.3 Formulation of extracts

Neither Agral nor LI–700 significantly reduced *B. cinerea* infection on leaf discs of cvs Berlo and Little Gem whilst $Y4^{A}$ and $Y4^{L}$ reduced disease by approximately 85% compared to

untreated controls (Figure 6). On whole plants (Figure 7) Agral did not reduce infection but LI-700 caused an increase of 22% and 19% in cvs Berlo and Little Gem respectively. $Y4^A$ reduced infection by 65% (Little Gem) and 68% (Berlo) when compared with untreated controls, whereas $Y4^L$ reduced infection by only 25% (Little Gem) and 22% (Berlo) compared to untreated controls, but by 37% (Little Gem) and 36% (Berlo) when compared to those treated with LI-700. Infection levels were higher in the glasshouse plants due to the presence of both *B. cinerea* and *R. solani*.

3.4 Effect of Y4 on fungal growth

Growth rate of both *B. cinerea* (Figure 8) and *R. solani* (Figure 9) was unaffected on DWA containing 10 μg ml^{-1} Y4 but was enhanced on DWA supplemented with 100 and 1000 μg ml^{-1} Y4. Microscopic examination of both fungi on DWA +/- 1000 μg ml^{-1} Y4 revealed no obvious differences in morphology resulting from the inclusion of Y4 in the media.

4. CONCLUSIONS

Spray application of water soluble resistance elicitors extracted from yeast cell walls protected lettuce leaf discs and intact plants against infection by *B. cinerea* and *R. solani*. Although Y4 and Y20 had higher phytoalexin elicitor activity than Y3, each treatment provided a similar level of disease control on leaf discs except on cv. Little Gem where Y4 and Y20 reduced *B. cinerea* infection by 90% and 85% respectively, whilst Y3 reduced infection by 50%. In the glasshouse the highest levels of disease control were obtained using Y4 which reduced *B. cinerea* and *R. solani* infection by approximately 70% on whole plants of cvs Little Gem and Patricia.

$Y4^L$ and $Y4^A$ each reduced *B. cinerea* infection on leaf discs by approximately 85%, whilst on whole plants $Y4^A$ reduced *B. cinerea* and *R. solani* infection by 65–68% compared with only 22–25% for $Y4^L$. A new formulation tested since the termination of funding reduced *B. cinerea* infection on cv. Patricia leaf discs (Plate 1) and whole plants (Plate 2) by approximately 92% and 85% respectively. These data demonstrate the importance of formulation and the considerable potential for further enhancing the performance of the yeast extracts. There were no phytotoxic or otherwise detrimental effects observed following application of any of the formulations containing yeast–derived resistance elicitors to any of the lettuce cultivars. Y4 showed no antifungal activity against either *B. cinerea* or *R. solani* on agar and, in contrast, actually encouraged growth at concentrations of 100 and 1000 $\mu g\ ml^{-1}$.

It is important to note that, unlike commercial practise, the lettuce in the glasshouse were deliberately grown under environmental conditions which would actively encourage disease in order to provide a stringent test for the yeast–derived elicitors. Therefore, the results obtained in this short study should not be taken as being the best that can be achieved using yeast–derived elicitors but rather as a demonstration of the principle of induced resistance as a viable means of disease control. The integrated use of yeast–derived elicitors in a spray regime with reduced rate fungicide may provide a viable means of reducing inputs in an integrated horticultural production system. This is an approach which has already been tested in field experiments on spring barley (Newton *et al.*, 1993; Reglinski *et al.*, 1993). Future research into the use of new formulations containing yeast–derived resistance elicitors, together with experimentation under commercial cultural conditions, would enable a more accurate assessment of the viability of this technique in terms of application rate, timing, and the frequency of application necessary for crop protection. Only under such conditions can

these elicitors be usefully compared with fungicide performance. Clearly this pilot project has demonstrated the principle of using non-toxic resistance elicitors derived from yeast cell-walls to be a viable crop protection strategy. The full potential of such an approach now needs to be investigated to determine whether adequate disease control can be achieved economically under conditions representative of normal horticultural practice.

Glossary

Elicitor – a substance that stimulates resistance mechanisms in plants.

Phytoalexins – low molecular weight antimicrobial compounds synthesized by and accumulated in plants in response to infection.

References

Cartwright D, Langcake P, Pryce R J, Leworthy D P, Ride J P, 1977. Chemical activation of host defence mechanisms as a basis for crop protection. Nature **267**, 511–513.

Coley-Smith J R, Ridout C J, Mitchell C M, 1991. Control of bottom rot disease of lettuce (*Rhizoctonia solani*) using preparations of *Trichoderma viride, T. harzianum* or tolclofos-methyl. Plant Pathology **40**, 359–366.

Elad Y, 1992. The use of antioxidants (free radical scavengers) to control grey mould (*Botrytis cinerea*) and white mould (*Sclerotina sclerotiorum*) in various crops. Plant Pathology **41**, 417–426.

Elad Y, Yunis H, Katan T, 1992. Multiple fungicide resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. Plant Pathology **41**, 41–46.

Forrest R S, Lyon G D, 1990. Substrate degradation patterns of polygalacturonic acid lyase from *Erwinia carotovora* and *Bacillus polymyxa* and release of phytoalexin–eliciting oligosaccharides from potato cell walls. Journal of Experimental Botany **41**, 481–488.

Griffin M J, Cox T, 1982. New controls for bottom rot. The Grower, 24 June 1982, pp 475–479.

Kuc J, 1987. Plant immunization and its applicability for disease control. In: Innovative approaches to plant disease control. Chet, I. (ed), John Wiley & Sons, New York. 255–274.

Lamb C J, Lawton M A, Dron M, Dixon R A, 1989. Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell **56**, 215–224.

Last F T, Hamley R E, 1956. A local lesion technique for measuring the infectivity of conidia of *Botrytis fabae* sardina. Annals of applied Biology **44**(3), 410–418.

LeClerg E L, 1941. Pathogenicity studies with isolates of *Rhizoctonia solani* obtained from potato and sugar beet. Phytopathology **31**, 49–60.

Lyon G D, Newton A C, Reglinski T, 1990. Pathogen control by defence mechanisms. Grower March 15th: 25–26.

Newton A C, Lyon G D, Reglinski T, 1993. Development of a new crop protection system using yeast extracts. Home–Grown Cereals Authority Project Report No. 78.

Reglinski T, Lyon G D, Newton A C, 1993. Assessment of the ability of yeast–derived resistance elicitors to control barley powdery mildew in the field. Journal of Plant Disease and Protection (In press).

Schonbeck F, Dehne H W, 1986. Use of microbial metabolites inducing resistance against plant pathogens. Microbiology of the Phyllosphere. Eds. Fokkema N J, Van den Heuvel J. Cambridge University Press 363–375.

Wareing P W, Zu–Nan Wang, Coley–Smith J R, 1986. Fungal pathogens in rotted basal leaves of lettuce in Humberside and Lancashire with particular reference to *Rhizoctonia solani*. Plant Pathology **35**, 390–395.

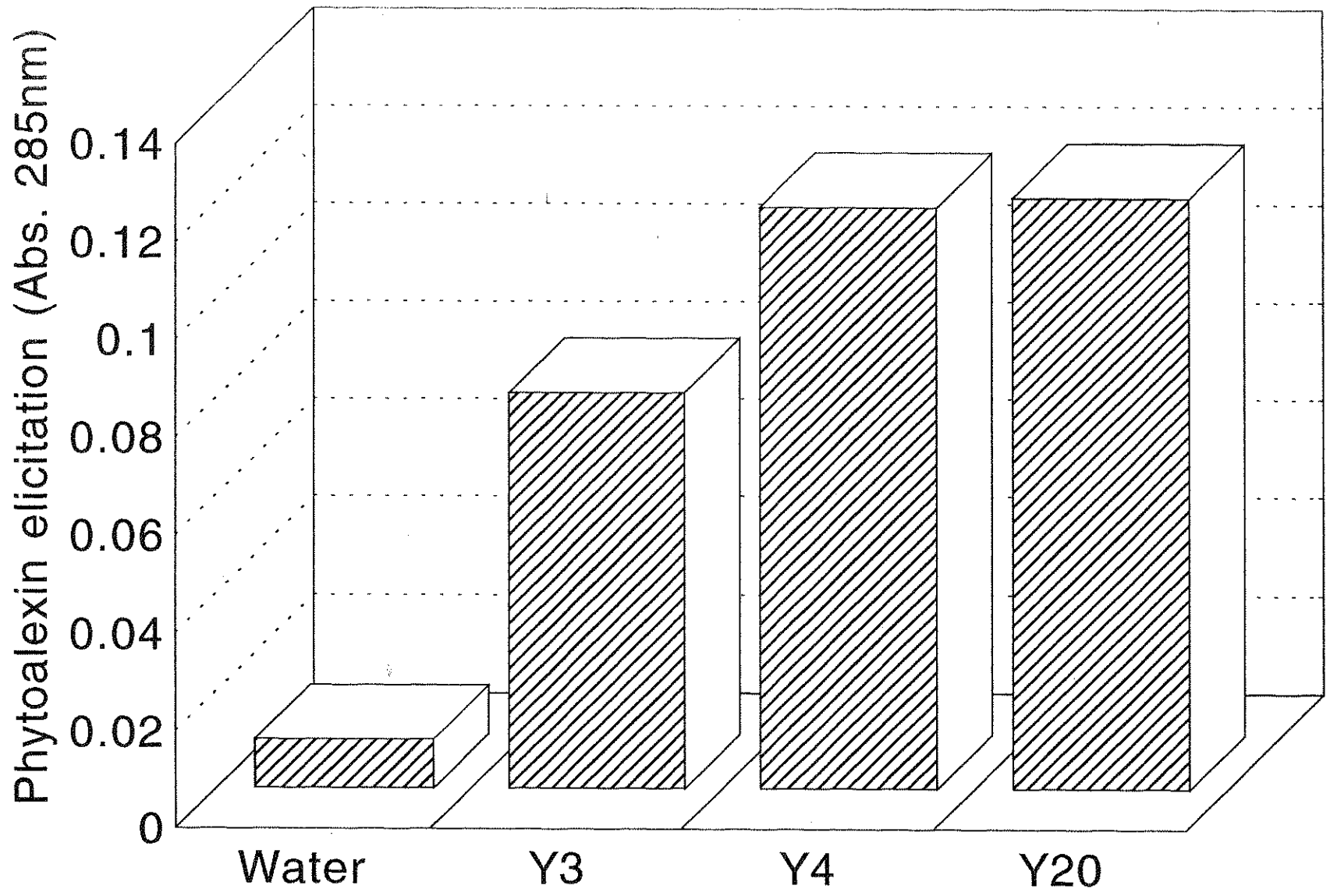

Fig. 1 Phytoalexin elicitor activity of yeast cell-wall extracts (50mg/l)

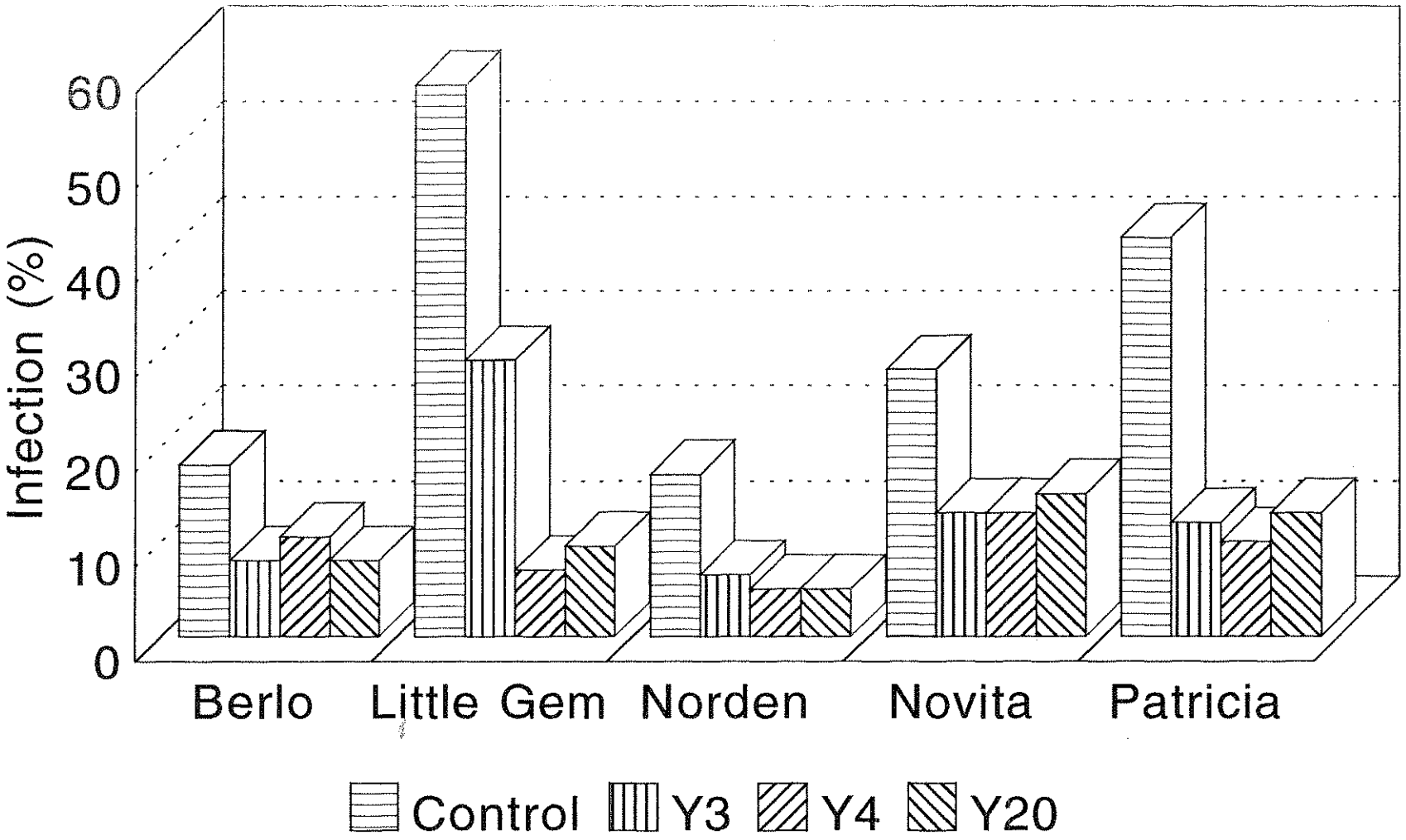

Fig. 2 Leaf discs were sprayed with yeast cell-wall extracts 24 hours before inoculation.
Leaves were inoculated with *B. cinerea* (5000 spores/leaf disc).
Infection (%) was assessd 7 days after inoculation. S.E.D.=3.67

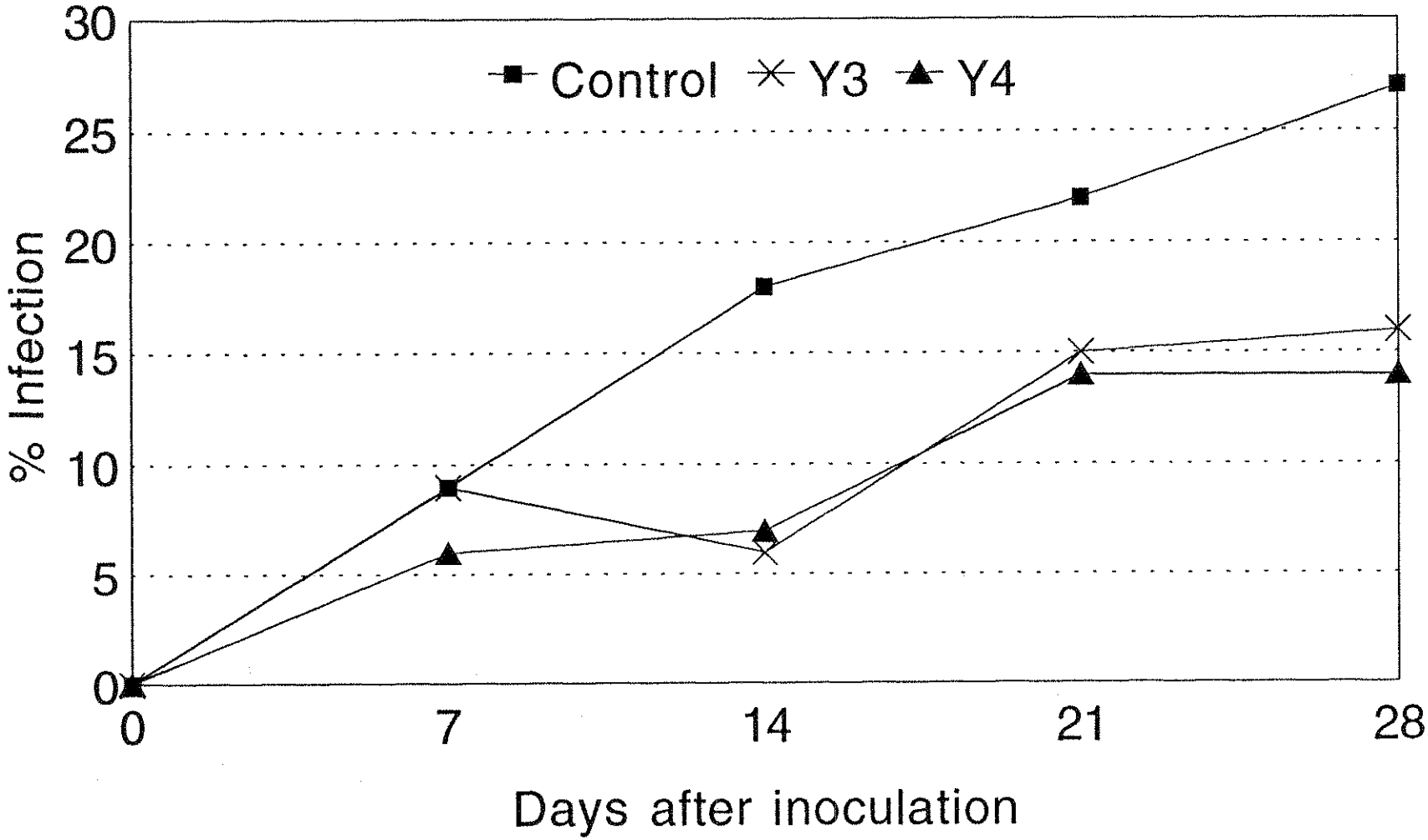

Fig.3 Disease progress of *B. cinerea* and *R. solani* on cv. Berlo in the glasshouse
Plants were treated 24 hours before inoculation and then again after 10 days and 20 days.

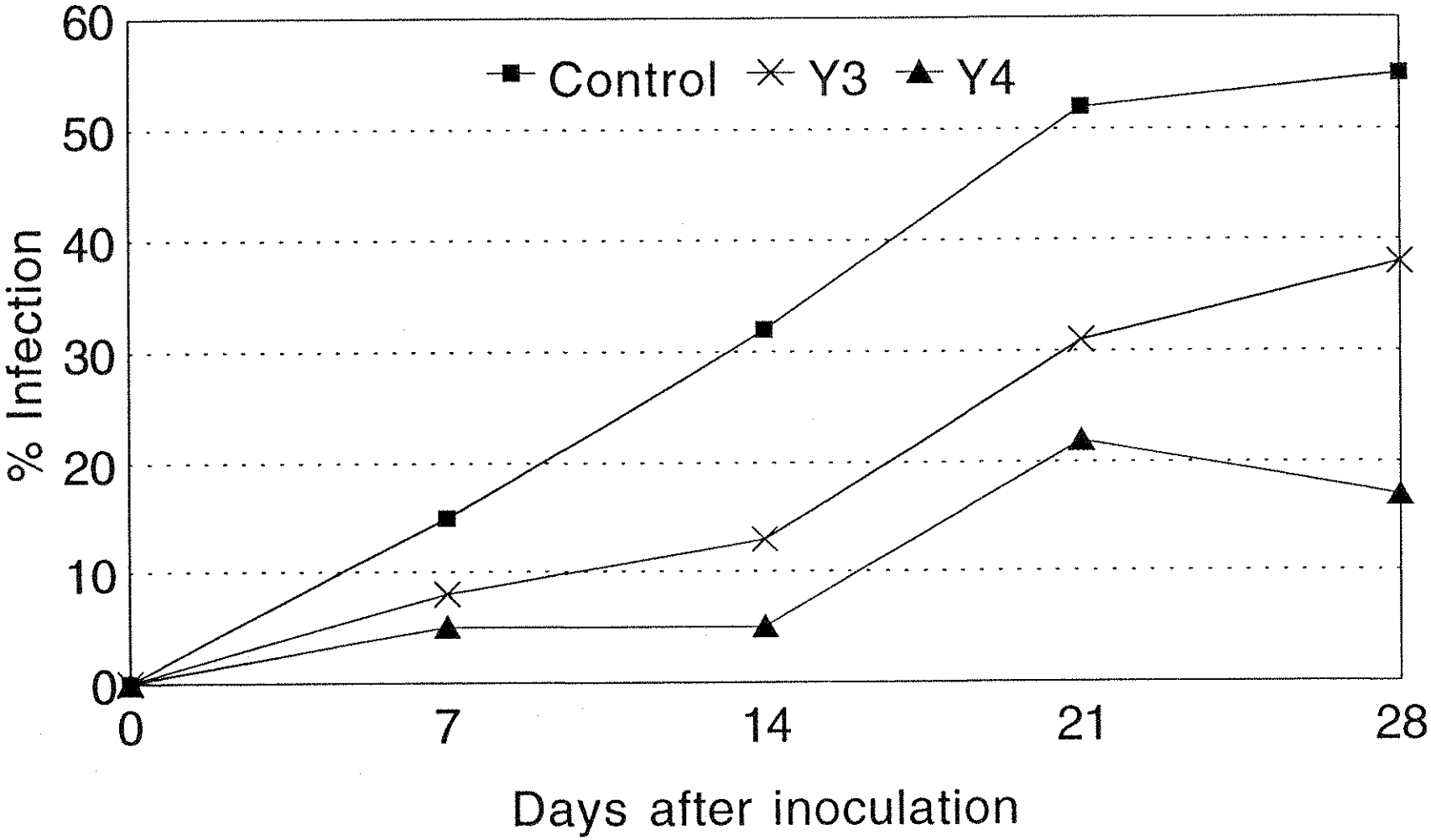

Fig.4 Disease progress of *B. cinerea* and *R. solani* on cv. Little Gem in the glasshouse
Plants were treated 24 hours before inoculation and then again after 10 days and 20 days.

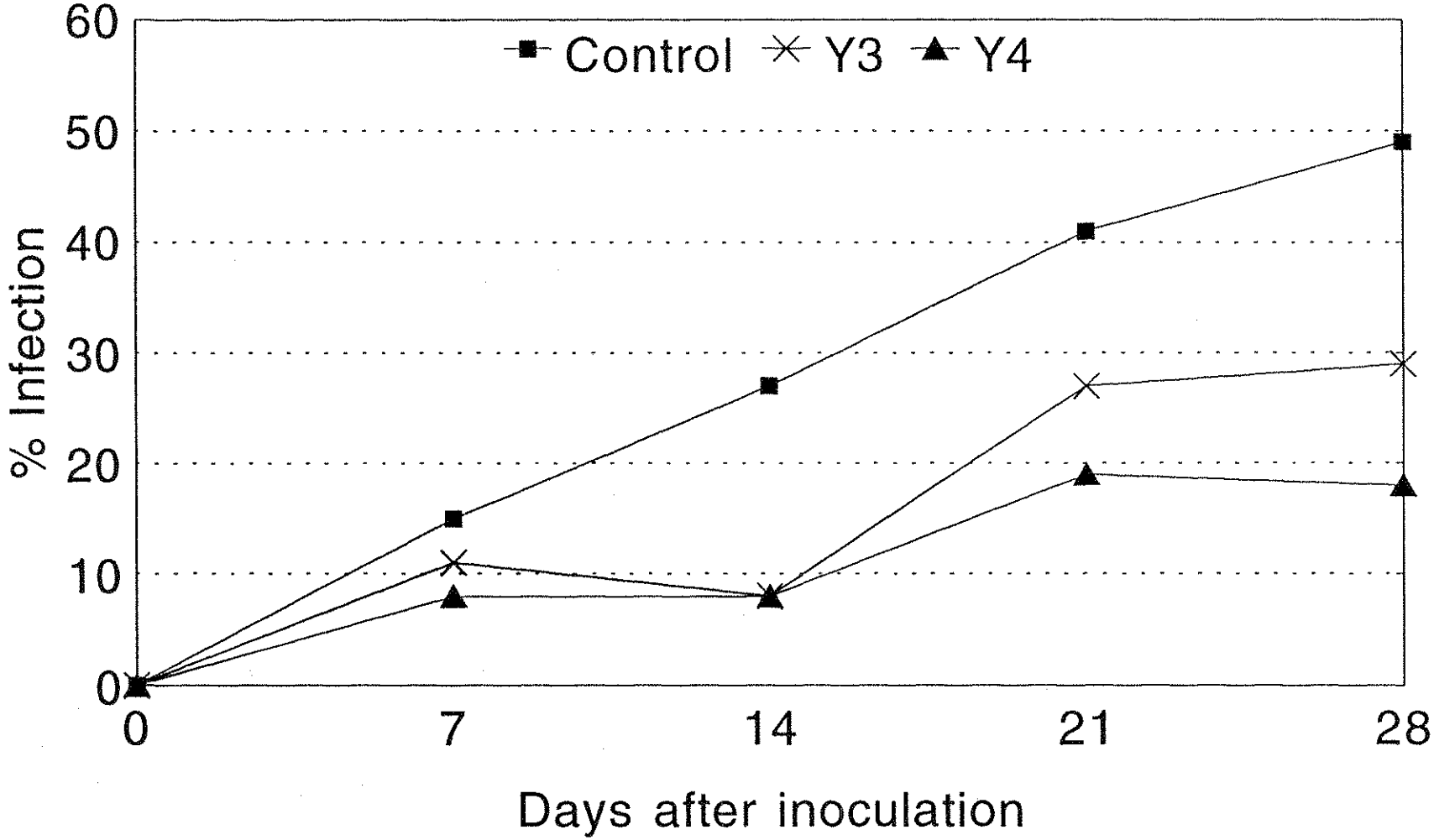

Fig.5 Disease progress of *B. cinerea* and *R. solani* on cv. Patricia in the glasshouse
Plants were treated 24 hours before inoculation and then again after 10 days and 20 days.

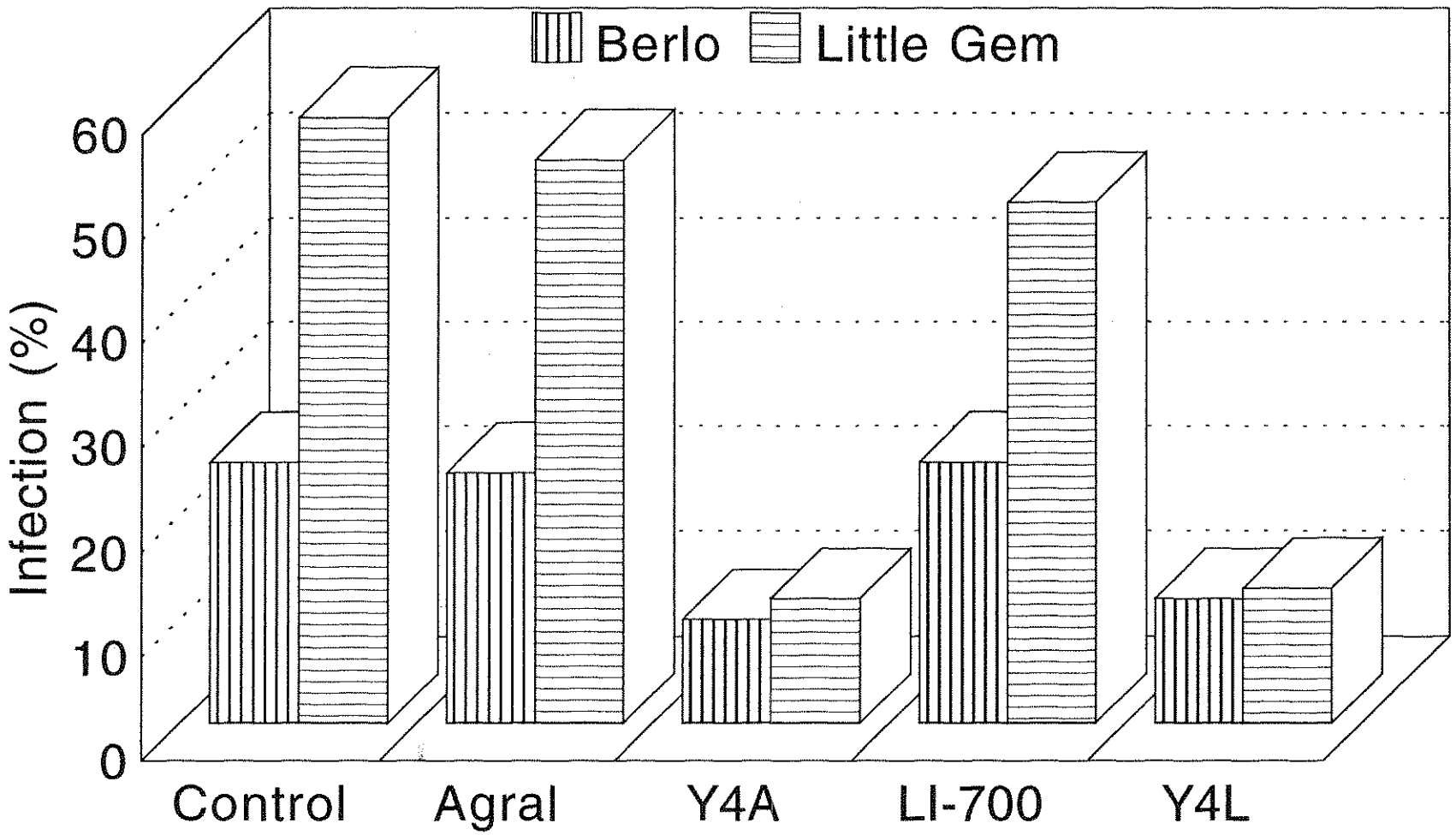

Fig.6 Effect of formulation on disease control on lettuce leaf discs
Leaves were treated 24 hours before inoculation with *B. cinerea* (5000 spores/leaf)
Infection(%) was assessed 10 days after inoculation. S.E.D.= 3.21

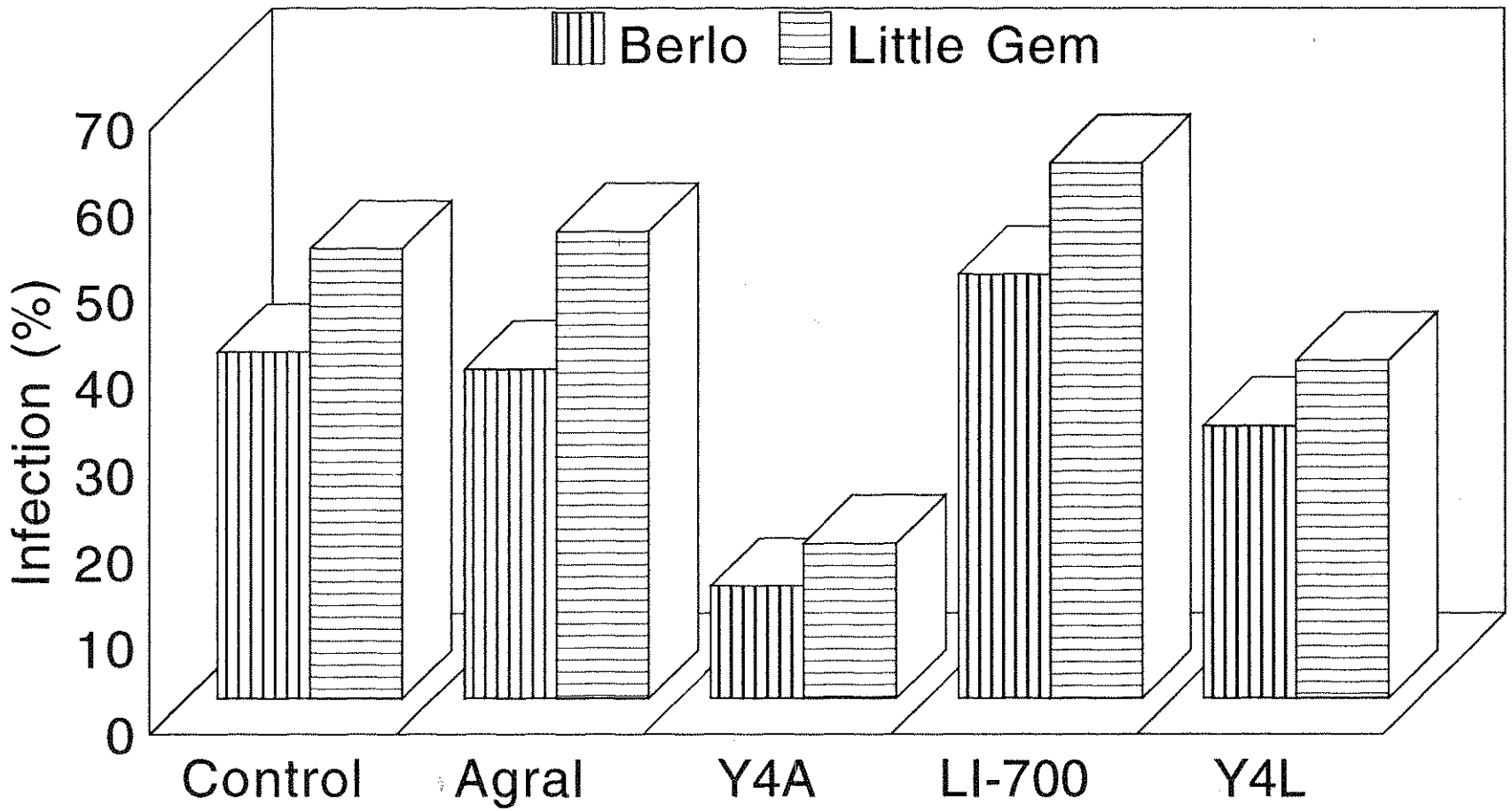

Fig.7 Effect of formulation on control of *B. cinerea* and *R. solani* in the glasshouse.
Plants were treated 24 hours before inoculation and then again 7 days and 14 days after inoculation.
Infection (%) was assessed 21 days after inoculation. S.E.D.=4.13

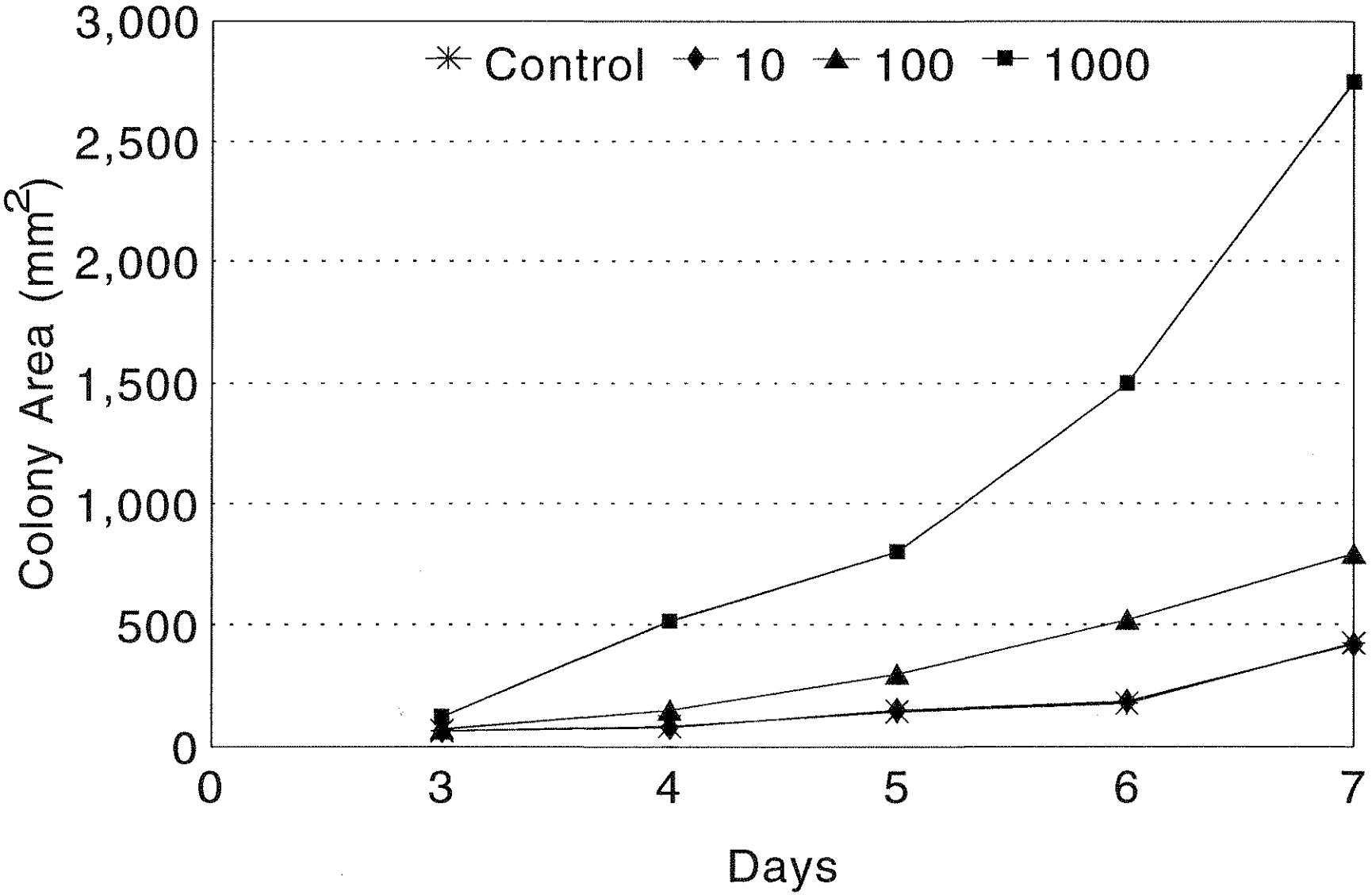

Fig. 8 Growth of *B. cinerea* on DWA supplemented with Y4 at at 10-1000 mg/l

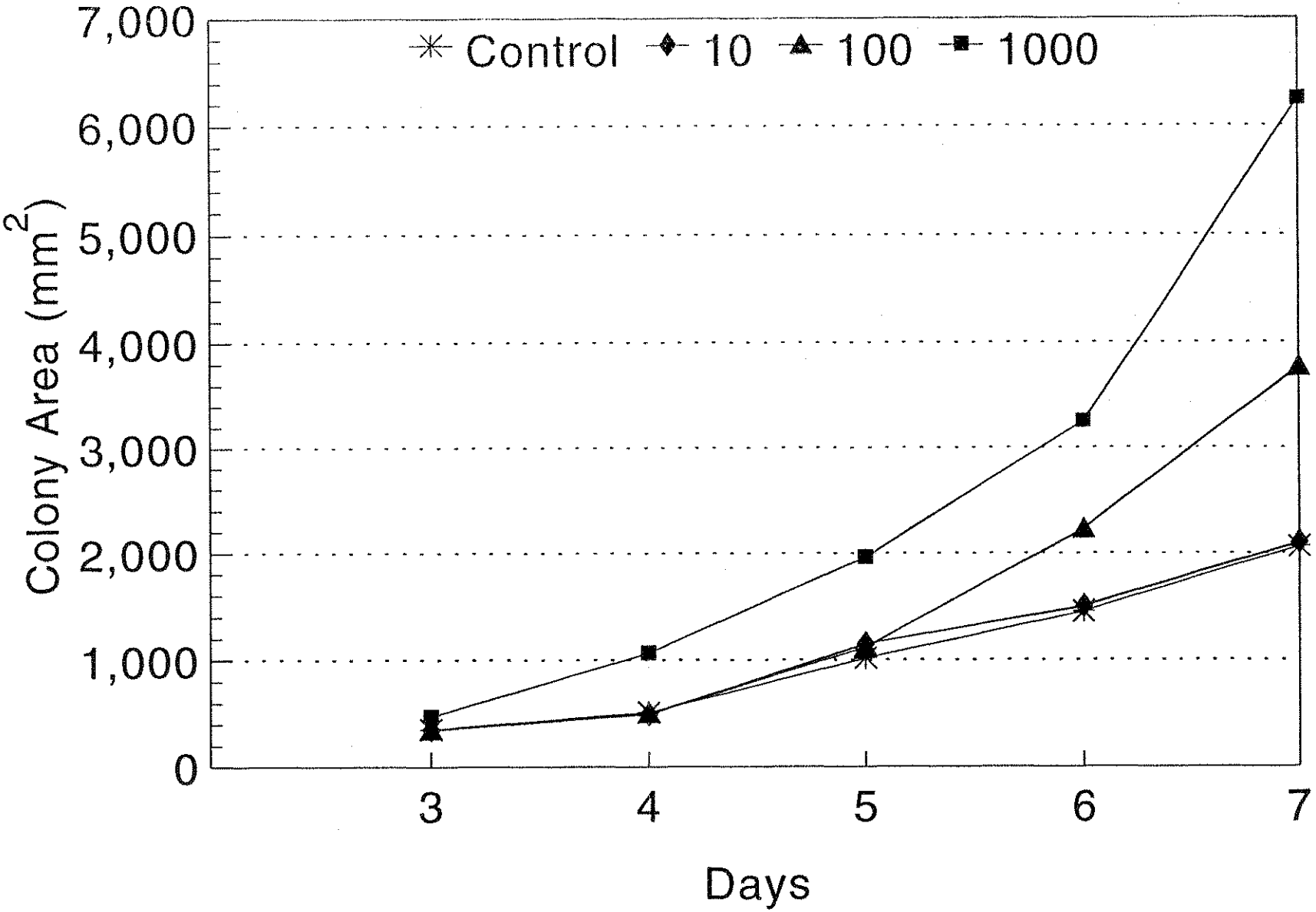

Fig. 9 Growth of *R. solani* on DWA supplemented with Y4 at 10-1000 mg/l

Appendix

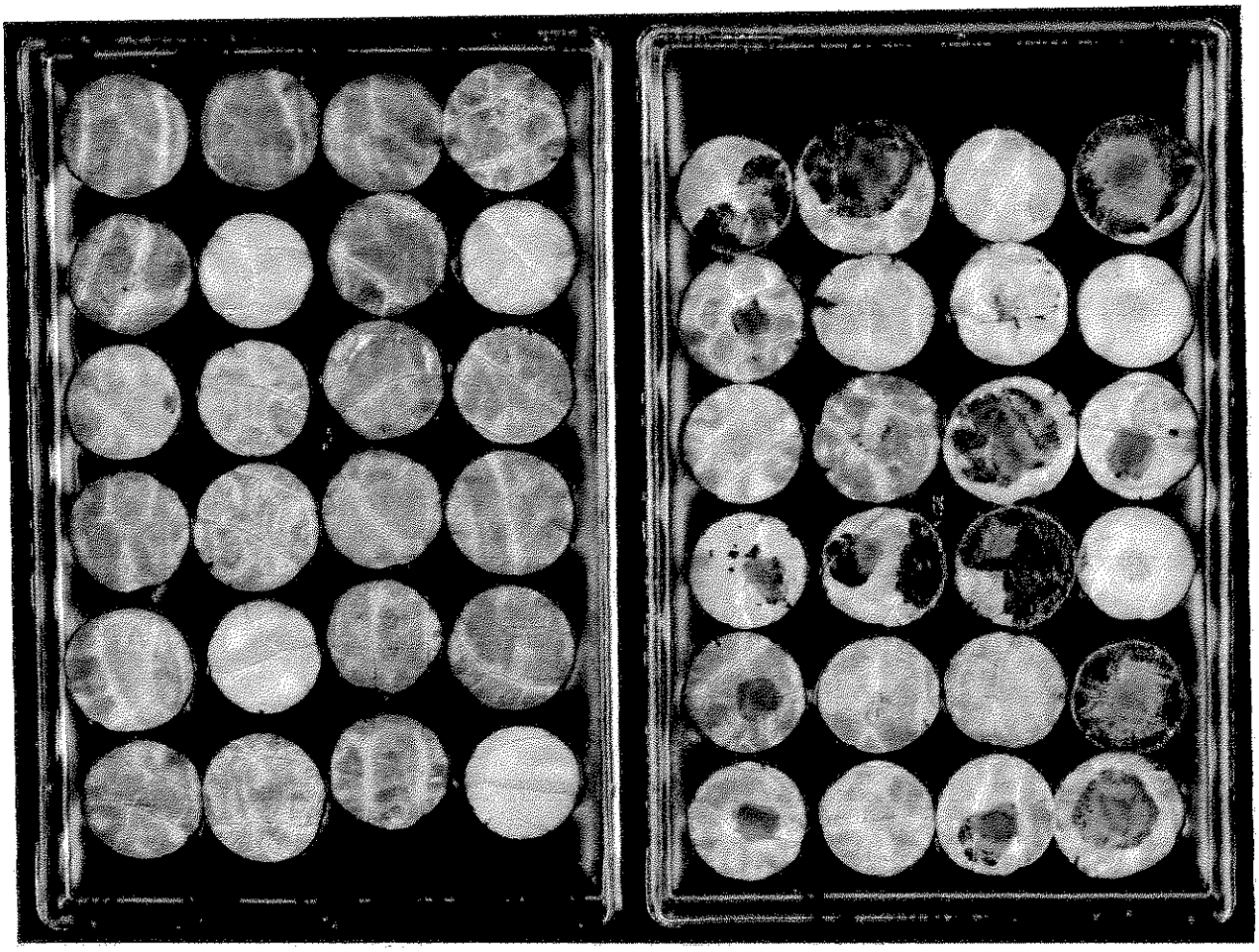

Plate 1

Leaf discs of cv. Patricia were treated then incubated at 15^{o}C for 24 hours in the light, and air dried to remove surface moisture. Inoculation with *B. cinerea* was carried out by placing 10 µl of a spore suspension containing 5 x 10^{5} spores/ml onto the centre of each leaf. Boxes containing the inoculated material were placed in polythene bags to maintain high humidity and incubated at 15^{o}C. The photograph was taken 10 days after inoculation and shows the treated leaves on the left and a control on the right.

Plate 2

Propagators containing cv. Patricia were treated then incubated at $15^{o}C$ for 24 hours (16h light/8h dark) in the growth cabinet. Inoculation with *B. cinerea* was carried out by spraying 10 ml of a spore suspension containing 2×10^{5} spores/ml evenly over each propagator. The photograph was taken 21 days after inoculation and shows the treated plants on the left and a control on the right.

1. TITLE OF PROJECT

Expected Contract Number: PV/FV 135

Lettuce: a non-toxic crop protection system for lettuce (and other vegetable crops)

2. BACKGROUND AND COMMERCIAL OBJECTIVE

A novel crop protection system is being developed at SCRI using extracts from yeast cell walls to stimulate plant resistance mechanisms thereby minimising the need for application of toxic fungicides. This mode of action means that pathogens are unlikely to build up resistance to the crop protectant. It will also enable the life of otherwise useful varieties which have lost their disease resistance to be extended. Field spray applications to spring barley have succeeded in reducing mildew infection by up to 70% and increase yield and quality levels to those of fungicide controls.

The work is funded by H-GCA and is therefore restricted to cereals. However the principles on which it is based are derived from our core strategic programme on resistance mechanisms and therefore we believe that the methods can be adapted to successfully control diseases of many other crop plants as we can demonstrate stimulation of the plant's physical and chemical resistance mechanisms. We have considerably enhanced resistance eliciting activity of extracts over the last two years in laboratory and glasshouse tests on cereals. In field tests the frequency of spray applications is still greater than desirable for cereal crops and not as effective as it is in the laboratory, probably due to lack of formulation, especially rainfastness. However, these problems do not affect many horticultural crops, particularly protected glasshouse crops with higher value and shorter life cycles.

We have selected lettuce as the most appropriate for a preliminary study for several reasons:

1. there is concern over the application of toxic fungicides to foliage which is for human consumption

2. relatively frequent spray applications are feasible

3. there are several disease problems which are likely to be controlled by resistance elicitors

4. a lack of alternative crop protection methods.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

We see the main benefit to the industry as being the ability to control pathogens without the use of toxic chemicals which are frequently perceived as unacceptable by the general public on food products where the sprayed component is eaten. Yield should be increased or wastage reduced in crops which currently use no control methods, and a price premium may be envisaged if this control method is deemed

'organic'.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

We propose to use a range of our extracts and formulations from yeast cell walls on some diseases of lettuce to:

1. determine whether control of the inoculated diseases is possible
2. find the most effective extract
3. assess which is the best disease control spray regime
4. ensure that there are no detrimental effects of the sprays.

For this purpose we propose to look at Downy mildew (*Bremia lactucae*) and grey mould (*Botrytis cinerea*).

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

The current work is funded by H-GCA and is therefore restricted to pathogens of cereals. In addition, PMB have agreed to fund a studentship to look at control of potato diseases. The work is underpinned by strategic studies (SOAFD funded) which indicate that these extracts should work on a wide range of crops against a variety of pathogens.

Our current H-GCA grant expires on 4 December 1992 and we are applying for funding from MAFF in conjunction with ADAS to continue the work from April 1993. This gives us the opportunity to spend the four month intervening period to investigate the wider potential of this approach to crop protection and to maintain continuity of the work overall.

6. DESCRIPTION OF WORK

Appropriate inoculation and assessment methods will be devised for downy mildew (*Bremia lactucae*) and grey mould (*Botrytis cinerea*) on control plants of a range of susceptibilities. One resistant and two susceptible varieties will be selected for testing with single sprays of a range of elicitor extracts. The best extracts will be tested with a range of formulations. Repeat spray regimes will be used in conjunction with continual inoculation for optimum application technique and to ensure that there are no detrimental effects of the sprays.

If results from this exploratory study look promising and potentially economically viable we would wish to discuss the future of this aspect of the resistance elicitors with the HDC to encourage support for its commercial application in the longer term.

7. COMMENCEMENT DATE AND DURATION

Commence 4 December 1992 for 4 months.

8. STAFF RESPONSIBILITIES

Project leaders:	Dr Adrian C Newton and Dr Gary D Lyon
Other staff:	Dr Tony Reglinski

9. LOCATION

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA