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MONITORING SENSITIVITY TO THE DMI GROUP OF FUNGICIDES IN POPULATIONS OF THE LEAF BLOTCH PATHOGEN (*Septoria tritici*) AND THE GLUME BLOTCH PATHOGEN (*Septoria nodorum*) OF WINTER WHEAT

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by

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Abstract

Leaf blotch, caused by the pathogen *Septoria tritici* is the most damaging disease recorded in the CSL/ADAS National Winter Wheat Disease Survey of England and Wales. Products in the 'DMI' group (e.g. propiconazole, prochloraz, triadimenol, flutriafol, flusilazole, cyproconazole, tebuconazole) now dominate, almost entirely, fungicide use in winter wheat. Most fungicides that offer effective control of leaf blotch belong to the DMI group and further base-line data on current sensitivity are required in order to help rapidly detect changes in sensitivity that may lead to resistance problems and failure of disease control. Leaves showing symptoms of leaf blotch from the 1990, 1991 and 1992 CSL/ADAS National Winter Wheat Disease Surveys were used to establish isolates of the fungus from all the cereal growing areas of England and Wales. During the three years of the study a total of 413 survey samples provided 1889 lesions from which 693 isolates were obtained. Of these, 687 isolates were tested successfully for their *in-vitro* sensitivity to three DMI fungicides, flutriafol, prochloraz, propiconazole and an MBC fungicide, benomyl, in 1990 (278 isolates) and to four DMI fungicides, flutriafol, prochloraz, propiconazole and triadimenol in 1991 and 1992 (409 isolates). In addition to isolates obtained from the survey samples during 1991 and 1992, 189 isolates were obtained from leaf tissue, sent to CSL by ADAS and other organisations, taken from crops in which the degree of leaf blotch control was much less than expected, i.e. instances of apparent disease control failure or partial failure. These 189 isolates were also tested successfully. Benomyl was excluded in 1991 and 1992 because it was obvious from the 1990 results that populations of *S. tritici* remained resistant to this fungicide.

Whilst collecting leaf tissue for isolation of *Septoria tritici*, lesions caused by *Septoria nodorum* on leaves and ears were also collected for isolation. During 1990 83 leaf samples provided 189 lesions that yielded 40 isolates all of which were tested successfully for their *in-vitro* sensitivity to the four fungicides used in 1990. Few lesions caused by *Septoria nodorum* were collected during 1991 and 1992 and no isolates were obtained for sensitivity-testing. Concentrations of 0.05, 0.5 and 5 µg ai/ml agar were used for flutriafol, prochloraz and propiconazole, 0.1, 1 and 10 µg ai/ml agar for triadimenol (1991 and 1992) and 10, 25 and 50 µg ai/ml for benomyl (1990). There were indications that *S. tritici* was less sensitive to triadimenol *in-vitro* compared to the other three DMI fungicides. Flutriafol appeared to be more inhibitory *in-vitro* during 1992 than to the first two years of the project. Sensitivity varied between isolates but showed no reduction over that found in earlier work for prochloraz and propiconazole. *In-vivo* testing using plant inoculations with pycnidiospores confirmed the *in-vitro* results with flutriafol, prochloraz and propiconazole providing 50, 60 and 80 % control of the disease at 5 µg ai/ml whilst triadimenol gave only 22 % control of the disease at 10 µg ai/ml. Results for benomyl in 1990 confirmed that the high incidence of resistance to this

fungicide had been maintained in populations of *S. tritici*. Results for *S. nodorum* at 0.05, 0.5, 5 µg ai/ml for all fungicides were similar to those for *S. tritici*; however, with the exception of benomyl, a greater percentage of *S. nodorum* isolates grew at a given concentration of each fungicide.

Introduction

Leaf blotch and glume blotch, caused by the fungi *Septoria tritici* Rob. and Desm. and *Septoria nodorum* Berk. respectively, are responsible for yield loss and reduction in quality in wheat crops throughout the world (Shipton *et al.*, 1971; Eyal *et al.*, 1987). During the 1970's and early 1980s these two diseases were recognised as increasingly important, particularly as pathogens reducing yield on dwarf and semi-dwarf wheat cultivars (King *et al.*, 1983). In the UK up to the early 1980's *S. nodorum* was the most important of the two pathogens but thereafter *S. tritici* predominated (Polley & Thomas, 1991). A similar trend has become apparent in other regions of the world where wheat is grown (Weise, 1987).

In England and Wales fungicides are widely used for the control of leaf and possible ear disease caused by the two *Septoria* species as well as other pathogens. During the period 1985-1992 up to 95% of the national winter wheat crop received one or more fungicide applications for leaf and ear disease control from the first node growth stage onwards (Thomas, 1985-89; Polley, 1990-92). The majority of these fungicide applications included at least one representative from the DMI group of fungicides. Since their introduction to the UK in 1985 when 48% of sprayed crops received one or more applications of a DMI fungicide an increasing number of crops have been similarly treated; during 1992 92% of crops received one or more applications of a DMI fungicide. Farmers and their advisers have recognised the valuable eradicant and protectant properties of this group of fungicides and therefore they are used extensively, particularly for the control of the diseases caused by *Septoria* spp. for which the infection event is difficult to identify with certainty.

To date there appears to be no published record of resistance to DMI fungicides in populations of either *Septoria* species but extensive use clearly increases the potential risk of pathogen resistance or reduced sensitivity to this valuable group of fungicides.

Sensitivity within populations of cereal crop pathogens has been monitored at Long Ashton Research Station for a number of years. It is only recently that rare, individual spore isolates have been found in populations of *S. tritici* that are capable of growing on agar amended with significant concentrations of a DMI fungicide (Hollomon, 1990). Failure to control either of the diseases caused by *Septoria* spp. in the field has yet to be demonstrated conclusively but

base-line data on sensitivity to DMI fungicides in current field populations of *S. tritici* and *S. nodorum* is required in order to detect any future shift towards insensitivity. Should this occur, alternative control strategies may be developed before the breakdown of control presents a serious threat.

The annual national survey of diseases in winter wheat, carried out by CSL and ADAS, affords an ideal opportunity to monitor the sensitivity of the two pathogens as up to 400 crops from throughout England and Wales are assessed at the milky-ripe growth stage (GS73). Sensitivity to four of the principal triazoles commonly used, either directly or indirectly, for septoria control - flutriafol, propiconazole, prochloraz and triadimenol have been examined in isolates established from crops grown in the 1990 - 1992 harvest years. Sensitivity testing of both *S. tritici* and *S. nodorum* to benomyl was performed in 1990 but excluded during 1991 and 1992 because the results from the 1990 survey clearly indicated that the populations of *S. tritici* remained resistant to this fungicide.

Objectives

To monitor populations of *Septoria tritici* and *Septoria nodorum* for and establish base-line sensitivity to DMI fungicides. Samples were obtained from winter wheat crops for a 3-year period (1990, 1991, 1992) as part of the CSL/ADAS National Winter Wheat Disease Survey.

Materials and methods

Sampling

Plant samples sent to Harpenden during mid-June to early August as part of the CSL/ADAS National Winter Wheat Disease Survey were assessed for the percentage leaf area affected by *S. tritici* and *S. nodorum* on the flag and second leaves. Leaves with symptoms characteristic of *S. tritici* and *S. nodorum* were removed, wrapped in damp tissue, placed in a polythene bag, labelled with the sample number and stored in a refrigerator. For samples with a low incidence of both species on the top two leaves, samples of leaves three and four with symptoms caused by septoria were also stored as described. Ears with symptoms of glume blotch were stored in a similar manner. Up to 10 leaves and ears per field sample were stored prior to isolation. In addition to the survey samples, leaf material, taken from crops where the degree of leaf blotch control was less than expected, i.e. instances of apparent disease control failure or partial

failure, was sent to CSL by ADAS and other organisations. These leaf samples were handled in the same manner as those from the survey.

Isolation

As soon as possible and certainly within 48 hours of selection and storage, leaf and ear glume samples were removed from the refrigerator and lesions of *S. tritici* and/or *S. nodorum* cut from them. Excess leaf tissue, which may have contained saprophytic fungi, was removed from around the lesion caused by the pathogens. Lesions were washed in sterile distilled water and placed onto sterile, damp filter paper in sterile plastic Petri plates. The latter were incubated under near ultra-violet light for 24 hours to encourage sporulation. The aim was to prepare a total of 10 lesions, one from each of 10 separate leaves or ears, resulting in one lesion per leaf or per ear (= one isolate and a total of 10 isolates per survey sample).

After 24 hours incubation the majority of lesions had sporulating pycnidia. The latter were examined under a binocular microscope for the presence of the pink-buff or buff-coloured cirrhi that were transferred aseptically, using a sterile needle, and 'streaked' onto potato dextrose agar (PDA) or V8-Czapek Dox (V8CD - *S. nodorum* only) amended with 100 µg ai/ml rifamycin and 125 µg ai/ml streptomycin. The plates were then incubated at 20 °C until growth was evident, i.e. pink yeast-like colonies, on the surface of the agar (*S. tritici*) or creamy-white mycelial growth with embedded pycnidia (*S. nodorum*). Isolates were confirmed as those of *S. tritici* or *S. nodorum* by examination under a binocular or compound microscope for the presence of the characteristic pycnidiospores.

Isolates confirmed to be those of *S. tritici* were transferred aseptically and streaked separately across unamended PDA. These plates were then incubated for 7 - 14 days, the resulting colonies flooded with a sterile 10% solution of skimmed milk and pycnidiospores removed from the agar surface by gentle rubbing with a sterile glass loop. This spore suspension was then decanted into sterile Eppendorf tubes, sealed with parafilm and stored in the freezer. Isolates confirmed as *S. nodorum* were sub-cultured, if necessary, to remove contaminants and the resulting plate flooded with 10% sterile skimmed milk once pycnidia were readily apparent. Pycnidiospores were released by rubbing the surface of the agar with a sterile glass loop and the spore suspension stored as for *S. tritici*.

An alternative method was employed for leaf and ear lesions typical of those caused by *S. nodorum* but which had no pycnidia apparent at the time of sampling or failed to produce pycnidia within 48 hours of incubation. All such samples were stored dry and labelled with the

sample number. At a later date these dried samples were incubated, after washing with alcohol and sodium sulphite, on damp filter papers sealed in plastic Petri dishes under near ultra-violet light. Samples were examined at intervals for the presence of pycnidia. When apparent the cirrhi were transferred aseptically to PDA and V8-CD and used directly as mycelial isolates or stored as frozen spore suspensions in 10% sterile skimmed milk.

This method of preservation enabled the pycnidiospores of *S. tritici* and *S. nodorum* to be stored successfully and to maintain their viability for at least six months or more. During the three years of the study a total of 413 survey samples provided 1889 lesions from which 693 isolates were obtained. Of these, 687 isolates were tested successfully for their *in-vitro* sensitivity to three DMI fungicides, flutriafol, prochloraz, propiconazole and an MBC fungicide, benomyl, in 1990 (278 isolates) and to four DMI fungicides, flutriafol, prochloraz, propiconazole and triadimenol in 1991 and 1992 (409 isolates). In addition to isolates obtained from the survey samples during 1991 and 1992, 189 isolates were obtained from leaf tissue from crops in which the degree of leaf blotch control was much less than expected, i.e. instances of apparent disease control failure or partial failure. These 189 isolates were also tested successfully. Benomyl was excluded in 1991 and 1992 because it was obvious from the 1990 results that populations of *S. tritici* remained resistant to this fungicide. Whilst collecting leaf tissue for isolation of *Septoria tritici*, lesions caused by *Septoria nodorum* on leaves and ears were also collected for isolation. During 1990 83 leaf samples provided 189 lesions which yielded 40 isolates all of which were tested successfully for their *in-vitro* sensitivity to the four fungicides used in 1990. Few lesions caused by *Septoria nodorum* were collected during 1991 and 1992 but no isolates were obtained for sensitivity-testing.

Sensitivity testing - *in-vitro*

The frozen spore suspensions were allowed to defrost and then streaked across the surface of PDA amended with fungicide to the appropriate concentration of 0.05, 0.5 and 5 µg ai/ml agar for flutriafol, prochloraz and propiconazole and 0.1, 1 and 10 µg ai/ml agar for triadimenol. In 1990 only, concentrations of 10, 25 and 50 g µg ai/ml agar were used for benomyl. Each isolate was also streaked across unamended PDA as a control. The Petri plates were sealed with parafilm to prevent desiccation, labelled with the sample number, isolate number and the fungicide concentration, then incubated at 20 °C under near ultra-violet light.

Petri plates were examined after seven days' incubation. Growth of *S. tritici* was recorded, on fungicide-amended agar plates, on a presence or absence basis (1990 only) and as a percentage of the total colony area of the unamended control agar occupied by colonies of *S. tritici*, i.e. an estimate of relative density or percentage of pycnidiospores able to germinate and grow at each

fungicide concentration relative to the control for that isolate (1991 and 1992). Atypical colony growth was confirmed as that of *S. tritici* by microscopic examination.

Sensitivity testing - *in-vivo*

An *in-vivo* experimental which measured sensitivity of isolates to the four triazoles and their pathogenicity was examined using plant inoculations with a sub-set of isolates of *S. tritici* that had grown at a range of fungicide concentrations *in-vitro*. Five seeds of winter wheat cv. Longbow or Riband were sown in 7.5 cm pots containing John Innes No. 2 potting compost. These were grown for twenty-one days and then all but one were removed, leaving only one plant per pot. Four isolates, representing the range of *in-vitro* sensitivity, and three fungicide concentrations plus an untreated control gave 16 treatments which were replicated three times. Plants were inoculated with 50 ml of a spore suspension containing 10^6 spores/ml obtained from separate sample isolates. The spore suspensions were separately sprayed on to the plants using a 'Humbrol Airspray' until maximum retention of discrete droplets. The inoculum was allowed to dry for one hour, and then the spore suspension was applied a second time, again to maximum droplet retention. Then the plants were well watered and placed under cloches to ensure a humid environment. After 48 hours, the cloches were removed and the plants were allowed to dry. Then, each group of three plants was treated with the appropriate concentration of the four fungicides at 0.05, 0.5 and 5 µg ai/ml for flutriafol, prochloraz and propiconazole and 0.1, 1 and 10 µg ai/ml for triadimenol. The plants were sprayed with the fungicide using a 'Humbrol Airspray' to maximum droplet retention. The control plants were similarly sprayed with distilled water. Twenty-one days after inoculation the percentage leaf area affected by *S. tritici* was assessed.

Results

Septoria tritici: 1990-1992

After five days, colony growth was seen on all the unamended PDA control plates. The growth of *S. tritici* on the agar appeared as mucose, pink-flesh coloured colonies. The colonies were circular, convex, smooth and had irregular margins. After 5 - 10 days mycelium was observed protruding from the margins of such colonies. If incubated further colonies turned dark brown in colour. However, on fungicide-amended PDA, particularly at the higher concentrations, colonies appeared folded and crystalline in texture. Microscopic examination of the pycnidiospores revealed the presence of nodulations and vesicles and the absence of germ

tubes. It appeared that such colonies were not viable. The development of such atypical colonies was adopted as the standard for non-viable growth. On the basis of the dose-response curves (Figure 1) generated in 1990 all tests of fungicide sensitivity were undertaken at 0.05, 0.5, 5 µg ai/ml for flutriafol, propiconazole and prochloraz and 0.1, 1, 10 µg ai/ml for triadimenol. For benomyl in 1990 only, concentrations of 10, 25 and 50 µg ai/ml were used (Figure 2).

1990

The results of testing the 1990 'population' of 278 isolates of *S. tritici* are shown in Figure 3. Prochloraz and propiconazole at 0.5 µg ai/ml inhibited growth of 80% or more of the isolates; flutriafol only achieved this at µg ai/ml, a concentration 10 times that of the other two triazole fungicides. Benomyl inhibited growth of 80% or more of the isolates but only at 25 µg ai/ml, 5 times that of flutriafol; even at 50 µg ai/ml a small percentage of the isolates were able to grow. No *in-vivo* results were obtained in 1990.

1991

The results of testing the 1991 survey 'population' of 266 isolates of *S. tritici* are shown in Figures 4 and 5. Prochloraz and propiconazole at 0.5 µg ai/ml inhibited growth of 40-50% or more of the isolates, the remainder growing to only 10% of that of the unamended controls; flutriafol only achieved this level of inhibition at 5 µg ai/ml although several isolates did grow to a degree similar to that of the unamended controls. Triadimenol was considerably less inhibitory than flutriafol with at least 25-30% of isolates growing to a degree of 50% or more of that of the unamended controls.

The result of testing the 156 isolates obtained from the 'non-survey' samples were very similar to those obtained from the survey samples although all fungicides appeared slightly less inhibitory of this 'population' from non-survey crops. A comparison between the two 'populations' (survey and non-survey) is shown in Figure 4 (propiconazole and prochloraz) and Figure 5 (flutriafol and triadimenol). Although there is slight variation in the shape of the corresponding (fungicide and concentration) histograms the growth distributions are very similar. No *in-vivo* results were obtained in 1991.

1992

The results for 1992 are shown in Figure 6. Prochloraz and propiconazole gave 98-100% inhibition of growth at 5 and 0.5 µg ai/ml. At 0.05 µg ai/ml there was considerable variation in

relative growth with 5-20% of isolates growing to 10-100% of the unamended controls. However prochloraz still maintained 100% inhibition in 50% of isolates compared to only 10% with propiconazole. Flutriafol and triadimenol exhibited still greater variation in the degree of inhibition. The majority of isolates grew to 50% or more of the unamended controls at 0.05 µg ai/ml flutriafol with only 5 µg ai/ml providing 100% inhibition. Triadimenol at 10 µg ai/ml provided 100% inhibition of only 45% of isolates and virtually no inhibition of growth at the other two lower concentrations.

A comparison of the *in-vitro* results for each of the fungicides, obtained using the same method of sensitivity assessment (1991 and 1992 only) at fungicide concentrations giving the greatest degree of variation in relative growth and percentage of isolates, is shown in Figures 7 - 10. There appears to be a very slight shift towards reduced sensitivity from 1991 to 1992 for all fungicides except flutriafol, for which there appears to a shift towards greater sensitivity.

In-vivo sensitivity tests were completed successfully using four isolates with varying degrees of *in-vitro* fungicide sensitivity obtained from the 1992 survey. Table 1 gives the results of these tests.

Table 1: Percentage leaf area affected by lesions of *S. tritici* relative to controls

Fungicide conc.(µg ai/ml)	Isolate number			
	481-9	415-1	11-3	233-5
triadimenol				
0.1	105	108	111	107
1.0	97	100	108	96
10.0	87	83	90	80
flutriafol				
0.05	106	108	121	109
0.5	75	75	107	100
5.0	55	81	59	83
propiconazole				
0.05	93	106	108	82
0.5	59	103	105	77
5.0	36	55	37	54
prochloraz				
0.05	98	105	120	113
0.5	66	53	101	78
5.0	65	53	52	71

Fungicides and isolates *in-vivo* gave results with a similar ranking to those obtained *in-vitro*. However the degree of disease control was generally less than would have been anticipated on

the basis of the *in-vitro* results particularly for the highest rates of the four fungicides.

Septoria nodorum: 1990

The results of the sensitivity testing of *S. nodorum* are shown in Figures 11-12. Mean percentage growth for the 'population' of 40 isolates of *S. nodorum* was markedly greater on benomyl compared to that on the other three fungicides at all concentrations except flutriafol at 0.05 µg ai/ml. However the latter failed to inhibit completely the growth of any of the isolates of *S. nodorum* whereas benomyl at 5 µg ai/ml inhibited growth of 63% of isolates. Similarly the mean percentage growth on flutriafol was markedly greater compared to that on prochloraz and propiconazole at all concentrations. Growth on propiconazole and prochloraz showed no marked differences except at 0.05 µg ai/ml. Both fungicides at 5 µg ai/ml inhibited growth of more than 60% of isolates. Growth distribution 'curves' (histograms) were very similar for the four fungicides except that propiconazole and prochloraz gave much greater inhibition of growth compared to flutriafol and benomyl. At the two lower concentrations of flutriafol and benomyl there appeared to be considerable growth stimulation.

Discussion

The results of the three years of this project indicate that there is some considerable variation amongst the isolates of *S. tritici* in their *in-vitro* sensitivity to the four DMI fungicides but much less so to the single MBC tested. Clearly the majority of 1990 isolates (72%) could be considered resistant to benomyl at 10 µg ai/ml, four times that of the theoretical concentration delivered onto field-grown plants. These results are similar to those obtained at Long Ashton (Hollomon, 1990). They are also similar to those obtained by Griffin & Fisher (1985) who showed that a limited number of isolates of *S. tritici*, resistant to benomyl at 1 µg ai/ml (as used in the compilation of the dose-response curve: Figure 2) were also resistant to 1000 µg ai/ml. The results from the first year of the survey confirmed the results from previous work and also confirmed that benomyl-resistance is a stable feature of field populations of *S. tritici* such that it seems likely that benomyl and other carbendazim-generating fungicides (MBC's) will give little or no control of *S. tritici* now or in the future.

The use of a simple presence/absence (of growth) assessment method in 1990 precluded a direct comparison of results across the three years of the project. However the only data lost during 1990 were the quantitative assessment of the relative growth of the isolates at the lowest concentration of prochloraz and propiconazole and the lower two concentrations of flutriafol. Triadimenol was not included in 1990.

Prochloraz and propiconazole gave similar results in each of the three years, particularly so at 5 and 0.5 µg ai/ml where 80-100% of the isolates were almost completely inhibited from growing to even 10% of the unamended controls. Variation in the apparent sensitivity to these two fungicides was discernible only at 0.05 µg ai/ml but even so at least 50% or more of the isolates exhibited relative growth rates of 50% or less compared to the unamended controls. The results of the tests with the other two DMI fungicides showed considerable contrast to those obtained with prochloraz and propiconazole. Flutriafol provided complete inhibition of growth or allowed less than 30% relative growth of all isolates only at 5 µg ai/ml, a concentration ten times greater than that of either prochloraz and propiconazole which achieved the same degree of inhibition. However, the flutriafol results were less consistent than those of any one of the other DMI fungicides, even triadimenol, which itself showed the greatest variation of *in-vitro* activity, achieving complete inhibition of only 45% of isolates at its highest concentration of 10 µg ai/ml.

The apparent sensitivity of the populations and isolates of *S. tritici* (up to a maximum of 10 µg ai/ml of triadimenol) found in this project may not reflect the true base-line sensitivity of *S. tritici* in England and Wales. The pathogen population has been subjected to the application of DMI fungicides since 1985, a period during which an unknown degree of shift towards reduced sensitivity may have already occurred. However the three years' results of this project indicate that the current base-line sensitivity may be stable or if not, it is shifting very slowly and as yet undetectably towards reduced sensitivity. This degree of reduced sensitivity appears unlikely to lead to problems of partial disease control failure at present. The opportunity to test isolates of *S. tritici* from winter wheat crops, in which the degree of disease control was less than expected, provided a valuable comparison with the 1991 and 1992 survey sample testing. The similarities in the growth distribution histograms for the survey and non-survey samples of 1991 (Figures 4 and 5) indicate that the apparent disease control failure or partial failure that originally prompted the testing of isolates from such sprayed crops is unlikely to be due to reduced sensitivity in the 'resident' population of *S. tritici*. Similarly the result of testing the small number of isolates from two 'suspect' instances of poor disease control in 1992 (Figure 13) provided no evidence of significant shifts towards reduced sensitivity.

Most of the suspect 'poor control' crops from 1991 and 1992 had been sprayed with triadimenol alone or in mixture with another fungicide. The fact that this fungicide achieved poor control may be far more a reflection of its inherent shortcomings when used for the control of *S. tritici* rather than any indication of reduced sensitivity to DMI fungicides in general. The poorer activity of triadimenol both *in-vitro* and *in-vivo* compared to the other three DMI fungicides but particularly prochloraz and propiconazole indicates that the timing of this fungicide may be fairly critical when it is used to control *S. tritici*. Any deviation in the

timing of fungicide application outside the product's inherent temporal capacity for marked biological (eradicant and protectant) activity may render it suspect in terms of performance and lead to implications of fungicide insensitivity. In this respect the current trend to use less than the recommended dose of fungicides on winter wheat crops may lead to an increase in such claims unless the principles behind such usage are fully appreciated.

A small percentage of both survey and non-survey sample populations appear to possess what may be reduced sensitivity *in-vitro* to flutriafol and triadimenol at 5 and 10 µg ai/ml respectively but there appears to be no significant incidence of reduced sensitivity to propiconazole and prochloraz detectable in the populations sampled in 1991 and 1992. These results are similar to those obtained, albeit on a smaller scale, by Mapstone and Hollomon (1991), Cooke (1991) and Griffin and Fisher (1985). However results of *in-vivo* tests indicate that despite virtual complete inhibition *in-vitro*, propiconazole, prochloraz and flutriafol at 5 µg ai/ml did not provide complete control of disease caused by *S. tritici* on inoculated wheat plants. Similarly triadimenol gave virtually no control even applied at 10 µg ai/ml. However, plant inoculation, high humidity and relatively warm glasshouse conditions bear only limited relation to field conditions for disease development and hence field performance of fungicides. Thus it may be that *in-vitro* test results should be used as the primary screening to select isolates exhibiting a range of sensitivity. This range of isolates should then be tested under real or simulated field conditions so as to reflect more accurately the field performance of fungicides against isolates of varying sensitivity. To date there are little published data on this subject, but work by Fehrmann *et al.* (1989) and Olvang (1988) indicates that there appears to be no shift towards reduced sensitivity in populations of *S. nodorum*, on a single site in Germany and across Sweden, for prochloraz, propiconazole or triadimefon.

The results from this survey emphasise the importance of regular monitoring of populations of *Septoria* species and other cereal crop pathogens for resistance to DMI fungicides. Flutriafol and triadimenol certainly appear to be less inhibitory against *S. tritici in-vitro* compared to the other two DMI-fungicides. What does this mean in terms of future use of these and other members of the DMI group? Clearly it would be most sensible to attempt to maintain the efficacy of this fungicide group recommending that they be applied as a co-formulation or as a tank-mix with an effective protectant fungicide such as chlorothalonil or at least in mixture with another fungicide to spread the burden of disease control particularly in terms of dose rate and timing. The trend towards the increased use of reduced doses for disease control in winter wheat demands an understanding of the biological activity of fungicides is crucial if such a strategy is to be used successfully. Such an understanding should markedly reduce the risk of selecting resistant or more resistant isolates or shifting the population towards reduced sensitivity as well as reducing the risk of spurious claims of the occurrence of such phenomena.

From the limited testing of isolates of *S. nodorum* it would appear that this pathogen may be behaving in a manner similar to *S. tritici* and should be treated in the same way.

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Figure 1 : Dosage response curves for fungicides used in the *in-vitro* study

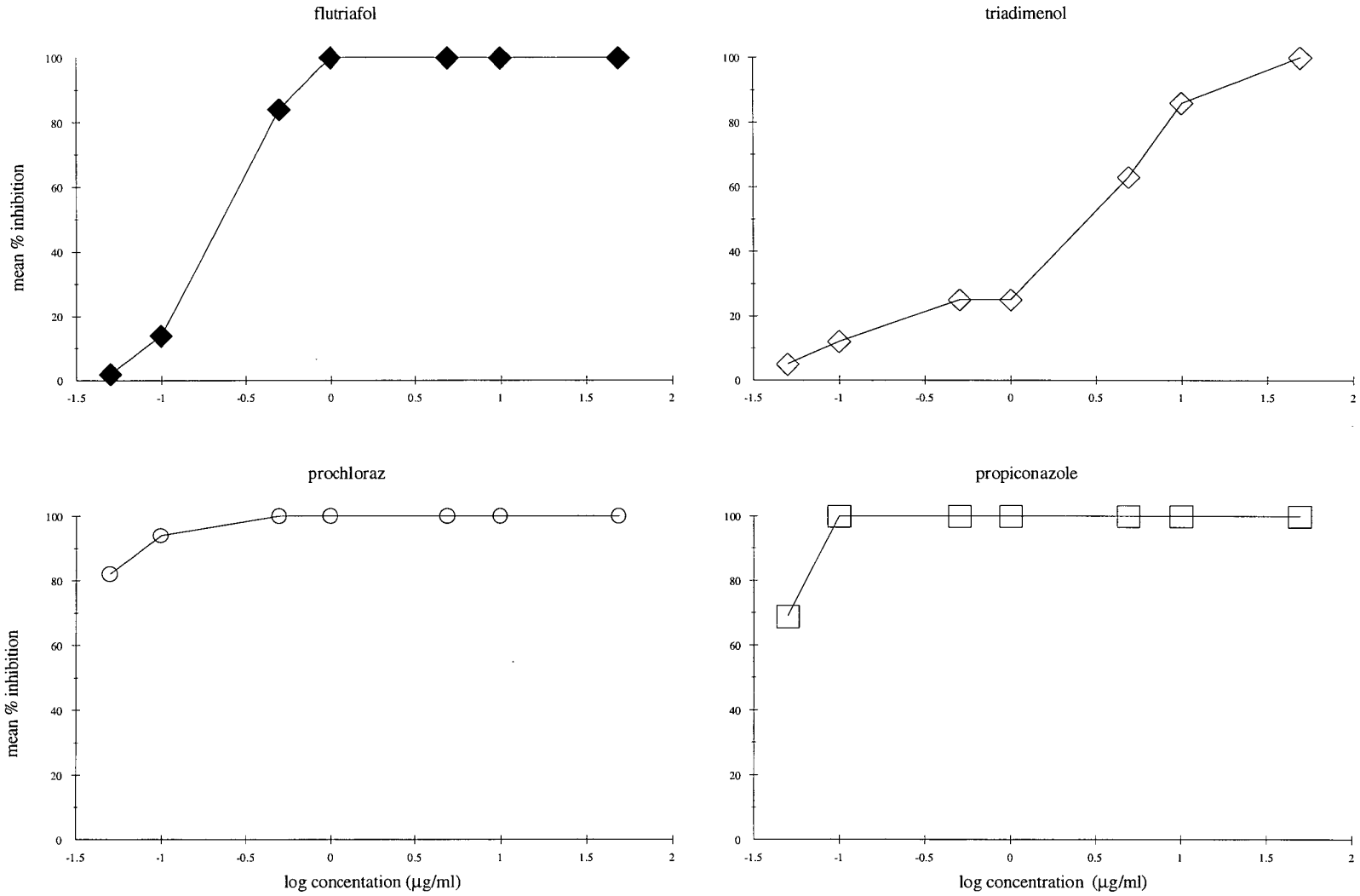


Figure 2: Dosage response curve for benomyl used in the *in-vitro* study

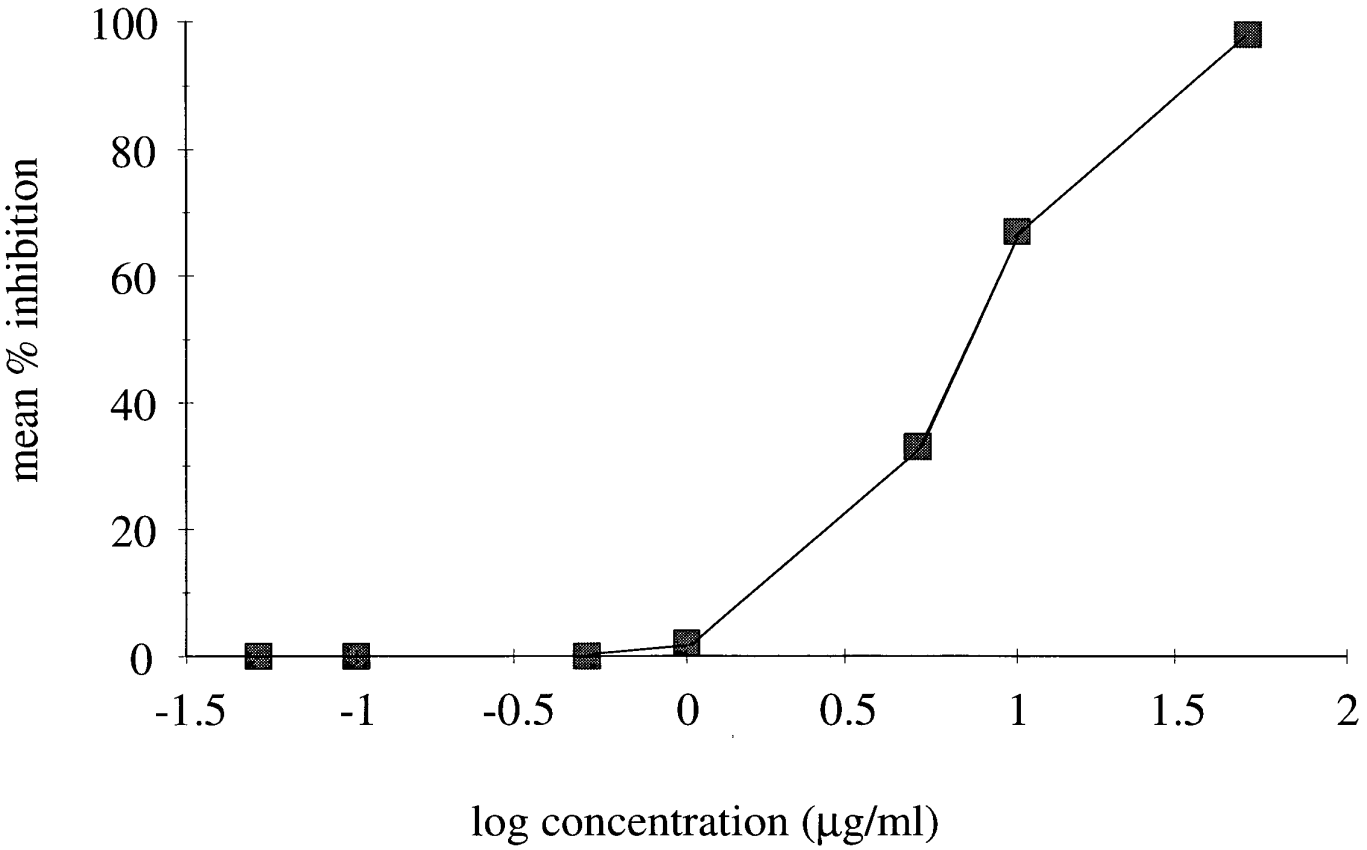
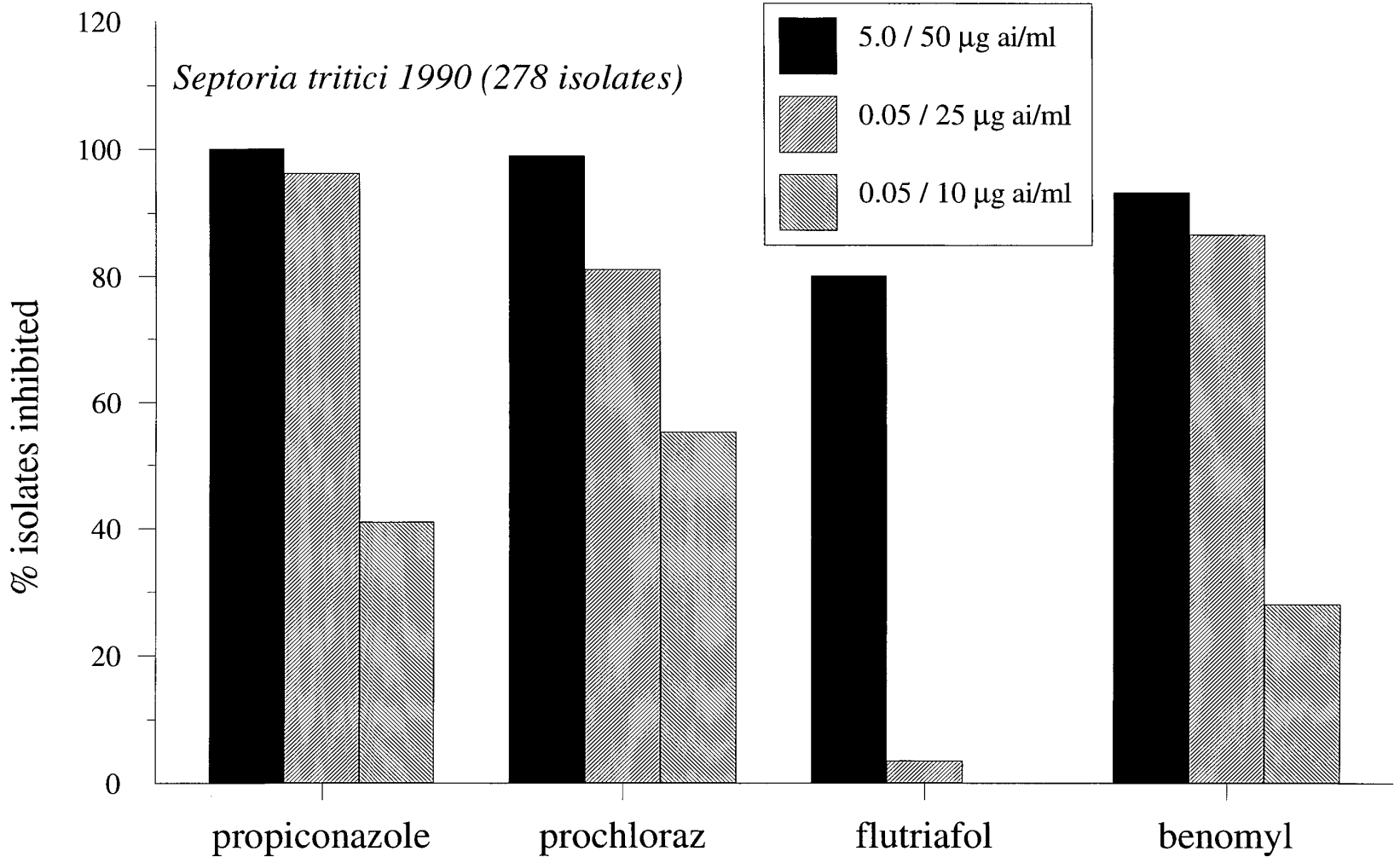


Figure 3: Growth of isolates inhibited by three fungicide concentrations



NB. only benomyl used at 50, 25 & 10 µg/ml

Figure 4.1: Results of 1991 testing of survey and non-survey isolates

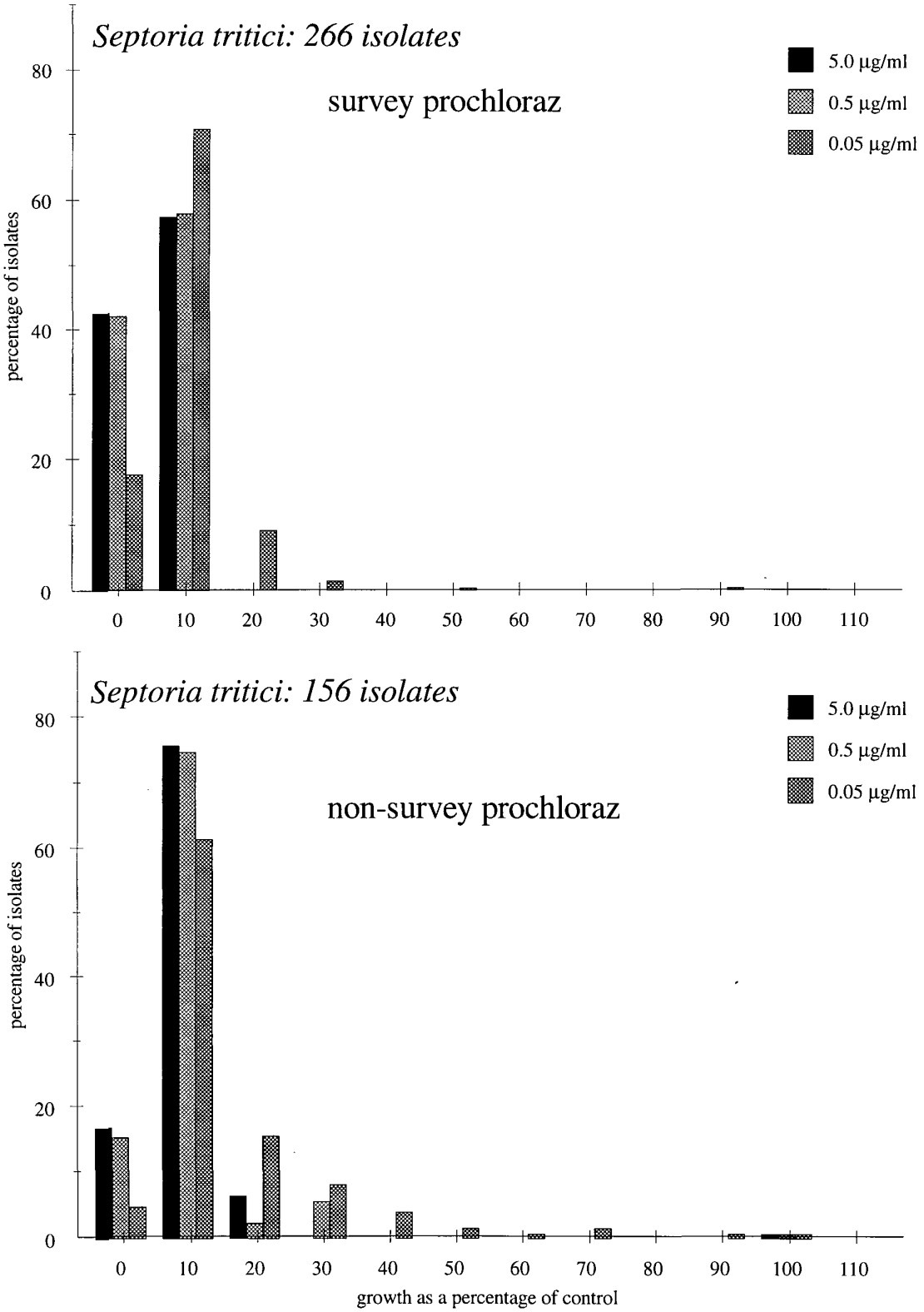


Figure 4.2: Results of 1991 testing of survey and non-survey isolates

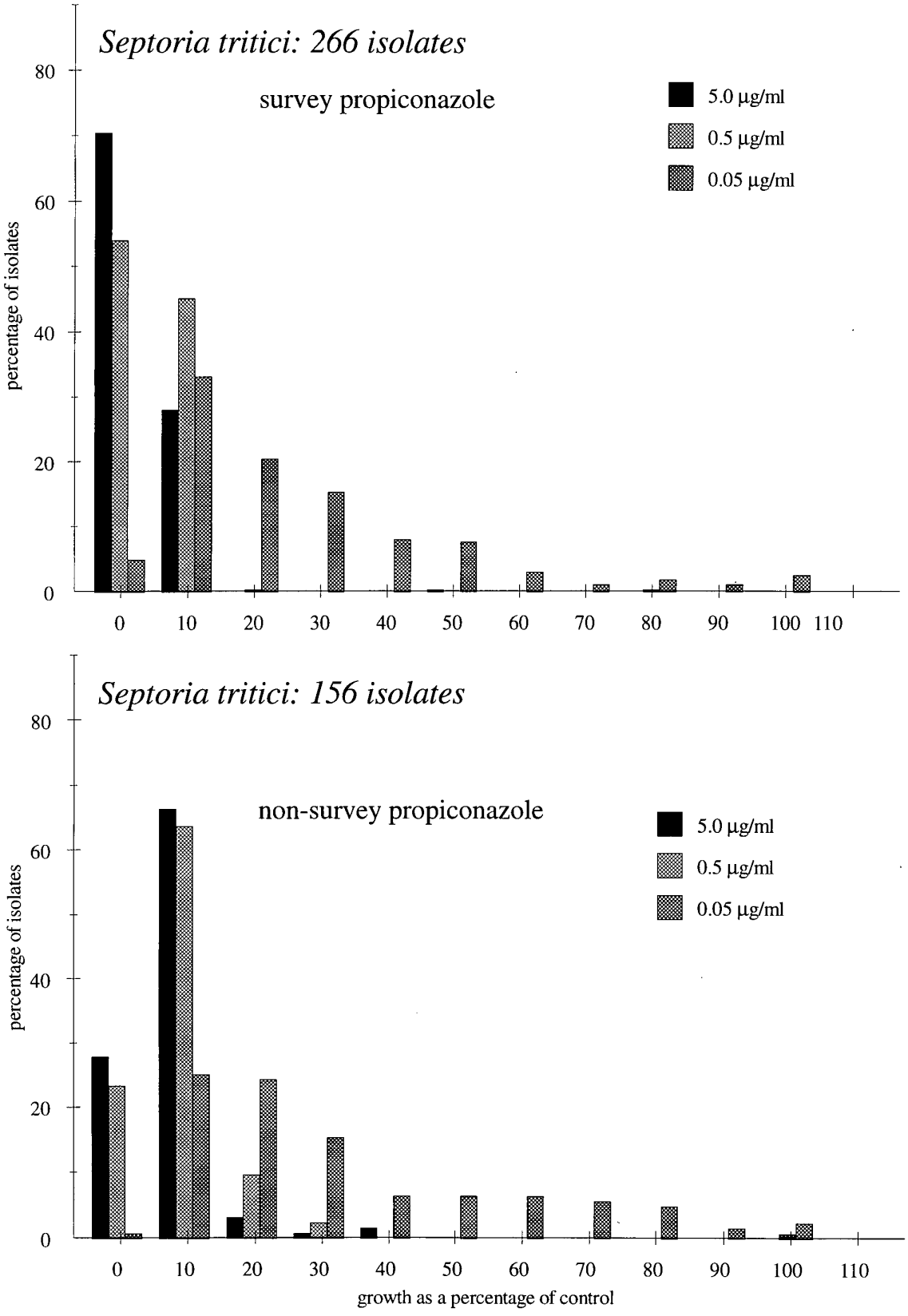


Figure 5.1: Results of 1991 testing of survey and non-survey isolates

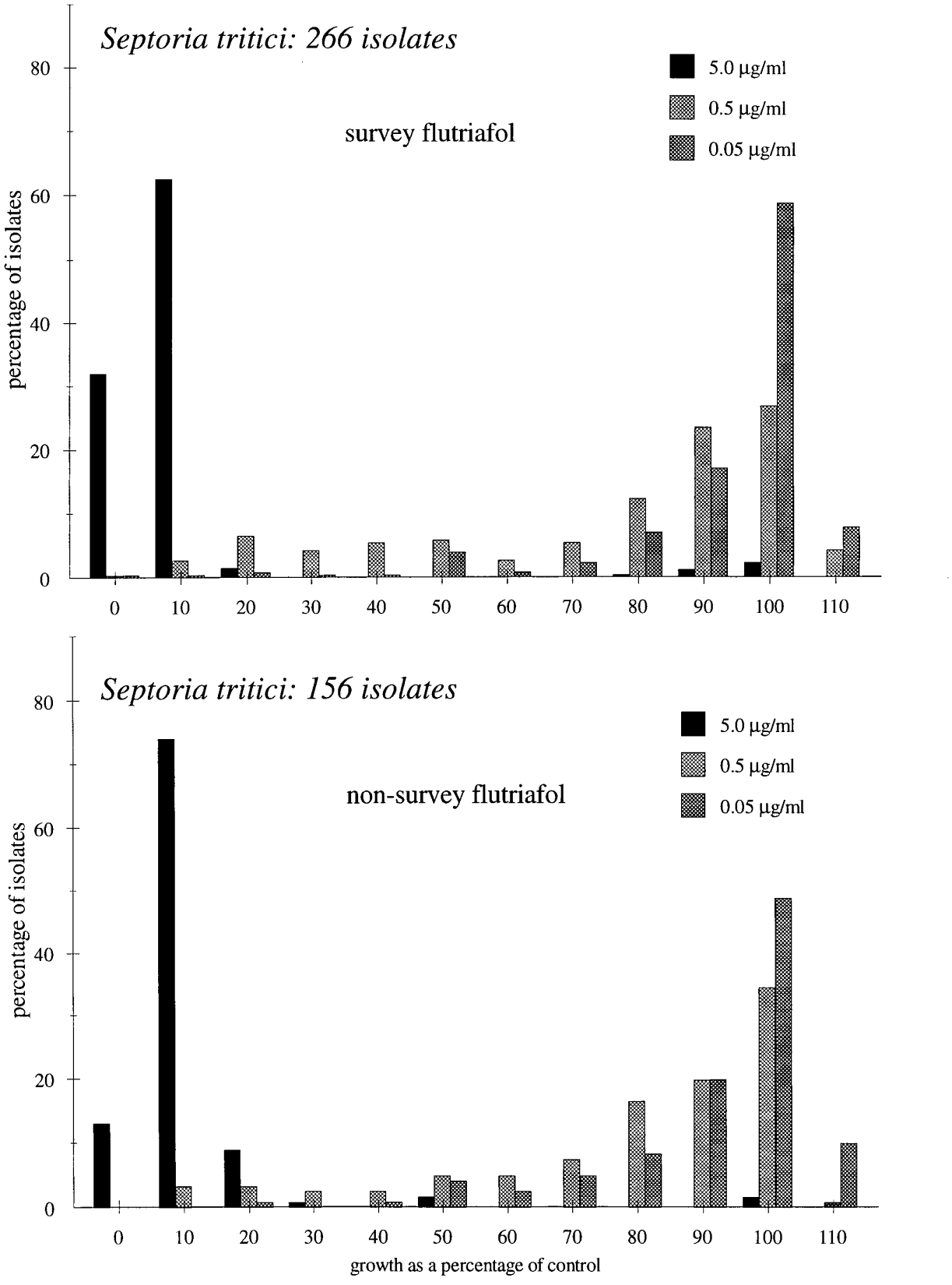


Figure 5.2: Results of 1991 testing of survey and non-survey isolates

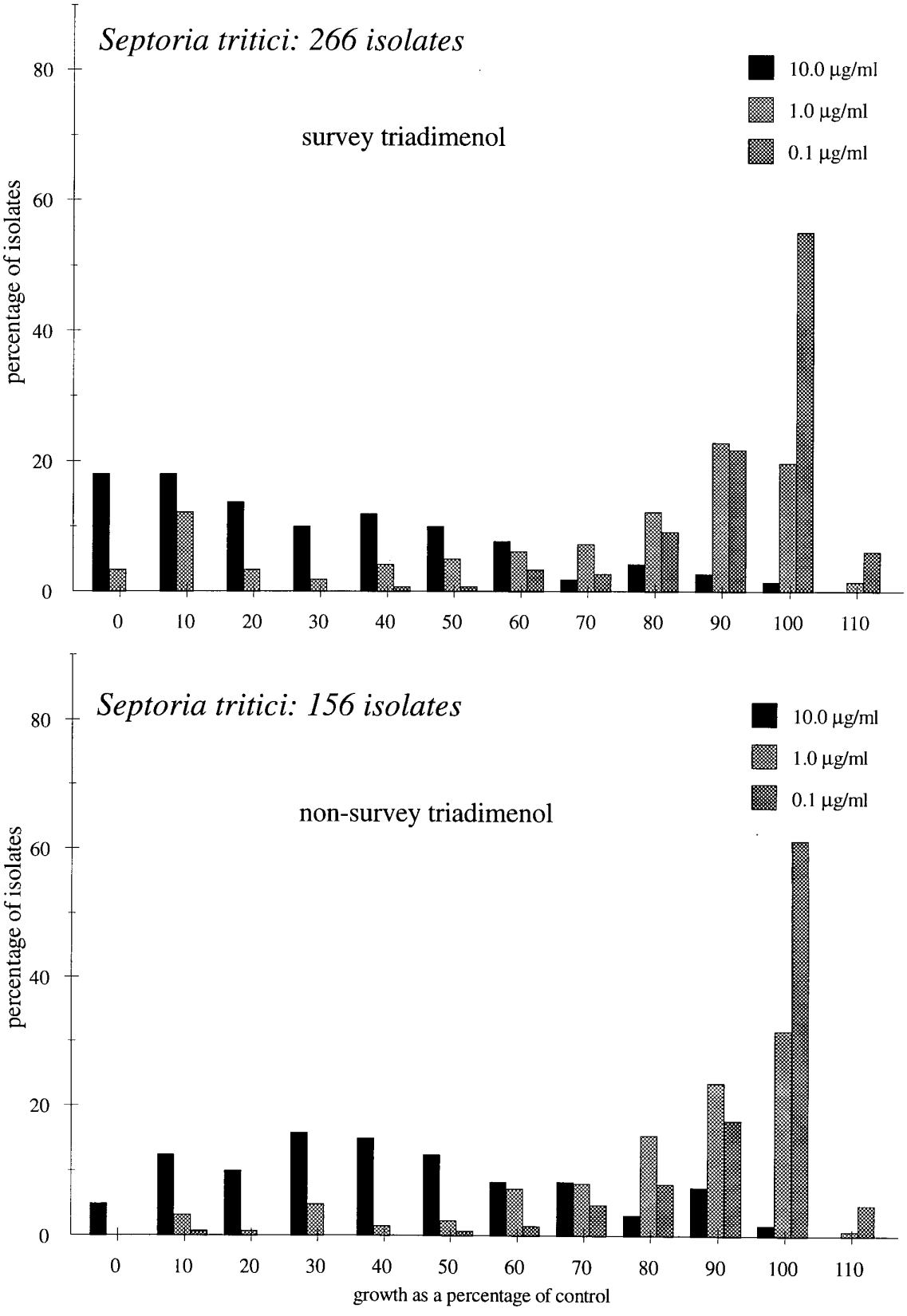
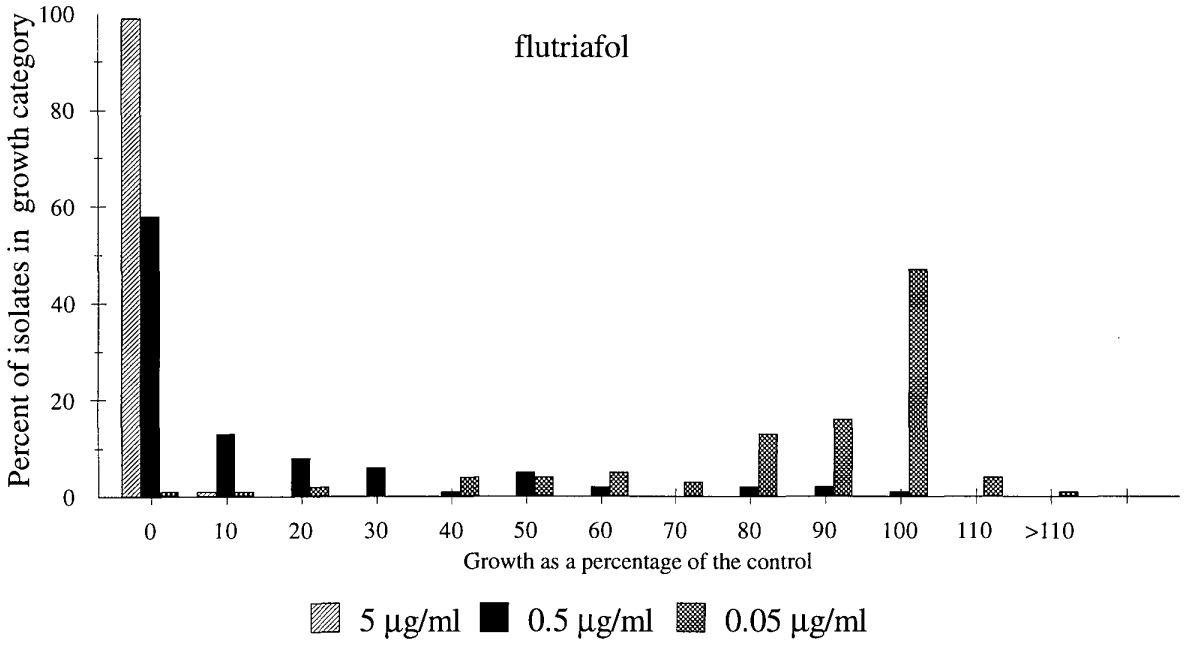


Figure 6.1: 1992 national sensitivity distribution

Septoria tritici 143 isolates in-vitro



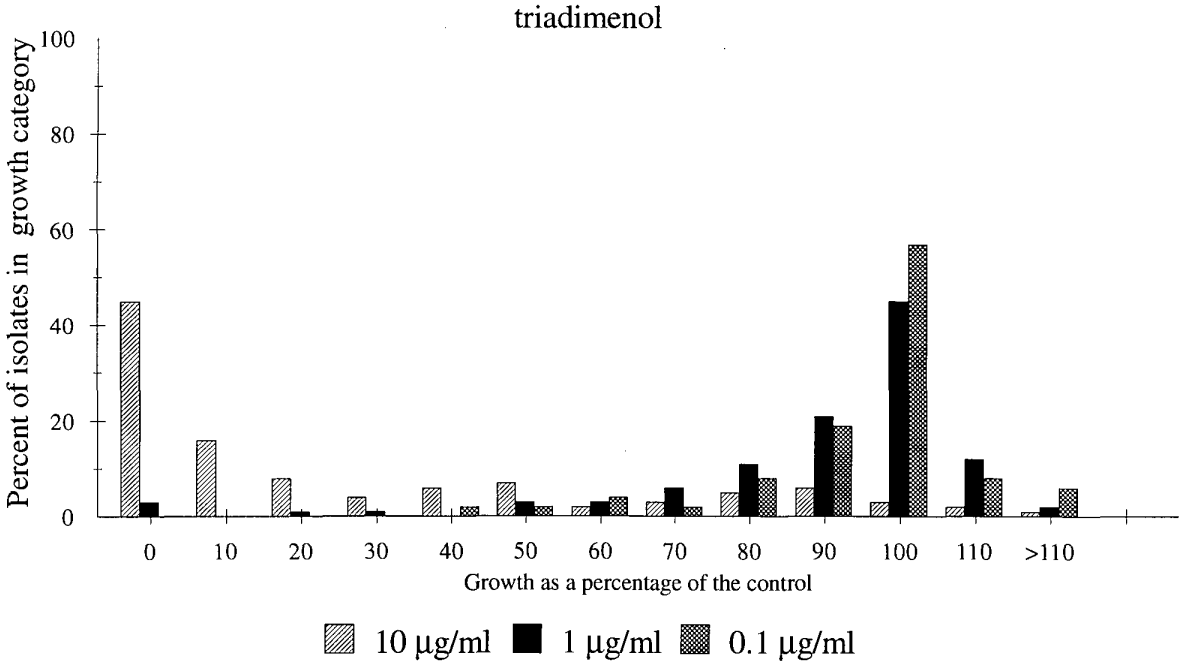
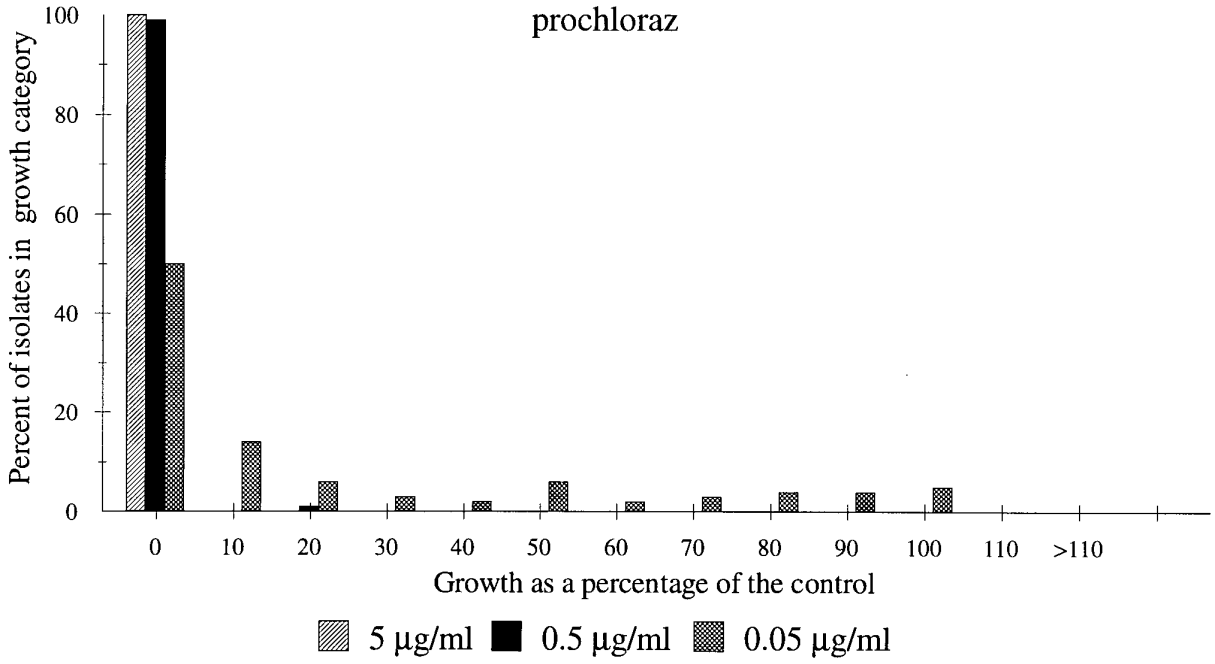


Figure 6.2: 1992 national sensitivity distribution

Septoria tritici 143 isolates in-vitro



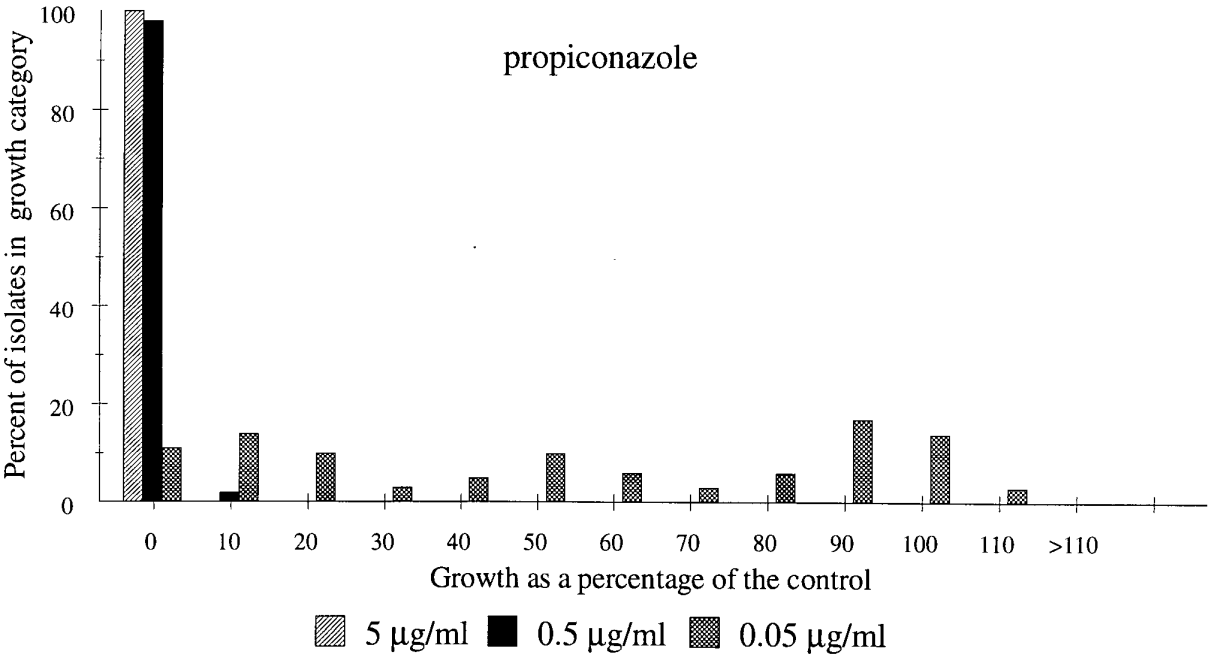


Figure 7: Comparison of 1991 and 1992 isolates - propiconazole 0.05 µg/ml

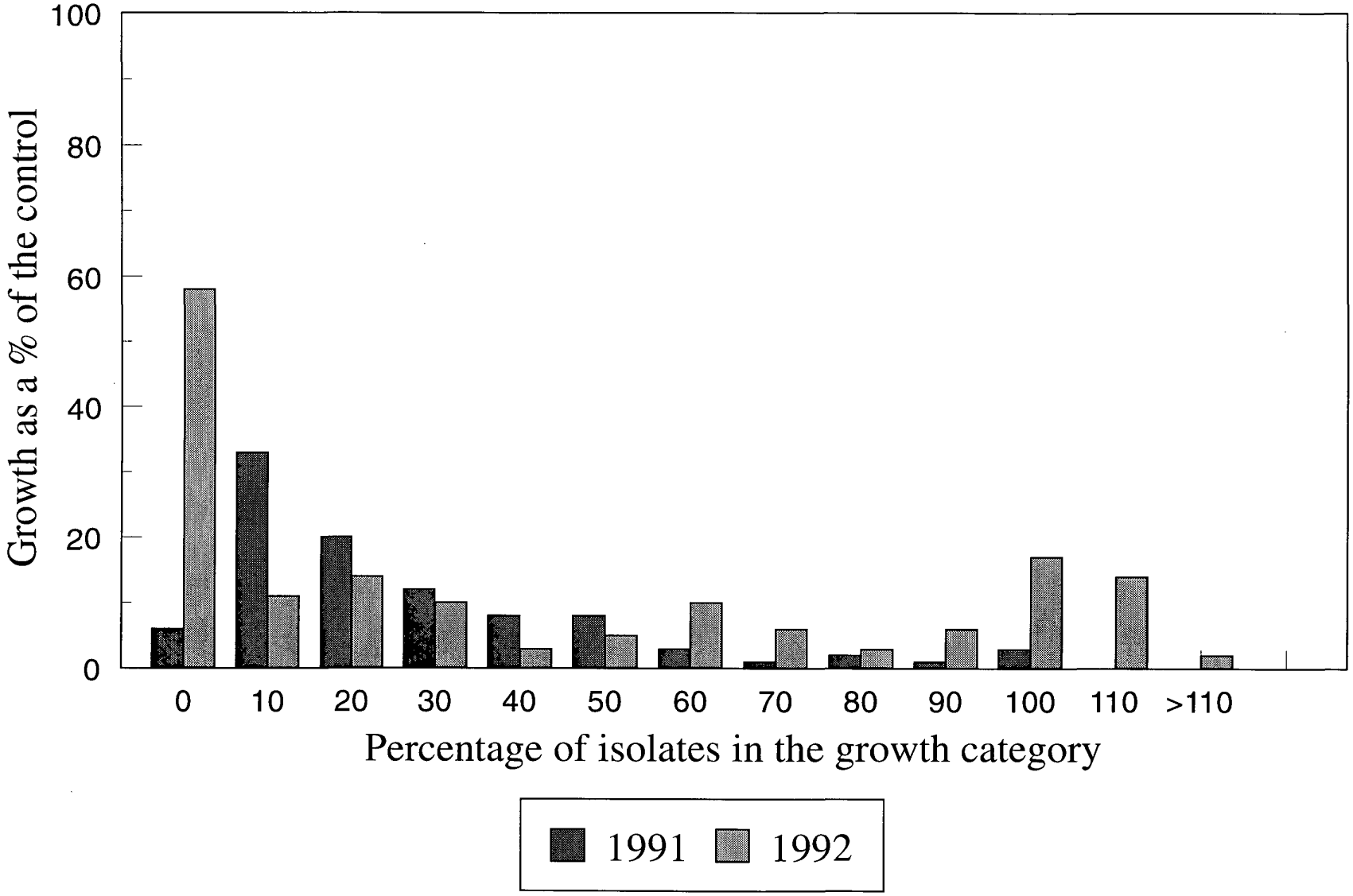


Figure 8: Comparison of 1991 and 1992 isolates - prochloraz 0.05 µg/ml

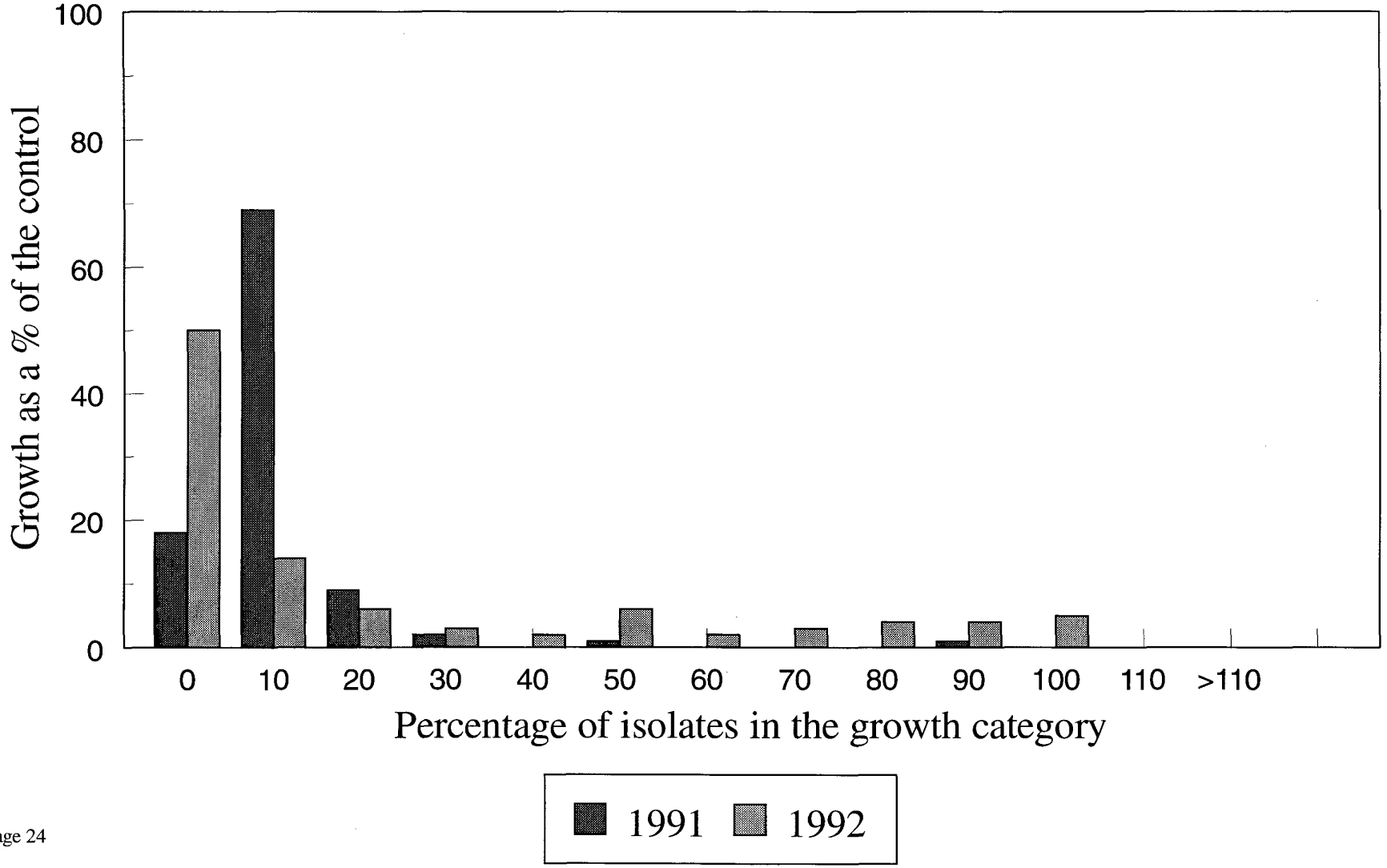


Figure 9: Comparison of 1991 and 1992 isolates - flutriafol 0.5 µg/ml

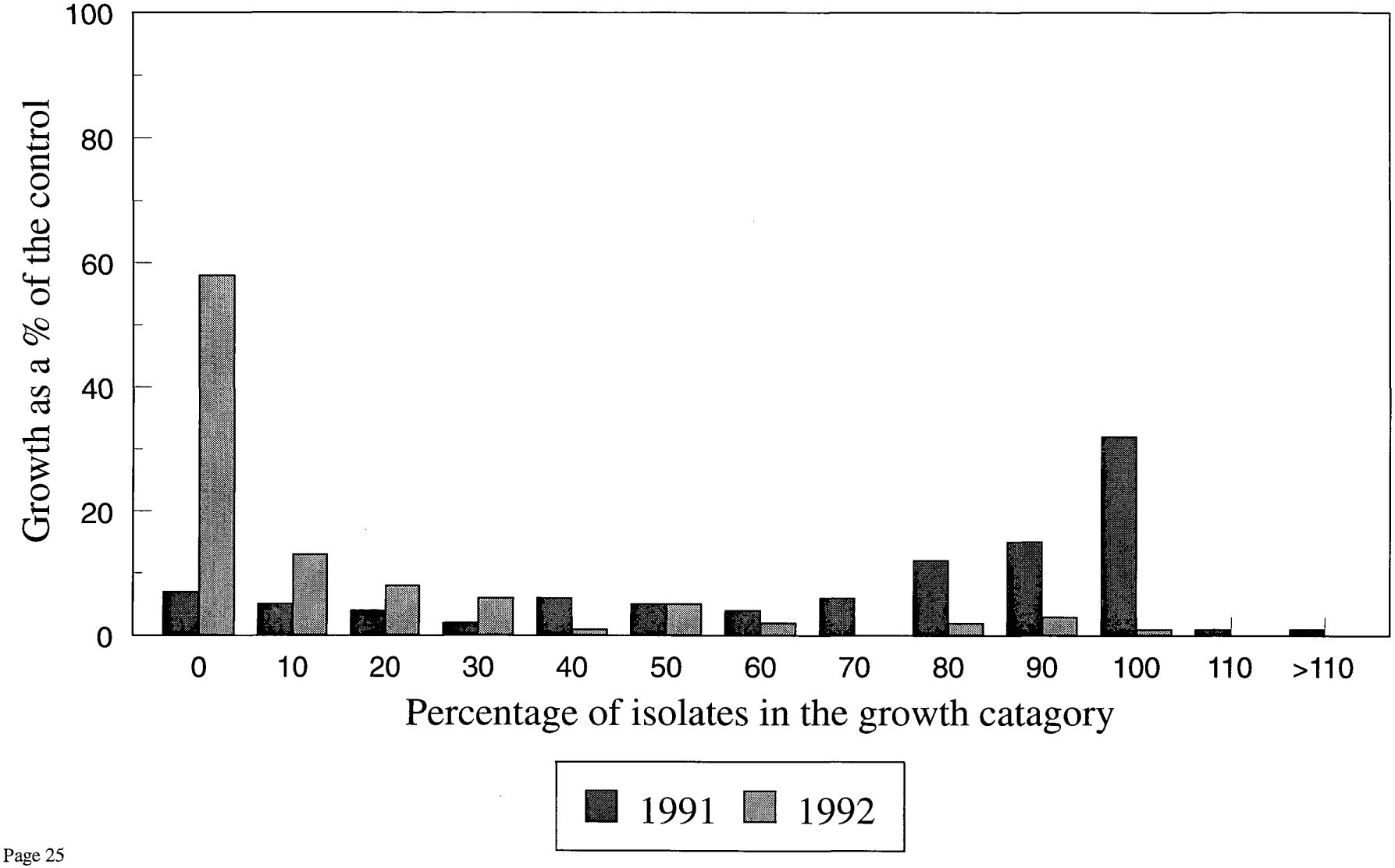


Figure 10: Comparison of 1991 and 1992 isolates - triadimenol 1 µg/ml

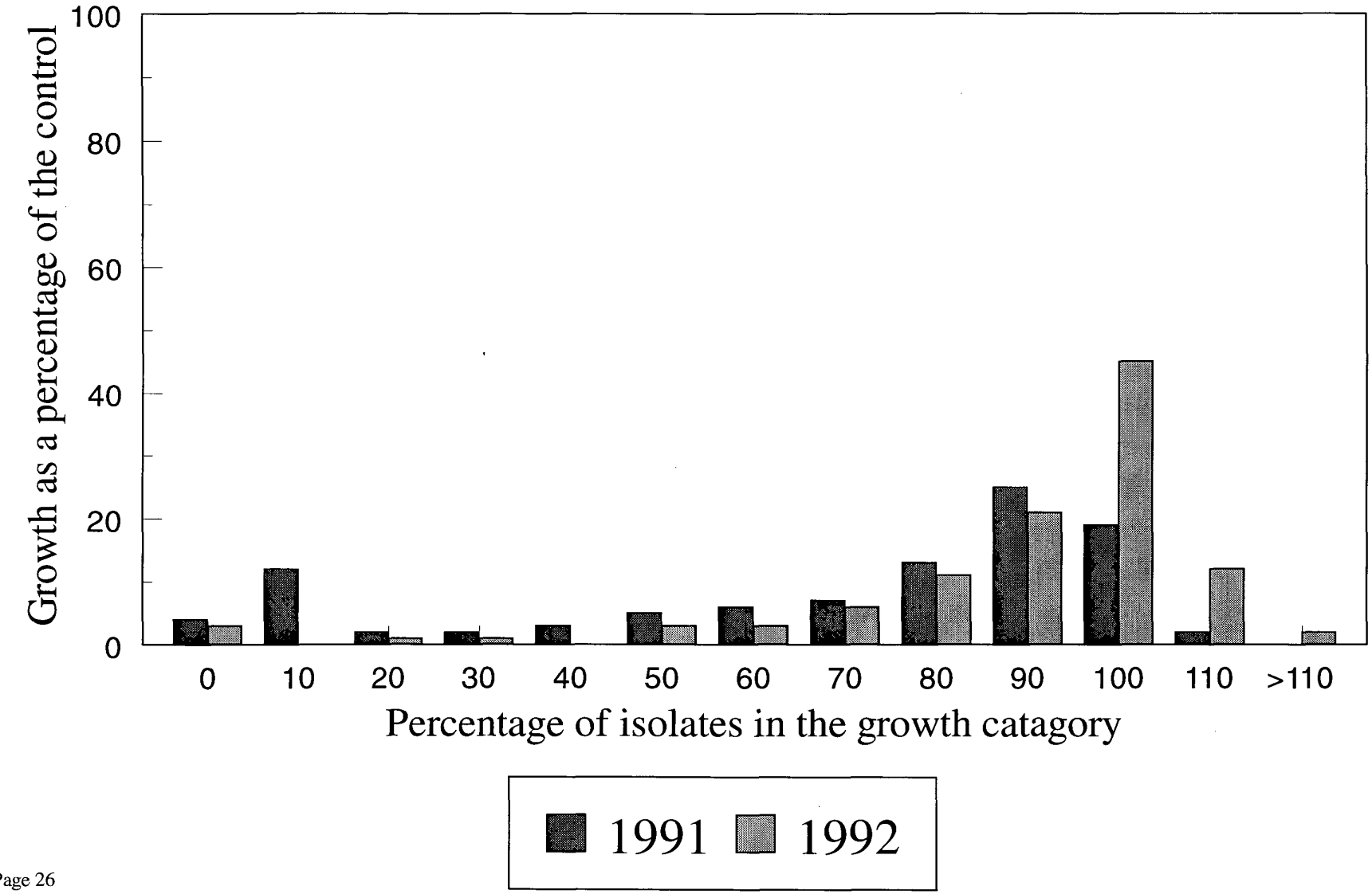


Fig 11.1 Growth of 40 isolates of *Septoria nodorum* on three concentrations of prochloraz *in-vitro*

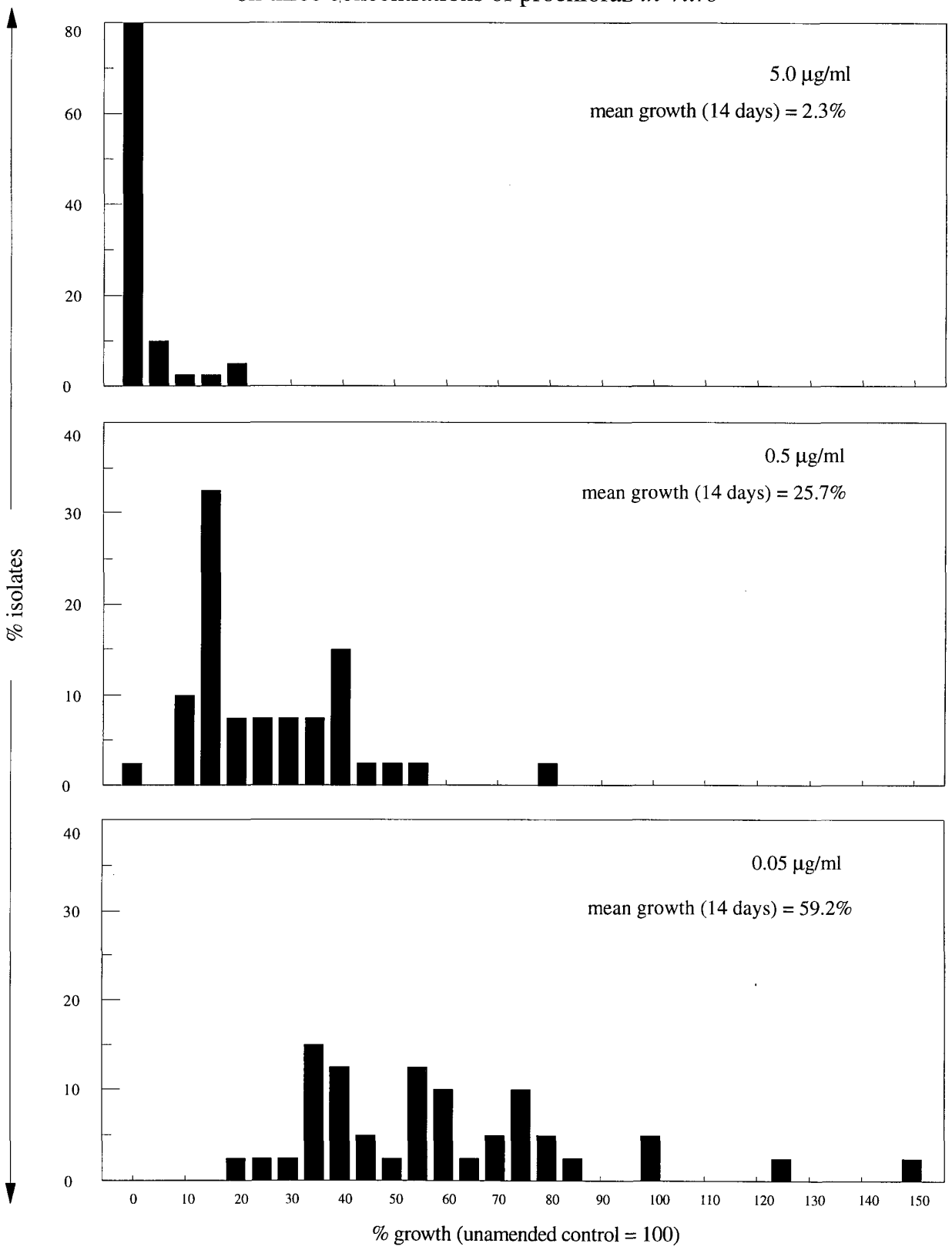


Fig 11.2 Growth of 40 isolates of *Septoria nodorum* on three concentrations of propiconazole *in-vitro*

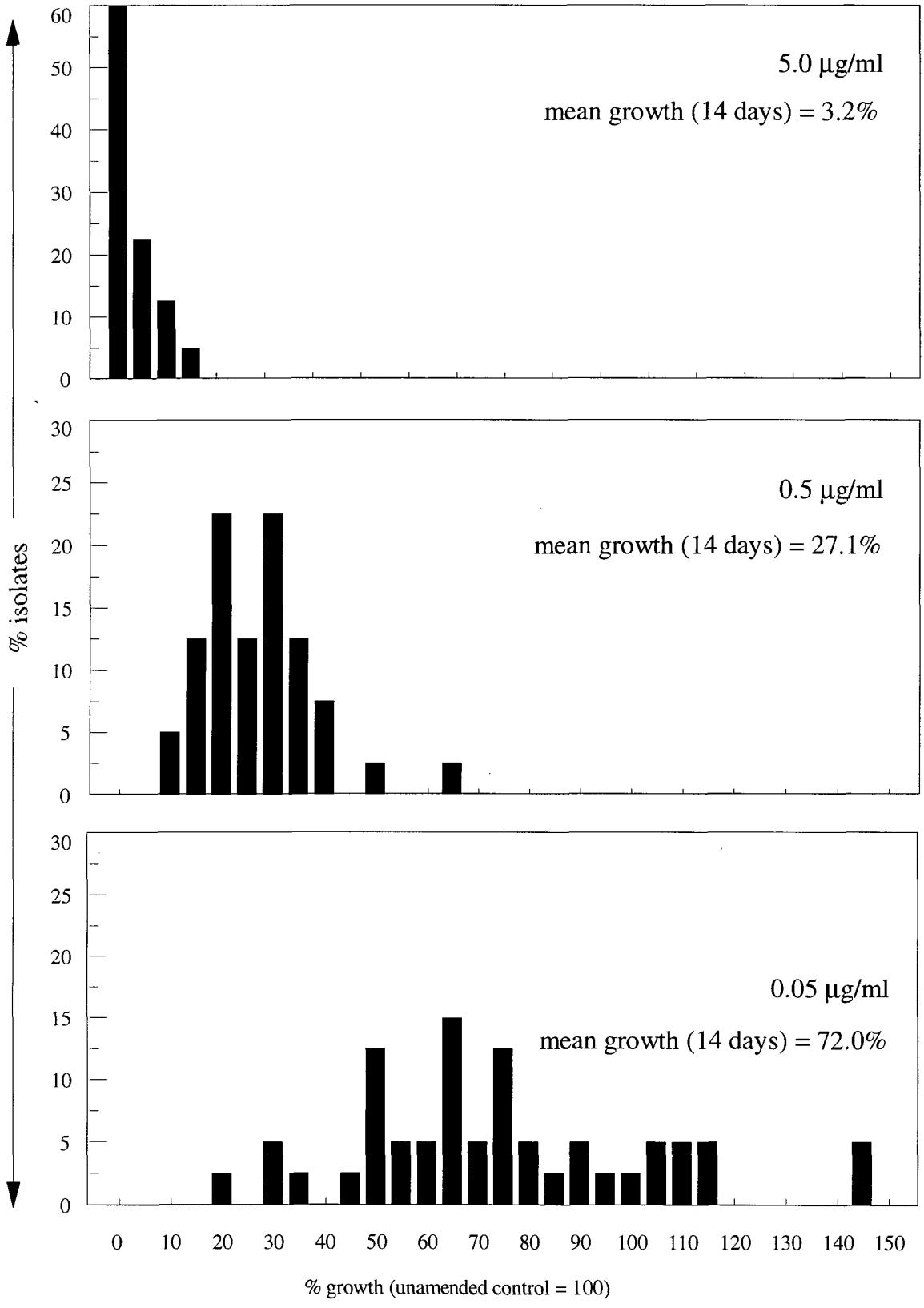


Fig 12.1 Growth of 40 isolates of *Septoria nodorum* on three concentrations of flutriafol *in-vitro*

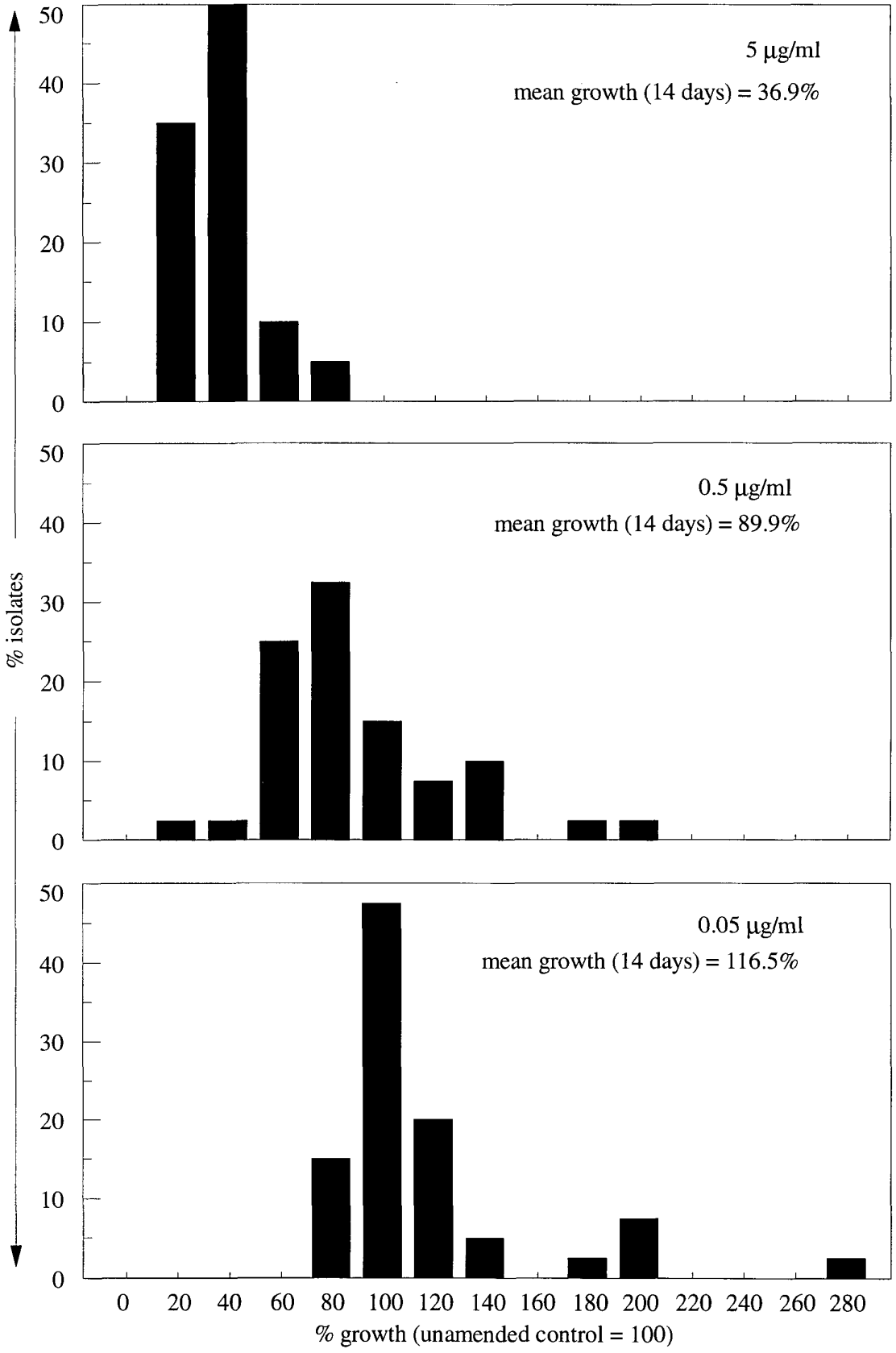


Fig 12.2 Growth of 40 isolates of *Septoria nodorum* on three concentrations of benomyl *in-vitro*

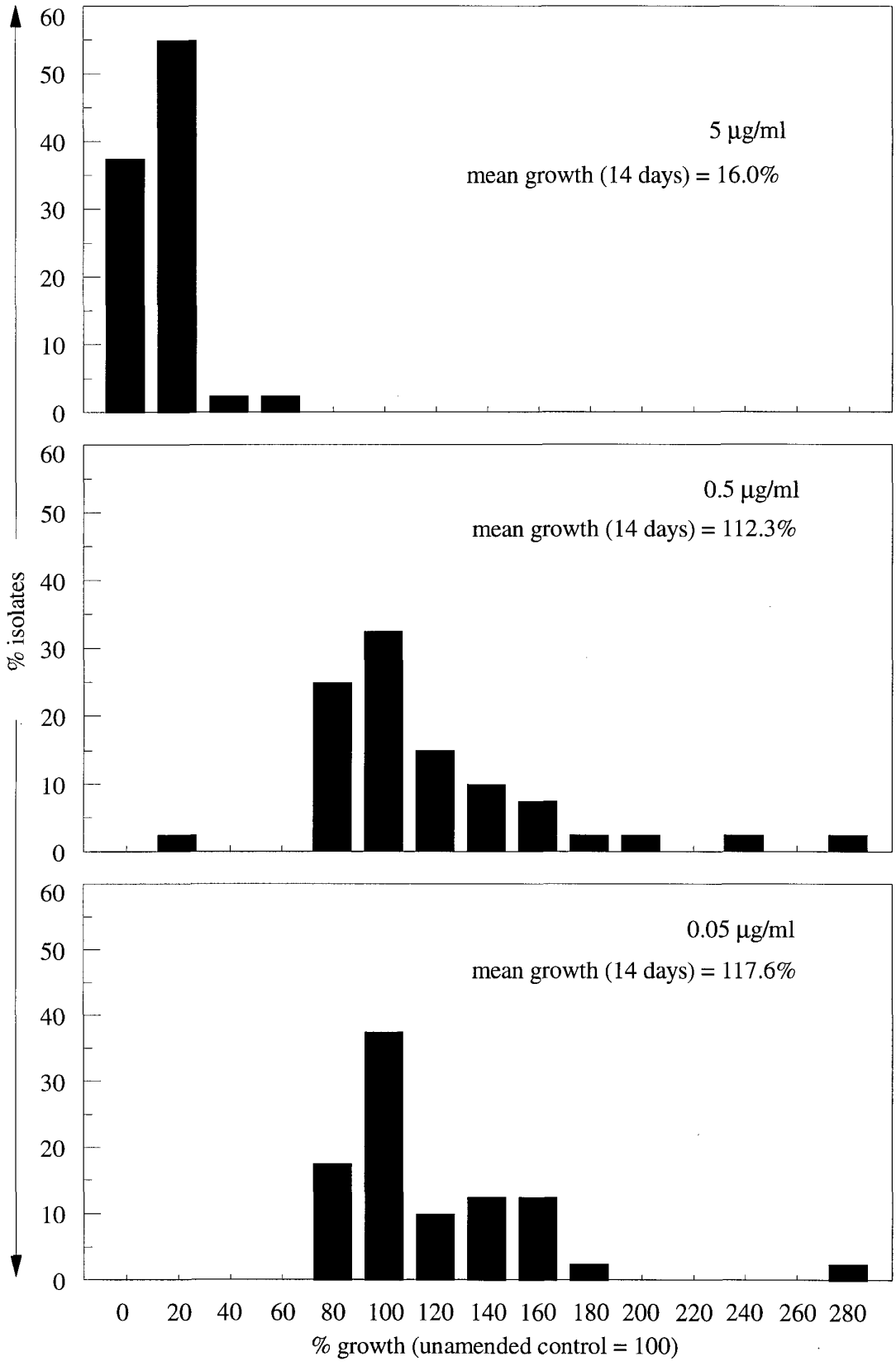


Figure 13: Sensitivity distribution of 33 isolates from crops with poor disease control in 1992