PROJECT REPORT 309

STRATEGIES FOR FUNGICIDAL CONTROL OF TAKE-ALL

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by

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PART 1: ABSTRACT

Strategies for fungicidal control of take-all

Fluquinconazole (Jockey F) seed treatment was applied or not applied, in all possible sequences, in up to six consecutive crops of winter wheat in field experiments. Take-all, caused by the root-infecting fungus *Gaeumannomyces graminis* var. *tritici*, was controlled effectively. Grain yield was usually increased by treatment when the disease was moderate or severe in the non-treated crops. Control of the most severe take-all did not result in acceptably high yields or adequate grain quality. Treatment of a second wheat with little take-all did not benefit the subsequent crop. There was, however, a small residual benefit in a second wheat following a treated first wheat (tested once only). Non-treatment of a crop grown after a treated, diseased crop usually resulted in a marked increase in disease. Take-all was controlled by treatment of a crop grown after a treated, diseased crop but the extent of control and of increased yield was often less than that in a treated crop grown after a non-treated crop in the same crop sequence.

It is recommended that seed treatment should be applied to second or third wheat crops (at risk from damaging take-all) and that a break crop should follow the treated crop. Seed treatment should not normally be used in longer sequences of wheat or on take-all decline sites.

Application of fluquinconazole seed treatment to crops grown successively on the same site for up to 4 years did not adversely affect the natural community of saprophytic fungi on the roots. No resistance or decreased sensitivity to fluquinconazole was found in populations of the take-all fungus in these crops.

A DNA probe and restriction-digestion were used to characterise isolates of the fungus from crops that were treated or non-treated in every year as RFLP types, T1, T1c and T2. On this basis, population structure was found to change from year to year but was not affected significantly by fluquinconazole seed treatment. A hypothesis that the greatest proportion of T2 isolates anticipated increased take-all was tested but only partially validated, which precluded the development of these markers for a risk assessment system. T1 types, often more predominant later in crop sequences, were frequently more melanised, suggesting a mechanism for enhanced survival. Other sub-groups of the take-all fungus (A and B), identifiable by PCR after DNA extraction directly from root tissue, were found to correlate closely with the RFLP types. Because of a correlation with insensitivity in the take-all fungus to the take-all-specific fungicide, silthiofam, and selection by host-plant species, the PCR test has potential value in future research.

PART 2: SUMMARY

Strategies for integrated control of take-all

Objectives

Take-all epidemics develop from year to year in sequences of susceptible crops and the disease is subject to suppression by natural biological control, including take-all decline. The introduction of seed treatment fungicides for controlling take-all has created a need to know at what stages in the epidemic they can be used most safely and effectively. It is also necessary to anticipate any disruption of the natural epidemic or other adverse side effects, and to assess disease risk more effectively. These considerations were the basis of the specific objectives:

1. To identify the best strategies for using seed treatment fungicides for controlling take-all in sequences of susceptible crops.
2. To identify any adverse or beneficial effects of seed treatment fungicides on populations of the take-all fungus (including alterations in fungicide sensitivity), and on other fungi, including those suppressive to take-all.
3. To seek confirmation of evidence (from a few sites) that population structure of the take-all fungus (determined by a DNA test) can provide the basis of a risk assessment method.

Methods

The effects of fluquinconazole seed treatment on take-all disease, take-all infectivity in soil and grain yield and quality were determined in field experiments in which wheat crops were grown successively. Treatment and non-treatment were compared in all sequence combinations in 2^{nd}-5^{th} wheats (three experiments), 1^{st}-6^{th} wheats (one experiment) and over four crops of continuous wheat on a take-all decline site. The effects of fungicide on fungal communities on the roots were determined by incubation on agar media. Populations of the take-all fungus were sampled by isolating on agar from wheat seedlings grown in soil from the seed treatment experiment and from four monitoring sites in which non-treated wheat crops were grown successively. Effects on sensitivity of the take-all fungus to fluquinconazole were determined in these isolates by agar-plate assay. They were also characterised by appearance in culture and by molecular methods (RFLPs determined by Southern blotting using diagnostic probes and, subsequently, by PCR).

Results and Discussion

Strategies for using seed treatment fungicides for controlling take-all in sequences of susceptible crops

Seed treatment usually decreased take-all severity by a significant amount except, usually, where only slight take-all occurred. Its incidence (% plants infected) was affected less often. Yield increases, actual and as a proportion of yields in non-treated crops, resulting from take-all control were usually greatest when there was most take-all in the non-treated crops, although an exception occurred in one experiment. Yield and quality in the most severely diseased non-treated crops were unacceptably low, however, and seed treatment did not improve them to an acceptable level.

The development of epidemics was plotted from the previous break crop in four experiments. In two or, possibly, three experiments, the progress of the epidemics was disrupted by sharp decreases in take-all that were unlikely to be explained wholly, if at all, as take-all decline, but rather by weather and late sowing. The epidemics in plots that grew treated crops every year took an almost parallel course, but with less disease, especially in peak take-all years, to those in plots that grew only non-treated crops. The yield benefits from growing treated crops successively on the same site were less than expected from the effects on take-all, suggesting a yield penalty, though unexplained, from repeated treatment. The epidemics in plots that remained non-treated after an initial treatment usually then took the same course as those in the plots that grew only non-treated crops. Treatment followed by non-treatment appeared to result in a delay in the later stages of the epidemic only in two experiments, and it is likely that this would have resulted in a delay in reaching effective take-all decline. A previous experiment indicated that the take-all peak can also be delayed in this way. This expected behaviour was apparently disrupted by the large decrease in take-all in the fourth wheat crops in two of the experiments.

Continuous wheat cropping is still practised in the UK, although the acreage involved has decreased since the late 1980s. The purpose is often to take advantage of take-all decline. These experiments suggest that seed treatment can decrease take-all and increase yield during take-all decline but, as in the pre-decline stages, withholding treatment to a crop grown after a treated crop will result in increased disease. Seed treatment should therefore not be used in take-all decline situations, unless it is proposed to follow the treated crop with a break crop. Similarly, seed treatment is unlikely to be worthwhile during the early stages of a take-all epidemic if the intention is to progress to, and exploit, take-all decline.

Cost benefits of treatment, assuming a marketable crop and that all other costs were equal, were calculated for the experiment considered the most representative. They reflect yield responses and confirm that optimum benefit resulted from treatment to only one crop in a sequence. A treated crop should not, therefore, be followed by another cereal crop. If that is unavoidable, then the following crop should also be treated.

Fluquinconazole seed treatment can delay the development of septoria disease but whether or not this obviates the need for a full septoria control programme later in the crop's

growth was not tested. Eyespot was not usually controlled by fluquinconazole seed treatment, in agreement with a previous report, despite the known sensitivity of one of the eyespot pathogens, *Tapesia yallundae,* to the fungicide. Occasional increases in eyespot in fluquinconazole-treated crops may have resulted from enhanced activity of *T. acuformis*, which is relatively insensitive, in the absence of competition from other, more sensitive fungi. These latter fungi are unlikely to include *T. yallundae*, since other evidence suggests that the two eyespot pathogens are not competitors.

Identification of any adverse or beneficial effects of seed treatment fungicides on populations of the take-all fungus and on other fungi, including those suppressive to take-all

There was no evidence of resistance to fluquinconazole, or of selection for resistance by the use of fluquinconazole, in populations of the take-all fungus in plots that had grown treated wheat crops for up to four consecutive years. Selection for resistance is therefore not expected to be a problem even where fluquinconazole is used repeatedly.

Fungal diversity was not affected by seed treatment, consistent with previous observations that most root-inhabiting, non-pathogenic fungi have low sensitivity to fluquinconazole. Any involvement of antagonistic, non-pathogenic fungi in take-all decline or other types of suppression is unproven, but changes in the development of the epidemic after seed treatment are clearly a consequence of altered amounts of the disease itself and not of changes in populations of other fungi.

A large increase in *Trichoderma* spp. in non-treated plots, after the third wheat crop, may have been a consequence of repeated wheat cropping and was possibly associated with the onset of take-all decline. This is consistent with the, sometimes, delayed increase in treated plots. The trend for year on year increases in *Trichoderma* spp. without seed treatment did not occur consistently in previous experiments at Rothamsted, in which wheat crops grown in different sequences on the same site were compared in the same year. *Trichoderma* spp. have been associated with take-all suppression (but not specifically take-all decline) in soils acidified with ammonium sulphate in Western Australia.

Fusarium culmorum was frequent and was not affected by seed treatment. Its incidence tended to be greatest when there was most disease, suggesting opportunistic colonisation of diseased roots. A role for *F. culmorum* in take-all suppression therefore seems unlikely, although its frequency on roots has previously been associated with continuous wheat growing on sites at Rothamsted. It is unimportant as a root pathogen in UK soils but the root-infecting phase may contribute to the reservoir of inoculum for foot rot and ear blight diseases.

Determination of population structure of the take-all fungus (determined by DNA tests) as the basis of a risk assessment method

Populations of the take-all fungus were analysed by determining the RFLP type of individual isolates using a DNA probe. Population structure was found usually to change from year to year but was not affected significantly by fluquinconazole. The hypothesis that the greatest proportions of RFLP type T2 in populations precede crops with the most severe take-all was tested. Although this sometimes occurred, its inconsistency, and large variations in population structure among sites, precluded the use of the RFLP test as the basis of a risk assessment method. An alternative to the RFLP method using a much simpler PCR method, which is suitable for DNA extracted directly from roots, was developed. The sub-groups of the take-all fungus identified by this method correspond closely to the RFLP types. Since the sub-groups are also correlated with sensitivity to the take-all-specific fungicide, silthiofam, as well as being selected by different host crop species, the PCR method is a potentially valuable tool for future research.

Conclusions

1. The seed treatment experiments led to a series of recommendations for the use of fluquinconazole in sequences of wheat crops, summarised here.

   i) First wheat crops will not develop take-all if they are grown after non-susceptible break crops free of cereal volunteers or grass weeds and so treatment is unnecessary. They should, however, be managed to minimise take-all development in second wheats, particularly by avoiding very early sowing.

   ii) Second (or third) wheat crops grown on sites known to be conducive to take-all will usually benefit from fluquinconazole. There will usually be an economic return, although control of the most severe take-all will still not produce an acceptable, or profitable, crop.

   iii) A break crop should follow a treated crop. If, however, a wheat crop is grown after a treated crop, then it should also be treated.

   iv) Fluquinconazole should not be used in long sequences of wheat. If the intention is to take advantage, subsequently, of take-all decline, treatment may delay the onset and decrease the intensity of take-all decline. It should not be used in take-all decline situations, unless the intention is to break the sequence and follow the treated crop with a non-cereal crop.

2. Repeated use of fluquinconazole on the same site is unlikely to lead to resistance in the take-all fungus or to have any adverse effects on the potentially antagonistic component of the fungal community in the root zone.

3. Changes in the population structure of the take-all fungus from year to year, determined by a molecular (RFLP) marker were confirmed. The extent of the change was not sufficiently consistent for this to be used as the basis of a universally applicable method of risk assessment. The development of a much quicker PCR procedure has provided a valuable research tool for use in further population studies.

PART 3: TECHNICAL DETAILS

1. Introduction

1.1 Background

Take-all, caused by the fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is one of the most important diseases of cereals, especially wheat, in the UK and worldwide and is of considerable concern to farmers. The best estimates of the damage caused by take-all are based on data collected in 1986-88 which showed average losses worth *ca* £40M per annum (D. Hornby, pers. comm.; Hornby & Bateman, 1991).

More than most diseases, take-all is not just the result of an interaction between a pathogenic fungus and a plant; it is a product of complex interactions between these and many components of the physical and biological environment. The latter includes a diverse community of micro-organisms that inhabit the root zone. Experimental studies on take-all, and effective disease control, are hampered particularly by its unpredictability and its occurrence in patches. All these problems have been the subjects of recent research and advances, particularly at Rothamsted.

Take-all has long attracted the interest of agrochemical companies but only recently was there a realistic prospect of effective fungicides, and two (Beale *et al.*, 1998; Löchel *et al*., 1998) have become available commercially since this project began. Because the disease is so widespread and damaging there may be a temptation for farmers to use such materials routinely, regardless of actual risk of disease.

The disease develops in consecutive cereal crops in a characteristic fashion. It often increases to a peak over 2-4 years, after which it typically declines in severity (`take-all decline'). Predicting the timing of severe disease is, however, difficult but necessary if chemical treatments are to be used effectively and efficiently. Some evidence was found in previous research that the population structure of the pathogen in a wheat crop, determined using a DNA probe to identify individual isolates as one of two main genetic types, was associated with the severity of take-all in a wheat crop grown immediately afterwards (Bateman *et al*., 1997). This observation has potential practical value in risk assessment.

1.2. Objectives

Seed treatment fungicides have the potential to decrease the losses caused by take-all but their effective and economic use depends on accurate targeting of those crops that will benefit from treatment. Take-all epidemics typically build up and decline over a number of years and so it is important that the use and effects of fungicides are studied in cropping systems (cereal sequences) as well as in individual crops. The effects of using fluquinconazole in sequences of wheat crops were studied to identify the most effective strategies for exploiting them and to determine whether there is any risk of adversely affecting the biotic environment or

compromising the development of take-all decline. The research was also designed to extend studies on population dynamics of the take-all fungus and other fungi, and so help to determine long-term effects of using seed treatment fungicides and the potential for developing risk assessment methods. The specific objectives were:

1) To identify the best strategies for using seed treatment fungicides for controlling take-all in sequences of susceptible crops.
2) To identify any adverse or beneficial effects of seed treatment fungicides on populations of the take-all fungus and on other fungi, including those suppressive to take-all.
3) To seek confirmation of evidence that population structure of the take-all fungus can provide the basis of a risk assessment method.

2. Materials and Methods

2.1. Field experiments testing seed treatment

Five field experiments tested the effects of fluquinconazole seed treatment (as Jockey F, in which fluquinconazole is the only active ingredient, supplied by Aventis CropScience), applied at 75 g a.i./100 kg seed, in sequences of winter wheat crops, cv. Hereward. The seed was treated using a small-scale Rotostat at Rothamsted. Three of the experiments (coded CS) were on silty clay loam with flints at Rothamsted Experimental Farm, Hertfordshire; the others were on sandy loam at the former Aventis trials farm in north Norfolk (East Winch) and on limestone brash at the Scott Abbott Agricultural Trust Farm (Sacrewell) in Cambridgeshire. Selected operations and applications, especially those with most potential to affect take-all, are summarised in Table 2.1. Other operations (e.g. herbicide applications) were done as necessary or were standard for the farm(s) on which the experimental crops were grown.

CS/476 (Long Hoos IV field) tested fluquinconazole seed treatment against no treatment in all combinations of years from first wheats, grown in 1997 (the date refers to the year of harvest and so experiments were usually sown in the autumn of the previous year), to sixth wheats, grown in 2002. The experiment was designed as four fully randomised blocks of eight plots (10 m x 3 m) to test effects of treatment in each of 3 years (i.e. 2^3), but there was effectively extra replication in the early years, before later treatments were applied. When the experiment was continued into the fourth and fifth years, it became, first, two blocks of 16 plots and then a single replicate. In the sixth year, the treatments repeated those applied in the first year, which were assumed to be no longer having an effect and were ignored in the analysis of the data.

The experiments at East Winch, on Stackyard field at Rothamsted (CS/508) and at Sacrewell tested seed treatment against no treatment in all combinations of years from second wheats (in 1999) to fifth wheats (in 2002). Four fully randomised blocks of 16 (2^4) plots (12 m x 3 m at East Winch, 10 m x 6 m in CS/508, and 14 m x 4 m at Sacrewell) were used.

CS/323 (West Barnfield) is a long-running crop-sequence experiment that was used to test sequences of different cereal species in three randomised blocks from 1988-1995. Winter wheat was sown in all plots (10 m x 3 m) from 1996, except in 1997 when the field was in set-aside. The experiment was incorporated into this project to determine effects of seed treatment in different take-all decline situations. Three groups of eight plots with different cropping histories were identified. Group I plots had been in continuous wheat since 1988 and so were expected to have well-established take-all decline. Group II plots had barley or triticale up to 1995 and so take-all decline was expected to be less well established. Group III plots had sequences up to 1995 that included oats and so take-all decline was expected to be weak or not established. Fluquinconazole seed treatment was tested against no treatment in all combinations of years from 1999 to 2002.

2.2. Field sites for take-all monitoring

Four further field sites were, like the field experiments, monitored for take-all incidence and severity, soil infectivity and take-all fungus population structure throughout the project. An area of about 2 ha in crops of continuous winter wheat was made available at each site. Site B was on a coarse loamy soil (over sandstone) near Radstock, Avon. Site C was on a brashy calcareous clay soil (over limestone) at Maperton, Somerset. Site L was on a deep well-drained sandy loam soil at Long Ashton Research Station, Avon. Site W was on sandy loam at Woburn Experimental Farm, Bedfordshire. Sampling started in 1999 in a second wheat crop on site B and in a pea crop on site C. Sampling on other sites (L and W) started in first wheat crops in 1999. Fluquinconazole was not applied to the seed used for any of the crops. The main agronomic operations and applications are shown in Table 2.2.

2.3. Field sampling

Plants from CS/476 were sampled to assess diseases affecting the roots and stem bases in the spring of each year at growth stage (GS) 30-34 (Zadoks *et al.*, 1974) (Table 2.3). Five or six 10-cm lengths of row were dug from random positions in each plot.

In CS/508, ten pairs of adjacent plants were dug, at the beginning of June in each year (GS 53, 45, 47-51 and 55, respectively, in 1999-2002) from random positions in the eight plots that were sown with untreated seed or with treated seed in all years. These plants were used to isolate and identify fungi from the roots.

The main plant samples to assess take-all and stem-base diseases were taken from all experiments in late June (GS 69-73). Ten 20 cm row-lengths were dug from each plot along two parallel zig-zag transects (Table 2.3). The plants from each transect, tied together in five bundles, were washed thoroughly, air-dried and stored for later assessment. At the same time, five soil cores (5.5 cm diam. x 12 cm deep) were taken from each plot for take-all infectivity bioassays and isolations of the take-all fungus.

Plant samples and soil cores were also taken from the four monitoring sites at about the same time (Table 2.4). Five 20 cm row-lengths of plants for disease assessments and five soil cores for infectivity bioassays (processed as above) were taken from each of 10 areas of 1 m^2 on each site in late June or early July (GS 71-75).

2.4. Disease assessments

The plants from the spring samples in CS/476 were examined immediately after washing them, and the total numbers of plants, and the numbers of plants and roots on each plant with take-all symptoms were recorded. Percentages of diseased plants and roots per plant were calculated. Percentages of plants and shoots with eyespot (*Tapesia* spp.), symptoms of other diseases on the stem base and with gout fly (*Chlorops pumilionis*) damage were also determined.

The disease assessments on stored plants from June/July samples from experiments and monitoring sites were made after soaking in water. Take-all was assessed on each plant, held under water against a white background, and scored on a 0-5 scale: 0 = no disease; 1 = slight take-all, less than 10% of the root system affected; 2 = slight take-all, 11-25% of the root system affected; 3 = moderate take-all, 26-50% of the root system affected; 4 = moderate take-all, 51-75% of the root system affected; 5 = severe take-all, 76-100% of the root system affected. We consider this more realistic than the widely used system in which category 5 has the range 61-100% (e.g. Schoeny *et al*., 1998) because yield losses in UK conditions tend to relate best to the upper part of this range in samples taken at this time (Gutteridge *et al*., 2003). From these scores, a mean take-all index (TAI) per plot (maximum 100) was calculated by summing the products of the percentages of plants in each score category by the corresponding score value and dividing the total by 5. TAI is referred to throughout the report as a measure of take-all severity although it is, more correctly, a measure of disease intensity, combining incidence and severity. TAI was used in preference to the somewhat cruder take-all rating (Dyke & Slope, 1978), which has a range of 0-300, although there is no consistent difference in the information they provide. The results presented here include only TAI values, total percentages of plants with take-all in any severity category (a measure of incidence) and percentage of plants with take-all in either the moderate or severe categories.

Stem-base diseases were assessed on plants from five of the 10 20-cm row lengths taken from each plot in the seed treatment experiments. Eyespot on each stem was assessed as slight (less than 50% girdling, no damage), moderate (more than 50% girdling, no softening) or severe (more than 50% girdling, stem soft). Sharp eyespot (*Rhizoctonia cerealis*) and brown foot rot (*Fusarium* spp. or *Microdochium nivale*) were assessed as slight or moderate-severe in a similar way.

2.5. Take-all infectivity bioassays

Each soil core (12 cm deep x 5.5 cm diam.) was inverted into a plastic beaker, with drainage holes drilled into its base that were covered with moist coarse sand. Ten wheat seeds (cv. Hereward) were placed on the soil surface and covered with horticultural grit. They were put in a controlled environment room (16-h day, day/night temperatures 15/10^{o}C and 70% r.h.) where they were watered to soil capacity and then twice weekly. After 5 weeks, the bioassay plants were removed from their pots, the roots were washed and the presence or absence of take-all lesions recorded on each main root axis. The mean percentage of roots infected in each soil core was determined as a measure of soil infectivity.

2.6. Fungal isolations

The method for assessing communities of root fungi, at a time of year when suppression is expected to be operating during take-all decline in continuously cropped wheat (Hornby, 1992), was as described previously (Bateman & Kwaśna, 1999). Root systems of plants taken in early June from plots in Rothamsted experiment CS/508 that were sown with either treated seed or untreated seed in every year were washed in running water. Six to eight randomly chosen 1-cm-long root pieces were cut from the upper parts of the root system of each plant, approx. 1.5 cm from their points of attachment. The root pieces from each plot were pooled and washed 20 times, for 3 min each time, by shaking vigorously in 10 ml sterile distilled water. Fresh sterile water, cooled to 5^{o}C, was used for each wash. The root pieces were dried in a sterile air-flow on sterile filter paper and each was cut into two 0.5-cm pieces. One piece was put onto potato dextrose agar (PDA; Oxoid) and one onto low nutrient agar (SNA: KH_2PO_4, 1 g l^{-1}; KNO_3, 1 g l^{-1}; $MgSO_4.7H_2O$, 0.5 g l^{-1}; KCl, 0.5 g l^{-1}; glucose, 0.2 g l^{-1}; sucrose, 0.2 g l^{-1}; Agar Technical (Oxoid), 15 g l^{-1}) (Nirenberg, 1976). Both media contained penicillin (30 mg l^{-1}), streptomycin sulphate (133 mg l^{-1}) and chloramphenicol (50 mg l^{-1}). The plate cultures were incubated for 3-5 days at 20^{o}C followed by 5-60 days at 5^{o}C. The plates were examined microscopically at intervals. Sporulating fungi were identified. Further subcultures onto PDA or SNA were made as necessary. Sporulation of some subcultures was encouraged by incubation under near-ultraviolet light at 15^{o}C, or in daylight.

2.7. Take-all fungus isolation and morphological characterisation

Pieces of root (*ca* 1 cm long) were cut from the upper parts of the root systems on six different bioassay plants grown in each soil core. Tests were done on bioassay cores from plots sown either with treated seed in every year or with untreated seed in every year in experiments at East Winch, Rothamsted (CS/508) and Sacrewell, and on those from all 1 m^2 sampling areas at the monitoring sites. The root pieces were surface-sterilised for 3-5 min in sodium hypochlorite (*ca* 2% available chlorine), rinsed twice in sterile distilled water and dried on filter paper in a sterile air-flow. They were then placed on PDA containing penicillin (30 mg l^{-1}), streptomycin sulphate

(133 mg l^{-1}) and chloramphenicol (50 mg l^{-1}) and incubated at 20°C for 3-6 days. Cultures resembling *G. graminis* were then transferred to fresh PDA containing the same antibiotics. Generally, three isolates were obtained after plating out six root pieces from six different bait plants grown in each soil core; each isolate was number-coded to indicate its origin. After 2 weeks at 20°C, any sectoring in the colonies was recorded. Colony pigmentation was scored on a 0-5 scale (0 = white, 5 = almost black), as was morphology (0 = flat, 5 = floccose), on 2-week-old cultures. They were then kept at 4°C.

2.8. Fungicide sensitivity testing

Isolates of *Ggt* from populations sampled annually from fungicide experiments (see sections 2.5 and 2.6) were tested for sensitivity to fluquinconazole to test whether selection for decreased sensitivity was occurring. Isolates from some populations were also tested, as part of the broader population characterisation study, for sensitivity to the other take-all fungicide, silthiofam, since differences among isolates in sensitivity to this fungicide have been reported (Joseph-Horne *et al*., 2000). Technical-grade fluquinconazole suspended in methanol, or silthiofam formulated as 125 g l^{-1} EC (Latitude) and diluted in sterile water, was added to sterile, molten (50°C) PDA at 1 ml 100 ml^{-1}. This was poured into Petri dishes to give final concentrations of 0.01 and 0.1 mg l^{-1} (fluquinconazole) and 0.5 or 1 mg l^{-1} (silthiofam). Control agar contained the same volume of non-amended methanol or water. Three 4-mm-diam. plugs from the edges of colonies of the take-all fungus isolated from soil bioassay plants were put onto each plate. In fluquinconazole tests, three replicate plugs of each isolate were placed on different plates of each fungicide concentration. In silthiofam tests, nine replicate plugs of each isolate on were placed on three plates of each fungicide concentration. Increases in colony diameter were measured after 4 days at 20°C.

2.9. Molecular characterisation of take-all fungus populations

Each isolate from the soil bioassay plants (see sections 2.5 and 2.6) was subcultured into *ca* 15 ml LB-broth (tryptone, 10 g l^{-1}; yeast extract, 5 g l^{-1}; NaCl, 5 g l^{-1}) in a universal bottle and grown at 22°C for 6 days. DNA extraction from LB-broth cultures was based on the method of Lee & Taylor (1990), as described previously (Ward & Bateman, 1994). Subsequent digestion with restriction enzyme *Eco*RI and Southern hybridisation, using a mitochondrial small-subunit rDNA probe, pEG34 or pGgtMS7, were as described previously (Bateman *et al*., 1997). The AlkPhos direct kit (Amersham Pharmacia Biotech UK Ltd, Little Chalfont, UK) was used for labelling and chemiluminescent detection, following the manufacturer's instructions, with a hybridisation temperature of 68°C. Isolates of *Ggt* were identified as RFLP type according to the presence (type T1) or absence (type T2) of a band representing a 4.0-kb DNA fragment to which pEG34 or pGgtMS7 had hybridised. A variant, T1c, produced a band in the 2.5-kb position rather than the 4.0-kb position.

Consensus fungal primers ITS4 and ITS5 (White *et al.,* 1990) were used for PCR, followed by RFLP analysis of the products as described in Ward & Akrofi (1994). These primers amplify ribosomal DNA (rDNA), specifically a region of DNA from the 3' end of the 18S-like gene to the 5' end of the 28S-like gene and including the 5.8S gene and the two internal transcribed spacer (ITS) regions. Each 25 µl PCR reaction contained 25 pmol of both primers**,** 0.25 units of *Taq* DNA polymerase (MBI Fermentas), buffer (Tris-HCl, 10 mM pH8.8; KCl, 50 mM; Nonidet P-40, 0.08%; BSA, 0.1 mg ml^{-1}; $MgCl_2$, 1.5 mM), 0.2 mM deoxyribonucleotide triphosphates and DNA (1 µl of 1:40 dilution of genomic DNA stock solution). Cycling conditions were 30 cycles of 94°C for 30 s, 42°C for 2 min and 72°C for 2 min followed by a final extension of 72°C for 10 min. PCR products (5 µl or 8 µl), were digested with *Hpa*II (Roche Diagnostics Ltd., Lewes, UK) in 1 x buffer (supplied as 10 x stock by the manufacturer) at 37°C for 2-4 hours. Restriction-digested PCR products were electrophoresed on agarose gels (2% NuSeive agarose + 1% standard agarose), in 1 x TBE, containing 0.5 µg ml^{-1} ethidium bromide. This method identified two sub-groups, A and B, within populations of the take-all fungus.

A *Ggt*-specific PCR that identifies the same sub-groups of *Ggt* whilst avoiding the need for restriction-digestion was developed in the course of this project. DNA was amplified using a common forward PCR primer (Ggtfwd), together with two reverse PCR primers (GgtArev and GgtBrev2). Each 12.5 µl PCR reaction contained 0.4 µM primers, 0.125 U Sigma REDTaq DNA polymerase (Sigma-Aldrich Company Limited, Poole, UK), buffer (Tris-HCl, 10 mM pH 8.3; KCl, 50 mM; gelatin, 0.01%), 1 mM $MgCl_2$, 0.2 mM deoxyribonucleotide triphosphates and DNA (1 µl of a 1:100 dilution of DNA stock solution). A touchdown PCR was used with an annealing temperature range of 72-67°C, decreasing by 1°C after every two cycles with 20 cycles at the minimum annealing temperature of 67°C. Cycling conditions were: a total of 30 cycles of 94°C for 30 s, annealing (as described above) for 1 min, 72°C for 1 min, followed by a final extension of 72°C for 10 min. PCR products were analysed on 2% agarose gels and the DNA stained with ethidium bromide. *Ggt* A-type and B-type isolates gave PCR products of 93 and 132 bp respectively.

The PCR was also tested on DNA prepared directly from wheat roots. Each preparation was made from three freeze-dried *Ggt*-infected root pieces, approximately 1 cm long. Grinding with metal rods (as for fungal mycelium) for approximately 1 min was sufficient to partially macerate tissue at the root surface, but not to obtain a powder, before DNA extraction. DNA was then extracted exactly as for fungal mycelium. As a control, DNA was extracted from non-infected wheat roots in the same way.

2.10. Statistical analyses

Statistical comparisons were made using Genstat. Most data from field experiments were tested by analysis of variance. Percentage values were usually analysed after transformation

to logits. Variations in the severity of take-all were especially large across the area occupied by experiment CS/476, and especially during the build-up of the disease. In the analyses of yields, this resulted in relatively large residual mean squares. Therefore, additional analyses were done using, as covariates, plot residuals derived from analyses of take-all indices in the same year. These gave significant improvements in precision, and the results of these analyses are the ones presented.

3. Objective 1: To identify the best strategies for using seed treatment fungicides for controlling take-all in sequences of susceptible crops

3.1. Results

3.1.1. Effects of seed treatment on take-all, grain yield and soil infectivity in the year of application of the treatment

Tables 3.1-3.11 show the incidence of take-all (% plants diseased) and its severity (number of roots diseased, % plants in different severity classes and take-all index) in each experiment. They also show residual infectivity in the soil (% diseased roots on soil bioassay plants; % diseased plants are not shown), and grain yields and quality measurements where available. The results are from plots that were treated or not treated in the harvest years shown and are averaged over treatments applied in all previous years. Interactions between treatments applied in different years were rarely significant.

Rothamsted (CS/476). Fluquinconazole was tested on the 1997 crop (first wheat) but there was negligible disease. In samples taken in April, disease incidence in 1999, 2000 and 2002 and severity in 1999 and 2002 were decreased by seed treatment; there was much less disease in the other years, 1998 and 2001 (Table 3.1). Disease incidence on plants in summer was decreased significantly by treatment only in 2001 and 2002 but severity was decreased in all years from 1998. Soil infectivity, not determined in the last two years, was affected significantly in 2000, when it was increased by treatment.

Grain yields were severely decreased by take-all in 1999 and 2000 (Table 3.2), when the peak of disease was reached. Yields increased in 2001 but fell dramatically in 2002 as a result of resurgent take-all. Plots treated with fluquinconazole gave larger yields than non-treated plots in all years but the difference was not always significant.

There were significant effects on plant establishment in 1998 (measured in March), when seed treatment resulted in fewer ($P = 0.03$) plants (353 plants m^{-2}) than did no treatment (397 plants m^{-2}).

East Winch. Samples taken from the non-treated first wheat crop in 1998 (i.e. before the experiment started) showed that it had negligible take-all. Fluquinconazole decreased take-all incidence in June in all years except 2000 (Table 3.3). It decreased take-all severity in all years. Soil infectivity was not affected. Grain yield, thousand-grain weight and specific weight were increased significantly by fluquinconazole only in 1999 (Table 3.4). The crop in 2002 was very poor and could not be harvested satisfactorily.

Rothamsted (CS/508). There was negligible take-all in the non-treated first wheat in 1998 that preceded the experiment. Take-all incidence in June/July was decreased significantly by fluquinconazole only in 2001 (Table 3.5). Take-all severity was decreased in all years except 1999, when it was generally slight. Soil infectivity was increased by fluquinconazole in 2000. Grain yield was increased significantly by fluquinconazole in 2001 and 2002 (Table 3.6). Fungicide increased thousand-grain weight and/or specific weight in 2000-2002 but not in 1999 when take-all was generally slight.

Sacrewell. Take-all incidence and severity in June were decreased by fluquinconazole in all years except for incidence in 2001, when there was very little disease (Table 3.7). Effects on soil infectivity were not significant. Grain yields were increased by treatment in all years except 2001 (Table 3.8). When measured, specific weights, but not thousand-grain weights, were increased by fluquinconazole. Yields were poor from 2000 onwards and the poor yield in 2002 was associated with a heavy infestation of cereal cyst eelworm (*Heterodera avenae*).

Rothamsted (CS/323: take-all decline site). In 1999, there was least take-all in June/July in the group of plots in which take-all decline was expected to be well-established and most where take-all decline was expected to be only just becoming established (Table 3.9). By 2001, amounts of take-all in the three groups of plots were very similar. Significant differences in grain yield reflected the amounts of take-all in 1999 and 2000 but not in 2001.

Fluquinconazole decreased take-all incidence in 2001 and 2002, and take-all severity in all four years (Table 3.10). Grain yields and thousand-grain weights were increased by fluquinconazole, except in 2000 (Table 3.11). There were no significant interactions between degree of establishment of take-all decline (group) and fungicide treatment (statistics not shown).

3.1.2. Effects of seed treatment on take-all and grain yield in the following crop

Tables 3.12-3.14 show the effects on take-all severity, soil infectivity and grain yield of seed treatment applied to the crop grown in the year of measurement and harvest or to the crop grown in the preceding year. The effects are averaged over treatments applied in all other years including the preceding year (for effects of treatments in the year of measurement) and the 'current' year, i.e. year of measurement (for effects of treatments in the preceding year). Interactions between treatments applied in different years were rarely significant, indicating that subsets of plots that were or were not treated in one of the two years showed similar effects of treatments applied in the other year.

Take-all severity was always decreased significantly, except when very slight, by seed treatment applied to the crop that was sampled (see section 3.1.1 and Table 3.12). It was often increased, however, sometimes significantly, by treatment of the preceding crop. The

effect tended to be greatest in the later stages of the epidemics or when disease was most severe. It occurred during take-all decline (experiment CS/323) as well as in pre-decline epidemics.

Soil infectivity was affected significantly by treatment of the preceding crop only in assessments made in 2001. In CS/508 and at Sacrewell, but not in the equivalent experiment at East Winch, infectivity in 2001 was greater in plots treated in 2000 than in plots not treated in 2000 (Table 3.13). In CS/508 (and CS/476), seed treatment in 2000 also increased soil infectivity measured in that year (see Tables 3.1 and 3.5).

Grain yield was affected in a corresponding way to disease (Table 3.14), with decreases in yield often resulting from seed treatment applied to the previous crop. There was a relatively large increase (+0.53 t ha^{-1}), however, in experiment CS/476 in 1998 following seed treatment applied to the first wheat in 1997. This is the only example of a significant positive effect on yield following treatment in the preceding year. Otherwise, the negative effects on yield were often greater than would be expected from the relatively small effects on take-all, for example in CS/508 in 2001 and 2002. A significant decrease in yield from treatment applied 2 years earlier occurred only once, in 2001 at Sacrewell, when the decrease was -0.27 t ha^{-1} (P<0.001).

3.1.3. Effects of seed treatment on the development of take-all epidemics from year to year

Figs 3.1-3.16 show the year-to-year progress of take-all epidemics in all experiments except CS/323 (the take-all decline experiment), based on take-all index in June/July. Complete graphs are shown only for those plots that grew non-treated crops every year, treated crops every year from the first (CS/476) or second wheat crop, or a sequence in which only the second crop was treated. The number of plots from which the means were obtained decreased by half in successive years as treatment or non-treatment was introduced. Epidemics in other sequences elaborate on these to a small extent only and are omitted for clarity.

Figs 3.1-3.4 compare mean amounts of take-all in all plots that had been repeatedly treated or non-treated up to the time of assessment at each site. In the Rothamsted experiment CS/476, severe take-all occurred in non-treated plots in the third (1999) and fourth (2000) crops (Fig. 3.1). The large decrease in take-all in the fifth wheat (2001) was indicative of take-all decline, although suppression was not sustained and there was a return to severe disease in the sixth wheat. A severe gout fly infestation may have exacerbated take-all in that year. Repeated application of the seed treatment resulted in a parallel, but less severe epidemic, the greatest effects on disease occurring in the years of the first peak. At East Winch, severe take-all occurred in the second wheat (1999) in non-treated plots and it reached a peak in the third wheat (Fig. 3.2). A steep decrease in the fourth wheat was associated with late sowing but take-all decline may have contributed. An increase to moderate take-all intensity occurred in the fifth wheat, which is not unusual after the onset of take-all decline.

The epidemic in the plots that grew treated crops every year from the second wheat was approximately parallel but less intense. The largest difference between the two graphs occurred in the second and fifth wheat crops, which had similar take-all intensities. In experiment CS/508 at Rothamsted, peak take-all occurred in the non-treated plots in the third wheat crop (2000), with progress towards take-all decline occurring subsequently (Fig. 3.3). Repeated treatment resulted in most disease reduction in the third wheat crop, with a smaller decline in the epidemic in the following year and an increase in take-all in the fifth wheat. This suggests that take-all decline had not developed in the treated plots. At Sacrewell, take-all did not become very severe, but the epidemic in non-treated plots reached a moderate peak in the second (1999) and third wheats, followed by a decline, to which late sowing undoubtedly contributed, and then an upsurge in the fifth crop (Fig. 3.4). Repeated treatment resulted in a similar epidemic, with the greatest suppression of disease in the peak years.

Figs 3.5-3.8 show the effects of applying fluquinconazole for the first time in each year of each experiment. In experiment CS/476 at Rothamsted, single treatments decreased take-all effectively except in the fifth wheat (2001), which had little take-all (Fig. 3.5). The effect was greater at the first take-all peak than after the fifth-wheat decline. At East Winch, treatment for the first time decreased take-all effectively except in the fourth wheat (2001), when there was little take-all (Fig. 3.6). In experiment CS/508 at Rothamsted, treatment for the first time decreased take-all in each year, but the decrease was very small in the second (1999) and fifth wheats (Fig. 3.7). At Sacrewell, first-time treatment resulted in decreased take-all, with the smallest decrease in the fourth wheat (2001), when there was little disease (Fig. 3.8).

Figs 3.9-3.12 show the effects of withholding fluquinconazole treatment after all previous experimental crops had been treated, in each year of each experiment. In experiment CS/476 at Rothamsted, withholding treatment always resulted in increased take-all compared to the repeatedly-treated plots (Fig. 3.9). At East Winch, withholding treatment similarly resulted in increased take-all except in the fourth wheat (2001), when there was little disease (Fig. 3.10). In experiment CS/508 at Rothamsted, withholding treatment resulted in increased take-all on each occasion (Fig. 3.11). At Sacrewell, withholding treatment resulted in increased take-all except in the fourth wheat (2001) when there was little disease (Fig. 3.12), as at East Winch.

Figs 3.13-3.16 show the epidemics in plots where only the second wheats were treated, compared with epidemics in plots that were treated or non-treated throughout, in each experiment. In experiment CS/476 at Rothamsted, this epidemic was similar to that in the non-treated plots from the third wheat (1999) onwards (Fig. 3.13). An indication of more severe take-all in the fifth wheat may be unreliable because of the minimal replication in the later stages of this experiment. At East Winch, the epidemic in plots that grew treated second wheats was similar, from the third wheat (2000) onwards, to that in the plots that were non-

treated throughout (Fig. 3.14). In experiment CS/508 at Rothamsted, treatment of only the second wheat resulted in a similar amount of take-all to that in the repeatedly non-treated plots in the third (2000) and fourth wheats. In the fifth wheat, however, an increase occurred (similar to that in repeatedly treated plots), suggesting that take-all decline had not become established as effectively as in the repeatedly non-treated plots (Fig. 3.15). At Sacrewell, the epidemic following treatment only of the second wheat was similar to that in repeatedly non-treated plots from the third wheat (2000) onwards (Fig. 3.16).

3.1.4. Effects of seed treatment on other diseases and pests

Eyespot was sometimes, but not usually, affected by fluquinconazole seed treatment. It was assessed in spring only in experiment CS/476 and was decreased significantly by treatment only in 2001, from 41% to 31% diseased plants ($P = 0.014$) and from 23% to 15% diseased shoots ($P = 0.037$) (percentages back-transformed from logits). When assessed in summer, it was increased significantly by treatment on five occasions, all at Rothamsted, and decreased on one, at Sacrewell (Table 3.15). Small but significant ($P = 0.02$) increases in eyespot from seed treatment applied to the preceding crop occurred on only two occasions (results not shown) and are considered unimportant.

Slight sharp eyespot and brown foot rot (caused by *Fusarium* spp. or *Microdochium nivale*) was evident in most samples taken in summer. Effects of treatments (not shown) were small and rarely statistically significant; such effects on sharp eyespot were usually the inverse of the effects on eyespot.

Septoria was assessed in CS/476 on 22 December 1997 because there were very obvious effects of treatments. Its incidence was decreased ($P<0.001$) by fluquinconazole from 64.8% to 16.6% of second oldest leaves affected.

Yellow rust (*Puccinia striiformis*) affected a few plots in experiment CS/476 in July 1997 and was much more severe in the non-treated plots than in adjacent fluquinconazole-treated plots (since it was localised, it was not assessed).

Damage by gout fly (*Chlorops pumilionis*) was common in the later years of experiment CS/476 when it probably contributed to the small yields. Symptoms were recorded on 26% of plants on 7 April 2000, and on 61% of plants and 32% of shoots on 25 April 2002. It was not affected by the seed treatment.

3.1.5. Economics of seed treatment

The margins over costs of different treatment regimes are shown only for Rothamsted experiment CS/508 (Table 3.20), because this was the only experiment (other than CS/323) in which yields remained moderately high and the grain of marketable quality throughout. They reflect the effects of treatment on grain yields. Substantial cost benefits occurred only in the fourth wheat crop, after the take-all peak, when disease and yield responded best to seed

treatment. The reason for the poor yield response in the third wheat, at the take-all peak, is unclear but is probably untypical. The greatest cost benefit of treatment was in plots growing a treated crop for the first time, or a treated crop after a non-treated crop. Plots growing successive treated crops showed less benefit.

3.2. Discussion

Fluquinconazole seed treatment usually decreased take-all severity by a significant amount except, usually, where only slight take-all occurred. Its incidence (% plants infected) was affected less often, as reported previously (Russell *et al*., 2002). Yield increases, actual and as a proportion of yields in non-treated crops, resulting from take-all control, were usually greatest when there was most take-all in the non-treated crops. An unexplained exception was in experiment CS/508. Yields in the most severely diseased non-treated crops were unacceptably low, however, and seed treatment did not improve them to an acceptable level. This emphasises the need to integrate all methods for minimising take-all (e.g. delayed sowing and maintaining an adequate nitrogen supply) with the use of seed treatment.

The development of epidemics was plotted from the first wheat crop in four experiments. In three of them (East Winch, Sacrewell and possibly CS/476), the progress of the epidemics was disrupted by sharp decreases in take-all in 2001 that are unlikely to be explained wholly, if at all, by take-all decline. It appears to have been a seasonal effect. The epidemics in plots that grew treated crops every year took an almost parallel course, but with less disease, especially in peak take-all years, than in those plots that grew only non-treated crops. The epidemics in plots that remained non-treated after a single treatment usually then took the same course as those in the plots that grew only non-treated crops. Such a once-only treatment appeared to result in a delay in the later stages of the epidemic only in CS/476 and CS/508. In those experiments, it may eventually have resulted in a delay in reaching effective take-all decline but progress to decline may have been disrupted in all experiments by the generally non-conducive conditions in 2001. A previous experiment indicated that the take-all peak can sometimes be delayed in this way (Dawson & Bateman, 2001a). Withholding treatment in plots that had previously been sown with treated seed for one or more years almost always resulted in an increase in take-all to a severity that was very similar to the mean severity in plots that had never been sown with treated seed. This seemed to be equally true at all stages of the epidemic that were studied and suggests that the use of the fungicide did not interfere in a fundamental way with the development of take-all decline. The increases in take-all where treatment was suspended also suggests that there was abundant inoculum in the treated plots and confirms previous evidence that there is often no correlation between amounts of the disease on the roots and amounts of inoculum that remain in the soil to affect a following crop.

A possible delay in the development of suppression, expressed fully during take-all decline, could account in part for relatively poor responses of disease to the extended use of fluquinconazole over several successive years. It does not, however, account for the yield penalty that we often detected in the year after the application of the treatment, and which is partly the basis of our recommendation that treatment of successive crops should be avoided and that a break crop should, where possible, follow a treated wheat crop. The explanation for this is uncertain. Visible take-all on the roots was often increased in the year following the application of fluquinconazole but the effects on take-all did not usually seem large enough to explain the effects on yield. Despite this, it seems more likely that the yield decreases are a consequence of disruptive effects of fluquinconazole on take-all epidemics than that they are a direct effect of residual fungicide. If so, other fungicides, with the same or different modes of action, may have similar effects. As far as we know, this has not been tested. Alternatively, fluquinconazole may affect micro-organisms that are antagonistic towards *Ggt*, although we have found no evidence to suggest this (Dawson & Bateman, 2000, 2001a, and section 4). If this is the explanation, fungicides with different modes of action might be expected to have different effects. The possibility that this yield penalty might be avoided by alternation of fluquinconazole with other treatments, silthiofam seed treatment or azoxystrobin spray (Jenkyn *et al*., 2000), is being investigated in further research.

Continuous wheat cropping is still practised in the UK, although the area involved has decreased since the late 1980s (Hornby *et al*., 1998). The purpose is often to take advantage of take-all decline. These experiments suggest that seed treatment can decrease take-all and increase yield during take-all decline, but presents the same risk to crops grown subsequently as it does during the pre-decline stages. Seed treatment should not, therefore, be used in take-all decline situations, unless it is proposed to follow the treated crop with a break crop. Whether or not seed treatment delays the onset of take-all decline, it is likely that avoidance of seed treatment in all crops in a sequence is the surest way of achieving the earliest and most effective take-all decline.

The cost benefits, assuming a marketable crop and that all other costs were equal, were calculated for experiment CS/508. They reflect yield responses and confirm that, at current prices, optimum benefit results from treatment of only one crop in a sequence. The aim should, therefore, be to target the fungicide and treat that crop in a sequence that is expected to have significant take-all, and can thus be expected to repay the cost of treatment, but not have such severe disease that yield and quality are so poor that the crop as a whole loses money. For the reasons explained above, a treated crop should not be followed by another take-all-susceptible cereal but, if that is unavoidable, then the following crop should also be treated. A decrease in the cost of treatment would alter the economic arguments but would not eliminate the risk of a yield penalty where a treated crop with significant take-all is followed by another wheat. However, because such a crop is likely to give only small yields

of poor-quality grain, a reduction in the price of fluquinconazole is, perhaps, more likely to encourage its use on first wheats to exploit its activity against foliar diseases (see below). Results from the one experiment, at Rothamsted (CS/476), that tested treatment of a first wheat, indicated a residual benefit, presumably reflecting a reduction in inoculum available to infect the second wheat that followed it. In this experiment there was, however, no visible take-all on the roots of the first wheat. If first wheats have more take-all, perhaps because cereal volunteers in the preceding break were not adequately controlled or because they follow set-aside (after a cereal), it is possible that the following, second, wheats will incur similar penalties to those that were often detected in third and subsequent wheats grown after treated crops. More research to test the effects of using fluquinconazole on first wheats, and the consequent effects on second wheats, is needeed.

Fluquinconazole seed treatment can delay the development of septoria and yellow rust, but whether or not this obviates the need for a full fungicide spray programme later in the crop's growth was not tested. Eyespot control by fluquinconazole seed treatment was not expected, on the basis of a previous report (Dawson & Bateman, 2001b), despite the sensitivity of one of the eyespot pathogens, *Tapesia yallundae,* to the fungicide (Dawson & Bateman, 2000). The occasional increases in eyespot may have resulted from enhanced activity of *T. acuformis*, which is relatively insensitive, in the absence of competition from other, more sensitive, fungi. These latter fungi are unlikely to include *T. yallundae*, since evidence suggests that the two eyespot pathogens are not competitors (Bierman *et al*., 2002).

4. Objective 2: To identify any adverse or beneficial effects of seed treatment fungicides on populations of the take-all fungus and on other fungi, including those suppressive to take-all

4.1. Results

4.1.1. Fungal sensitivity to fluquinconazole

Annual applications of fluquinconazole over 4 years did not affect the sensitivity of isolates of the take-all fungus to the fungicide at any site (Table 4.1). There were apparent differences in sensitivity from year to year, but these, and differences between sites, can not be tested because the data were obtained in different assays. Isolates from CS/508 in 1999 and from East Winch and Sacrewell in 2000 grew significantly ($P<0.05$) more in 4 days when they came from treated plots than when they came from non-treated plots. This was not associated with their response to fluquinconazole in the agar.

4.1.2. Fungal communities

Fungal diversity was not affected by treatment with fluquinconazole in experiment CS/508 at Rothamsted (Table 4.2). There was a suggestion of a greater number of different fungi on root pieces of treated plants in 2000 (not statistically significant), associated with less take-all on plants grown from treated seed than from non-treated seed.

An average of 27 different fungi per plot were identified to species or genus. Small effects of treatment or year on some fungi that are not considered to be important were noted (results not shown). *Trichoderma* spp. and *Fusarium culmorum* may have more significance in take-all epidemics (see 4.2) and their frequencies are shown. *Trichoderma* spp. were more frequent on roots of treated than non-treated plants in 2000, but more frequent on roots of non-treated plants in 2001 (Table 4.2). Frequency of *Trichoderma* spp. increased year on year throughout the experiment. *F. culmorum* was not affected by seed treatment, but was most frequent in 2000 and 2002, the years in which there was most take-all.

4.2. Discussion

There was no evidence of resistance to fluquinconazole, or of selection for resistance by the use of fluquinconazole, in populations of the take-all fungus. Selection for resistance may be considered unlikely to be a problem in such a situation because a fungicide applied to the seed will make contact with only a very small part of the total pathogen population. However, much of the population that survives from a treated crop to provide inoculum for the following crop will have come from the residues of the treated plants and so a disproportionate part of the surviving population will, in all probability, have been in close proximity to the fungicide. Monitoring sensitivity to fluquinconazole in populations of the take-all fungus is therefore advisable.

Fungal diversity was not affected by seed treatment, consistent with previous observations that most root-inhabiting, non-pathogenic fungi have low sensitivity to fluquinconazole (Dawson & Bateman, 2000). Any involvement of antagonistic, non-pathogenic fungi in take-all decline or other types of suppression is unproven, but changes in the development of the epidemic after seed treatment are apparently a consequence of altered amounts of the disease itself and not of changes in populations of other fungi.

The large increase in *Trichoderma* spp. in non-treated plots, after the third wheat crop, may have been a consequence of repeated wheat cropping and was possibly associated with the onset of take-all decline. This is consistent with the delayed increase in treated plots. The trend for year on year increases in *Trichoderma* spp. without seed treatment did not occur consistently in previous experiments, in which wheat crops grown in different sequences on the same site were compared in the same year (Bateman & Kwaśna, 1999). *Trichoderma* spp. have been associated with take-all suppression (but not specifically take-all decline) in soils acidified with ammonium sulphate in Western Australia (Simon & Sivasithamparam, 1988).

Fusarium culmorum was abundant and was not affected by seed treatment. Its incidence tended to be greatest when there was most disease, suggesting opportunistic colonisation of diseased roots. A role for *F. culmorum* in take-all suppression therefore seems unlikely although its frequency on roots has previously been associated with continuous wheat growing (Bateman & Kwaśna, 1999). It is unimportant as a root pathogen in UK soils but the root-infecting phase may contribute to the reservoir of inoculum for foot rot and ear blight diseases.

5. Objective 3: To seek confirmation of evidence that population structure of the take-all fungus can provide the basis of a risk assessment method

5.1. Results

5.1.1. Effects of fluquinconazole seed treatment on population structure of the take-all fungus

The population structure of the take-all fungus, determined as percentage of T2 isolates, was not affected significantly by the repeated use of fluquinconazole seed treatment at any of the sites at which population analyses were made (Table 5.1). The results for % T2 in populations are shown in comparison with those for take-all index, which was usually decreased by treatment (although there was less statistical significance than when all plots were included in the analyses, *cf.* Tables 3.3, 3.5, 3.7), and soil infectivity, which was also unaffected by treatment. Other characteristics (colony pigmentation and morphology, colony sectoring and presence of dsRNA) were similarly unaffected by seed treatment (results not shown).

5.1.2. Population structure of the take-all fungus in relation to epidemic development

Epidemic development based on annual disease assessments, in relation to the frequency of T2 isolates in the preceding crops, was followed in the seed-treatment experiments (see description in 3.1.3 and Figs 3.1-3.16; Figs 5.1-5.6) and at the monitoring sites (Figs 5.7-5.10).

At East Winch, only T2 isolates were found in samples preceding the second and third wheats in treated and non-treated plots (Table 5.1; Figs 5.1, 5.2). The percentage of T2 isolates then decreased before the decrease in take-all in the fourth wheat. T2 remained the predominant type.

At Rothamsted (CS/508), the percentage of T2 isolates decreased between the first and third wheats, during which period take-all severity increased and then decreased (Table 5.1; Figs 5.3, 5.4). The only evidence of an increase (which was small) in the percentage of T2 isolates before an increase in take-all was in the seed-treated plots between the fourth and fifth wheats.

At Sacrewell, the population of the take-all fungus was predominantly T2 (Table 5.1; Figs 5.5, 5.6). There was a gradual increase throughout most of the experiment despite both increases and decreases in take-all severity.

At monitoring site B, take-all was severe in the second wheat crop (the first crop sampled, in 1998), declined markedly in the third wheat and then stabilised at a level of greater severity (Table 5.2; Fig. 5.7). This is typical of take-all decline. Soil infectivity remained at a moderate level. The smallest incidence of the T2 type in the pathogen population occurred in the severely diseased second wheat crop and preceded a marked decrease in take-all in the following crop. The increased proportion of the T2 type in the third

and subsequent crops preceded a consistently moderate level of take-all. Soil infectivity was not related to pathogen population structure or to take-all in the following crop.

At site C, take-all became severe in the second wheat crop (2000), and then decreased in the third wheat before increasing again (Table 5.2; Fig. 5.8). Soil infectivity reached a maximum in the second wheat (Table 5.2). The population structure of the take-all fungus in the pea crop (1998) was based on too few isolates to be reliable but, thereafter, population structure remained almost constant, despite fluctuations in take-all. It was not clearly related to soil infectivity or to take-all in the following crop, although a small decrease in the proportion of T2 isolates preceded the large increase in take-all severity in 2002, after the first build-up phase and following the first indications of possible take-all decline in 2001.

At site L, wheat volunteers in the break crop had allowed the development of some take-all, which was unevenly distributed (as indicated by the high standard error), in the first wheat crop, in 1998 (Table 5.2). A take-all peak was reached in the second wheat crop (1999) (Table 5.2; Fig. 5.9), followed by decline to a low level in the fourth wheat and a subsequent increase. The highest incidence of the T2 type preceded the most severe take-all during the build-up stages (second and third wheat crops), but not the severe take-all in the fifth wheat, after decline had apparently begun.

At site W, take-all did not increase between the second (1999) and third wheat crops (Table 5.2; Fig. 5.10). The low level of take-all in the fourth wheat (2001) was unlikely to have resulted from strong take-all decline, since severe take-all had not occurred previously. In this case, the most severe take-all, in the fifth wheat, followed an increased incidence of the T2 type and decreased soil infectivity in the preceding crop.

Increases and decreases in take-all in 2001 and 2002 at sites C, L and W were similar to those in the fungicide experiments at East Winch, Rothamsted (CS/476) and Sacrewell (Figs 3.1-3.8 and 3.13-3.16).

There were no consistent changes from year to year in fungal morphology or dsRNA in pathogen populations in the seed treatment experiments or at the monitoring sites (see Tables 5.3-5.5).

5.1.3. Association of molecular and morphological characters

Associations between molecular and morphological characteristics of isolates of the take-all fungus are shown for those sites and years that had sufficient isolates of more than one T-type (i.e. data from East Winch and Sacrewell are excluded).

T1 isolates were usually more darkly pigmented than T2 isolates. This difference was significant at three of the five sites in 1999 (Table 5.3), two sites in 2000 (Table 5.4) and one site in 2001 (Table 5.5). In 2001, T1c isolates were significantly less darkly pigmented than other isolates at two sites, and T2 isolates were the most darkly pigmented at one site (Table 5.5). Aerial growth from the colonies was similarly associated with T-type, but with fewer

significant effects. There were few significant differences between T types in colony sectoring, and differences in dsRNA were inconsistent.

5.1.4. Molecular characterisation by PCR

Isolates from Rothamsted (CS/508) and Sacrewell for 1999, 2000 and 2001 (a total of 957 isolates) were typed by PCR amplification using consensus fungal primers ITS4 and ITS5, digestion with *Hpa*II, and electrophoresis on agarose gels. The two sub-populations of *Ggt* identified (A- and B-type) were similar, but not identical, to the sub-populations identified by T-typing (Table 5.6). Results from different experiments were similar to those obtained using the T-typing method (data not shown).

Results from preliminary tests using primers ITS4/ITS5 had indicated a possible correlation between A/B-type isolates of *Ggt* and insensitivity/sensitivity to silthiofam, determined by amended-agar assay, with most B-type isolates being sensitive (data not shown). This was tested further using the *Ggt*-specific PCR (primers Ggtfwd, GgtArev and GgtBrev2). *Ggt* was isolated from bioassay plants from plots in the experiments at Rothamsted (CS/508; 118 isolates) and Sacrewell (111 isolates). These isolates, together with isolates from Rothamsted experiment CS/323 (135 isolates), were identified as A or B type using this PCR assay and tested for sensitivity to silthiofam using the agar plate assay. A regression analysis of the percentage of silthiofam-sensitive isolates on the percentage B-type isolates for each plot (total of 25 plots) showed that the regression was highly significant ($P < 0.001$) (Fig. 5.11).

When DNA prepared directly from wheat roots infected with A- or B-type isolates of *Ggt* was analysed using the PCR assay, it was possible to determine which of the two subgroups was present (Fig. 5.12).

5.2. Discussion

Fluquinconazole seed treatment did not affect significantly the population structure of the take-all fungus in wheat-field soil when determined late in crop growth by the methods used here. Associations between epidemic development and changes in pathogen population structure remain unproven. The changes that occurred may be a consequence of epidemic development or may be causal, but they may be co-incidental. If causal, then the results suggest that fluquinconazole is unlikely to have much, if any, influence on epidemic development by affecting population structure or, usually (since effects on soil infectivity were small), the amount of fungal inoculum.

Previous observations on populations of the take-all fungus in cereal crops grown in different sequences showed that one type (T2) was selected, relative to the other main type (T1), in barley when compared with wheat (Bateman *et al*., 1997). Additionally, there was evidence that the population structure of the take-all fungus, analysed as T types by RFLPs

using probe pEG34, changed from year to year and that the direction of change tended to anticipate the direction of change in disease development. The hypotheses that population structure changes from year to year as the epidemic develops and that the direction of change anticipates the course of the epidemic have now been tested. Whilst population structure was shown, usually, to change from year to year, only on a few occasions did the greatest proportions of the T2 type precede crops with increased take-all intensity (as anticipated). This occurred, for example, at site B between the third and fourth wheats and at site W between the fourth and fifth wheats. Conversely, a decrease in the proportion of the T2 type sometimes preceded decreased take-all, notably at East Winch between the third and fourth wheats. Because of the inconsistency and, additionally, very different proportions of the two T types in populations at the start of wheat crop sequences at different sites, the T-typing method does not provide the basis of a universal method of risk assessment. It remains possible, nevertheless, that the differences between sites and changes over time in the relative frequencies of the two types do have epidemiological significance.

The new PCR method is able to identify A and B types of the take-all fungus that correspond closely to the RFLP types T1 and T2, even though they are based on different target sequences. A and B types can be identified in DNA samples extracted directly from root tissue. As with the T types, and for the same reasons, it is unlikely that this method can be used as the basis of a risk assessment method. Regressions of percentage sensitive to silthiofam on percentage B-type demonstrated that molecular typing of isolates can, however, be used to assess the proportion of silthiofam-sensitive isolates in field populations of the take-all fungus. It should also be possible to make this assessment directly from the roots of field plants rather than from isolates obtained after culturing the fungus from field plants or from bait plants used in bioassays. Further testing would be needed to confirm this. The method therefore has considerable potential as a research tool. This potential is enhanced because of the association of the types with different host plants, possibly including grass weeds (for which there is some evidence but, as yet, incomplete and unpublished).

Non-molecular characteristics of individual isolates within populations of the take-all fungus have been associated, either strongly or weakly, with the development of take-all epidemics (Hornby *et al*., 1998). Some of these characters (colony morphology, including sectoring, and dsRNA/virus content) were investigated here. None was found to be affected significantly by fungicide and none produced robust data that would make their routine analysis suitable for risk assessment.

6. Conclusions and recommendations

The following recommendations for using fluquinconazole (Jockey) seed treatment for managing take-all in sequences of winter wheat crops have been developed from a series of five field experiments in eastern England, each running for a minimum of 4 years, in which treatment and no treatment in successive years (crops) were compared in all possible combinations.

- Fluquinconazole should not be applied to a first wheat crop since, if the break crop was properly managed, e.g. to control volunteers and grass weeds, there will be no take-all. Residual beneficial effects in a following wheat crop, if they occur (as in the one test made here), are unlikely to repay the cost of treatment at current prices. If a sequence of wheat crops is planned, the first crop should be managed to minimise take-all development, e.g. by avoiding very early sowing.
- Fluquinconazole can be expected to be effective and economic when applied to a second or third wheat, when take-all is building up. Very severe take-all, which usually occurs at the peak of the epidemic, will be decreased by fluquinconazole. This will usually be accompanied by a large proportional yield response, but the total yield and quality are likely to be poor and the crop unprofitable.
- Ideally, a treated, diseased crop should be followed by a break crop. A non-treated wheat crop should not be grown, since take-all will continue to build up when the treatment is withheld.
- If growing a wheat crop after a treated, diseased crop is unavoidable, seed for the new crop should also be treated. Although treatment will be effective, the yield benefit may not be commensurate with the amount of disease control.
- During the take-all decline phase, treatment is effective and economic where sufficient take-all is present, but decline may be less effective in the following year.
- Normal progress to take-all decline may be delayed by treatment and so there is no advantage in using it where the intention is to exploit the benefits of take-all decline.

No evidence of resistance or altered sensitivity to fluquinconazole, or of adverse effects on potentially antagonistic fungi was found after 4 years of application to the same field plots.

A DNA probing method for analysing populations of the take-all fungus has now been superseded by a quicker and easier PCR method with similar diagnostic characteristics. The fungal types identified show associations with crop species, sensitivity to the take-all fungicide silthiofam, and possibly grass weed species (important in the carry-over of take-all inoculum), indicating its potential value in future population research and diagnosis.

Associations between changes in population structure of the pathogen and changes in disease were sometimes evident. However, it is not yet possible to devise a universal risk

assessment method because of large differences between field sites in the initial population structure (proportion of fungal isolates with the DNA marker) and overriding effects on disease of external factors, notably sowing date and weather, in some years.

The results relate to only one of the fungicides available for controlling take-all. Recommendations for the other seed treatment, silthiofam, or for foliar treatment with the strobilurin fungicide azoxystrobin may be different. It is possible that the relative inefficacy of fluquinconazole applied to a crop following one that was also treated may not occur with the other fungicides, or if more than one fungicide is used in the same crop sequence. Different recommendations may be needed for controlling take-all in sequences of cereals that include barley. These issues are being addressed in continuing research.

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Table 2.1. Agronomy in wheat crops used for seed treatment experiments (all cv. Hereward)

Year of harvest	Sowing date[a]	Seeds/m^{2}[b]	N application (kg N/ha)[c]		Fungicides (litres or kg formulation /ha in 200 litres)[a,d]		Harvest[a]
			Early	Late	T1	T2/T3	
Rothamsted (CS/476)							
1997	17/10	380	11/3 (41)	14/4 (160)	None	Folicur (0.5)	19/8
1998	1/10	400	19/2 (41)	29/4 (159)	None	None	19/8
1999	12/10	380	12/3 (50)	14/4 (150)	None	27/5, Opus (0.75)	29/7
2000	22/9	380	8/3 (80)	4/5 (118)	27/4, Opus (0.5) + Unix (0.5)	22/5, Opus (0.75)	7/8
2001	5/10	350	20/3 (60)	5/5 (150)	8/5, Opus (0.5) + Unix (0.5)	5/6, Opus (0.7)	15/8
2002	23/9	300	-	25/4 (140)	15/4, Opus (0.5)	27/5, Opus (0.75)	12/8
East Winch							
1999	28/9	N/A	17/2, 2/4, 10/5 (200)		-	19/5, Opus (1.0) + Bravo (1.0)[e]	
2000	4/9	N/A	N/A		-	30/5, Opus (0.75) + Bravo (0.5)	
2001	13/10	170	N/A		-	15/5, Sportak Delta (N/A) + Mistral (N/A)	24/8
2002	11/10	170	N/A		-	-	14/8
Rothamsted (CS/508)							
1999	15/10	380	10/3 (50)	16/4 (150)	30/4, Opus (0.5)	25/5, Opus (0.75) + Rover (1.0)[e]	16/8
2000	14/10	380	7/3 (80)	19/4 (150)	24/4, Opus (0.5) + Unix (0.5)	22/5, Opus (0.75)[e]	11/8
2001	25/9	250	20/3 (60)	5/5 (150)	8/5, Opus (0.5) + Unix (0.5)	6/6, Opus (0.7)	17/8
2002	22/9	300	8/3 (60)	24/4 (120)	14/4, Opus (0.5) + Unix (0.4)	27/5, Opus (0.75)	12/8
Sacrewell							
1999	24/9	168	5/3(47), 5/4(109), 1/5(46)		24/4, Opus (0.5) + Bravo (1.0)	16/5, Opus (0.5) + Atlas Cropguard (1.0)	30/7
2000	6/10	250	27/2(46), 19/3(52.9), 3/5(74)		9/5, Opus (0.5) + Bravo (1.0)	31/5, Opus (0.5) + Bravo (1.0)	11/8
2001	14/11	275	14/3(43), 24/4(76), 5/5(71)		23/5, Opus (0.2) + Bravo (0.8)	4/6, Opus (0.5) + Bravo (1.0)	24/8
2002	26/9	240	10/4(10), 29/5(102)		15/5, Opus (0.5) + Bravo (1.0)	1/6, Opus (0.6) + Bravo (1.1)	28/8
Rothamsted (CS/323)							
1999	25/9	380	12/3 (50)	15/4 (150)	1/5, Opus (0.7)	29/5, Folicur (1.0) + Rover (1.0)[e]	29/7
2000	13/10	380	7/3 (80)	27/4 (120)	27/4, Opus (0.5) + Unix (0.5)	22/5, Opus (0.75)	11/8
2001	6/10	350	20/3 (60)	5/5 (150)	10/5, Opus (0.5) + Unix (0.4)	6/6, Opus (0.7)	17/8
2002	23/9	350	26/3 (50)	25/4 (140)	14/4, Opus (0.5) + Unix (0.4)	27/5, Opus (0.75)	13/8

[Footnotes on next page]

Table 2.1. continued.

[a]Dates are shown as day/month.
[b]Straw of previous crops was baled and removed before ploughing the soil.
[c]As 33.5% or 34.5% N in CS experiments; urea at East Winch and Sacrewell.
[d]Active ingredients: Opus, epoxiconazole; Unix, cyproconazole; Rover and Bravo, chlorothalonil; Folicur, tebuconazole; Sportak Delta, cyproconazole + prochloraz; Mistral, fenpropimorph; Atlas Cropguard, chlorothalonil.
[e]Plus T3 spray, Folicur (0.25 - 0.5 l) in late June.
N/A, information not available.

Table 2.2. Agronomy in wheat crops grown on the monitoring sites

Year of harvest	Cultivar	Sowing date[a]	Seeds/m^2	N (kg ha^{-1})	Fungicides (litres formulation ha^{-1})[b,c]
Site B (Radstock)					
1999 (2nd wheat)		8/10	250	200	-
2000		18/10	250	200	Opus+Bravo, T1; Mantra, T2
2001		14/10	250	200	Landmark, T1; Opus+Twist, T2
2002		9/10	250	200	Twist+Opus, T1; Twist+Opus, T2; Amistar, T3
Site C (Maperton)					
2000 (1st wheat)	Claire	22/10	300	208	Opus+Bravo, T2; Landmark, T3
2001	Claire	14/01	300	240	Landmark, T1; Epic, T2; Amistar, T3.
2002	Claire	10/10	300	240	Opus, T1, T2; Amistar, T3
Site L (Long Ashton)					
1999 (1st wheat)	Charger	5/10	302	182	Unix+Alto+Tilt, T1; Opus, T2; Sanction +Bravo+Standon Fenpropimorph, T3
2000	Shamrock	11/10	272	220	Unix+Alto, T1; Landmark+Corbel+Ensign+ Opus, T2; Corbel+Sanction, T3
2001	Claire	11/1	377	184	Alto+Fortress, T1; Standon Kresoxim-methyl Epoxiconazole, T2; Opus, T3
2002	Claire	27/9	250	191	Corbel+Alto+Fortess+Poraz T1; Landmark+Corbel, T2; Opus, T3
Site W (Woburn)					
1999 (1st wheat)	Hereward	12/10	380	180	Folicur, T2; Folicur+Rover, T3
2000	Hereward	1/10	350	130	Opus, T2
2001	Hereward	3/10	350	200	Opus, T2
2002	Hereward	22/9	300	180	Opus, T1, T2

[a]Dates are shown as day/month.

[b]Active ingredients: Amistar, azoxystrobin; Alto, cyprodinil; Bravo, chlorothalonil; Corbel, fenpropimorph; Ensign, fenpropimorph + kresoxim-methyl; Epic, epoxiconazole; Folicur, tebuconazole; Fortress, quinoxyfen; Landmark, epoxiconazole + kresoxim-methyl; Opus, epoxiconazole; Poraz, prochloraz; Rover, chlorothalonil; Sanction, flusilazole; Tilt, propiconazole; Twist, trifloxystrobin; Unix, cyproconazole.

[c]Approximate fungicide application times: T1, leaf 3 emergence/ start of stem extension; T2, flag leaf emergence; T3, ear emergence.

N/A, information not available.

All crops were sown after ploughing the sites.

Table 2.3. Sampling dates[a] and growth stages (GS) of plants used for disease assessments and when soil cores (June/July only) were taken for infectivity bioassays in field experiments

Year of harvest	Rothamsted (CS/476)				East Winch		Rothamsted (CS/508)		Sacrewell		Rothamsted (CS/323)	
	Date	GS	Date[c]	GS	Date	GS	Date	GS	Date	GS	Date	GS
1998	16/3[b]	23	24/6	71	[17/6]	[69]	[15/7]	[77]	-	-	-	-
1999	12/4	30	25/6	71	21/6	73	1/7	73	23/6	73	8/7	77
2000	7/4	22-30	27/6	73	19/6	71	27/6[d]	71	20/6	69	28/6	73
2001	26/4	30	21/6	69	25/6	69	28/6[d]	69	27/6[e]	69	3/7	71
2002	25/4	31	25/6	71	24/6	71	27/6[d]	71	26/6	71	1/7[f]	73

[a]Dates are shown as day/month. Dates and growth stages in square brackets are for pre-experiment samples for take-all assessments.
[b]Also sampled 22/12/97, GS 27.
[c]Plant samples only in 1998-2000; the soil cores were taken after harvest, on 28/8/98, 27/8/99 and 21/8/00.
[d]Plant samples only; soil cores were taken on 3/7/00, 16/7/01 and 4/7/02.
[e]Plant samples only; soil cores were taken on 18/7/01.
[f]Plant samples only; soil cores were taken on 4/7/02.

Table 2.4. Sampling dates[a] and growth stages (GS) of plants used for disease assessments and when soil cores were taken for infectivity bioassays at monitoring sites

	Site B		Site C		Site L		Site W	
Year of harvest	**Date**	**GS**	**Date**	**GS**	**Date**	**GS**	**Date**	**GS**
1999	20/7	83	-	-	13/7	83	12/7	77
2000	18/7	83	18/7	83	20/7	83	6/7	73
2001	23/7	85	23/7	83	24/7	77	12/7	73
2002	8/7	73	8/7	73	9/7	73	11/7	75

[a]Dates are shown as day/month. Soil cores only were taken from site C in 1999, then growing a pea crop.

Table 3.1. Effects of fluquinconazole seed treatment on take-all incidence and severity and on soil infectivity, averaged over treatments applied in all previous years, at Rothamsted (CS/476)

Treatment	1998	1999	2000	2001	2002[a]
Logit % plants with take-all in March/April (back-transformed mean)					
None	-0.97(10.8)	0.16(57.7)	1.30(92.6)	-0.19(40.4)	0.81(94.0)
Fluquinconazole	-1.09(10.6)	-0.28(35.8)	1.23(91.6)	-0.35(32.9)	1.42(83.0)
SED[d.f.]	0.201[25]	0.154[21]	0.153[15]	0.128[6]	0.110[6]
P	0.95	0.009	0.64	0.26	0.001
No. infected roots per plant (March/April)[b]					
None	0.31	1.32	4.34	0.71	5.21
Fluquinconazole	0.28	0.56	2.96	0.50	2.50
SED[d.f.]	0.163[25]	0.192[21]	0.315[15]	0.113[6]	0.307[6]
P	0.84	<0.001	<0.001	0.11	<0.001
Logit % plants with take-all in June (back-transformed mean)					
None	-0.15(42.0)	1.15(90.4)	1.97(97.6)	-0.10(44.7)	2.02(97.8)
Fluquinconazole	-0.60(22.6)	0.96(86.7)	1.49(94.7)	-0.30(34.8)	1.62(95.7)
SED[d.f.]	0.244[25]	0.177[21]	0.273[15]	0.075[6]	0.141[6]
P	0.08	0.30	0.10	0.03	0.03
Logit % plants with moderate-severe take-all in June/July(back-transformed mean)					
None	-0.43(29.3)	0.43(69.7)	0.93(86.1)	-1.12(9.1)	1.04(88.3)
Fluquinconazole	-1.09(9.6)	-0.17(40.9)	-0.08(45.5)	-1.92(1.6)	0.10(54.7)
SED[d.f.]	0.283[25]	0.125[21]	0.239[15]	0.145[6]	0.200[6]
P	0.03	<0.001	<0.001	0.002	0.003
Take-all index (0-100) (June/July)					
None	36.6	69.0	76.8	17.1	78.4
Fluquinconazole	20.2	45.0	48.0	9.5	51.5
SED[d.f.]	7.34[25]	4.21[21]	5.21[15]	1.32[6]	4.83[6]
P	0.04	<0.001	<0.001	<0.001	0.001
Logit % roots with take-all in soil bioassay (back-transformed mean)					
None	-0.14(42.6)	0.20(59.4)	-0.28(35.7)	NT	NT
Fluquinconazole	-0.36(32.1)	0.31(64.6)	-0.08(45.4)	NT	NT
SED[d.f.]	0.124[25]	0.105[21]	0.082[15]	-	-
P	0.09	0.31	0.03	-	-

[a]A full replicate of 2^5 treatments was made possible in 2002 by ignoring treatments applied to the first wheat in 1997; it was assumed that they were having no residual effects.
[b]% roots with take-all was also analysed, with similar result.
NT, not tested.

Table 3.2. Effects of fluquinconazole seed treatment on grain yield and quality, averaged over treatments applied in all previous years, at Rothamsted (CS/476)

Treatment	1997	1998	1999	2000	2001	2002[a]
Grain yield (t ha^{-1})						
None	10.02	7.78	5.77	4.14	7.35	3.61
Fluquinconazole	10.31	8.24	6.35	4.91	7.45	4.72
SED [d.f.]	0.225[27]	0.211[24]	0.297[20]	0.190[15]	0.116[6]	0.321[6]
P	0.22	0.04	0.07	0.001	0.43	0.01
Thousand-grain weight (g)						
None	NT	38.9	37.8	30.9	38.1	33.3
Fluquinconazole	NT	40.1	38.5	32.9	38.7	33.8
SED [d.f.]	-	0.65[25]	0.68[21]	0.80[15]	0.52[6]	0.73[6]
P	-	0.08	0.31	0.02	0.26	0.54

[a]A full replicate of 2^5 treatments was made possible in 2002 by ignoring treatments applied to the first wheat in 1997; it was assumed that they were having no residual effects.
NT, not tested.

Table 3.3. Effects of fluquinconazole seed treatment on take-all incidence and severity in June and on soil infectivity, averaged over treatments applied in all previous years, at East Winch

Treatment	1999	2000	2001	2002
Logit % plants with take-all (back-transformed mean)				
None	0.93(86.0)	1.83(97.0)	-0.14(42.5)	0.67(78.7)
Fluquinconazole	0.45(70.4)	2.06(98.0)	-0.29(35.2)	0.26(62.2)
SED [d.f.]	0.121[59]	0.146[57]	0.072[53]	0.079[45]
P	<0.001	0.11	0.038	<0.001
Logit % plants with moderate-severe take-all (back-transformed mean)				
None	0.08(53.5)	0.86(84.3)	-1.29 (6.6)	-0.16(41.8)
Fluquinconazole	-0.68(20.1)	0.53(73.6)	-2.09(1.0)	-1.13(8.9)
SED[d.f.]	0.108[59]	0.181[57]	0.135[53]	0.093[45]
P	<0.001	0.07	<0.001	<0.001
Take-all index (0-100)				
None	53.4	77.2	14.9	44.6
Fluquinconazole	28.8	62.8	9.2	20.7
SED [d.f.]	3.10[59]	4.01[57]	1.45[53]	2.25[45]
P	<0.001	<0.001	<0.001	<0.001
Logit % roots with take-all in soil bioassay (back-transformed mean)				
None	0.183 (58.5)	0.193(59.0)	-0.483(27.1)	NT
Fluquinconazole	0.077 (53.4)	0.143(58.5)	-0.510(26.9)	NT
SED [d.f.]	0.0669[59]	0.0396[57]	0.0601[53]	-
P	0.12	0.21	0.65	-

NT, not tested.

Table 3.4. Effects of fluquinconazole seed treatment on grain yield and quality, averaged over treatments applied in all previous years, at East Winch

Treatment	1999	2000	2001
Grain yield (t ha^{-1})			
None	8.13	4.94	4.06
Fluquinconazole	8.90	5.18	4.44
SED[d.f.]	0.222[59]	0.316[57]	0.282[53]
P	0.001	0.45	0.18
Thousand-grain weight (g)			
None	39.1	35.4	35.2
Fluquinconazole	40.5	34.7	35.3
SED [d.f.]	0.37[59]	0.53[57]	0.42[53]
P	<0.001	0.19	0.71
Specific weight (kg hl^{-1})			
None	71.9	68.5	NT
Fluquinconazole	72.4	68.6	NT
SED [d.f.]	0.19[59]	0.92[57]	-
P	0.008	0.87	-

NT, not tested.
Yield could not be measured in 2002.

Table 3.5. Effects of fluquinconazole seed treatment on take-all incidence and severity in June/July and on soil infectivity, averaged over treatments applied in all previous years, at Rothamsted (CS/508)

Treatment	1999	2000	2001	2002
Logit % plants with take-all (back-transformed mean)				
None	-0.14(42.8)	1.19(91.1)	0.79(82.5)	1.40(93.8)
Fluquinconazole	-0.28(36.0)	1.02(88.0)	0.26(62.2)	1.33(93.0)
SED[d.f.]	0.128[59]	0.182[57]	0.144[53]	0.112[45]
P	0.28	0.35	<0.001	0.52
Logit % plants with moderate-severe take-all (back-transformed mean)				
None	-0.83(15.5)	0.56(74.7)	0.02(50.4)	0.68(79.0)
Fluquinconazole	-1.06(10.3)	0.02(50.5)	-0.65(20.8)	0.06(52.7)
SED[d.f.]	0.162[59]	0.164[57]	0.142[53]	0.123[45]
P	0.16	0.002	<0.001	<0.001
Take-all index (0-100)				
None	24.2	67.7	49.8	68.5
Fluquinconazole	20.6	48.8	28.4	50.8
SED[d.f.]	3.32[59]	4.21[57]	4.48[53]	2.79[45]
P	0.29	<0.001	<0.001	<0.001
Logit % roots with take-all in soil bioassay (back-transformed mean)				
None	-0.20(39.4)	0.44(70.0)	0.31(64.5)	NT
Fluquinconazole	-0.23(38.5)	0.64(77.8)	0.20(67.2)	0.42(69.2)
SED[d.f.]	0.091[59]	0.063[57]	0.078[53]	-
P	0.82	0.002	0.16	-

NT, not tested; soil infectivity after fluquinconazole treatment is shown for comparison with other years.

Table 3.6. Effects of fluquinconazole seed treatment on grain yield and quality, averaged over treatments applied in all previous years, at Rothamsted (CS/508)

Treatment	1999	2000	2001	2002
Grain yield (t ha^{-1})				
None	9.72	6.23	5.24	5.82
Fluquinconazole	9.74	6.61	6.03	6.64
SED [d.f.]	0.149[59]	0.265[57]	0.273[53]	0.163[45]
P	0.86	0.16	0.006	<0.001
Thousand-grain weight (g)				
None	43.9	35.8	38.8	37.2
Fluquinconazole	43.7	36.9	39.3	38.9
SED [d.f.]	0.37[59]	0.48[57]	0.36[53]	0.42[45]
P	0.55	0.03	0.15	<0.001
Specific weight (kg hl^{-1})				
None	76.3	78.1	77.3	70.2
Fluquinconazole	76.3	78.7	78.0	71.4
SED [d.f.]	0.17[59]	0.25[57]	0.20[53]	0.32[45]
P	0.96	0.03	<0.001	<0.001

Table 3.7. Effects of fluquinconazole seed treatment on take-all incidence and severity in June and on soil infectivity, averaged over treatments applied in all previous years, at Sacrewell

Treatment	1999	2000	2001	2002
Logit % plants with take-all (back-transformed mean)				
None	0.26(62.0)	0.76(81.7)	-0.798(16.6)	0.30(64.1)
Fluquinconazole	0.02(50.4)	0.51(72.8)	-0.88(14.2)	-0.12(43.4)
SED [d.f.]	0.100[59]	0.110[57]	0.083[53]	0.102 [45]
P	0.02	0.02	0.28	<0.001
Logit % plants with moderate-severe take-all (back-transformed mean)				
None	-0.14(42.6)	0.00(49.6)	-1.92(1.6)	-0.51(26.0)
Fluquinconazole	-0.53(25.4)	-0.72(18.7)	-2.18(0.8)	-1.46(4.6)
SED [d.f.]	0.102[59]	0.097[57]	0.120[53]	0.128[45]
P	<0.001	<0.001	0.03	<0.001
Take-all index (0-100)				
None	43.6	48.4	6.1	33.0
Fluquinconazole	30.5	27.9	4.5	14.2
SED [d.f.]	3.53[59]	2.62[57]	1.00[53]	2.69[45]
P	<0.001	<0.001	0.13	<0.001
Logit % roots with take-all in soil bioassay (back-transformed mean)				
None	-0.02(48.6)	-0.16(41.6)	-0.35(32.6)	NT
Fluquinconazole	-0.20(39.6)	-0.23(38.3)	-0.34(33.0)	-0.21 (39.0)
SED [d.f.]	0.100[59]	0.058[57]	0.080[53]	-
P	0.07	0.24	0.91	-

NT, not tested; soil infectivity after fluquinconazole treatment is shown for comparison with other years.

Table 3.8. Effects of fluquinconazole seed treatment on grain yield and quality, averaged over treatments applied in all previous years, at Sacrewell

Treatment	1999	2000	2001	2002
Grain yield (t ha^{-1})				
None	7.28	3.87	3.06	4.74
Fluquinconazole	7.50	4.51	3.01	4.95
SED [d.f.]	0.068[59]	0.082[57]	0.075[53]	0.072[45]
P	0.002	<0.001	0.43	0.005
Thousand-grain weight (g)				
None	42.0	NT	33.1	37.6
Fluquinconazole	42.3	NT	32.8	38.1
SED [d.f.]	0.24[59]	-	0.28[53]	0.33[45]
P	0.24	-	0.27	0.09
Specific weight (kg hl^{-1})				
None	81.0	70.2	NT	NT
Fluquinconazole	81.2	71.6	NT	NT
SED [d.f.]	0.11[59]	0.56[57]	-	-
P	0.05	0.01	-	-

NT, not tested.

Table 3.9. Effects of differences in intensity of take-all decline (as a result of growing different crop sequences from 1988) on take-all severity in June/July and grain yield at Rothamsted (CS/323)

	Take-all index (0-100)				Grain yield (t ha^{-1})			
Take-all decline group	1999	2000	2001	2002	1999	2000	2001	2002
I (established)	48.8	31.1	30.4	67.7	9.73	9.06	6.41	7.07
II	51.6	28.4	29.4	68.1	9.37	9.25	6.65	7.32
III (recent)	55.8	35.9	32.4	68.9	9.41	8.84	6.30	6.87
SED	2.86	2.40	2.97	2.07	0.160	0.142	0.129	0.189
[d.f.]	[64]	[58]	[51]	[46]	[64]	[58]	[51]	[46]
P	0.05	0.01	0.59	0.85	0.05	0.02	0.03	0.07

Table 3.10. Effects of fluquinconazole seed treatment on take-all incidence and severity in June/July, averaged over treatments applied in all previous years, on a take-all decline site at Rothamsted (CS/323)

Treatment	1999	2000	2001	2002
Logit % plants with take-all (back-transformed mean)				
None	1.42(94.0)	0.71(80.0)	0.78(82.1)	2.14 (98.1)
Fluquinconazole	1.21(91.3)	0.59(76.0)	0.43(69.6)	1.87 (97.2)
SED [d.f.]	0.113[64]	0.095[58]	0.085[51]	0.100[46]
P	0.07	0.22	<0.001	0.01
Logit % plants with moderate-severe take-all (back-transformed mean)				
None	0.32(65.0)	-0.37(32.0)	-0.35(32.5)	0.88 (84.8)
Fluquinconazole	-0.33(33.4)	-0.97(12.1)	-1.16(8.5)	0.20 (59.1)
SED [d.f.]	0.079[64]	0.096[58]	0.107[51]	0.071[46]
P	<0.001	<0.001	<0.001	<0.001
Take-all index (0-100)				
None	63.4	37.8	38.9	76.8
Fluquinconazole	40.7	25.8	22.6	53.9
SED [d.f.]	2.33[64]	1.96[58]	2.42[51]	1.74[46]
P	<0.001	<0.001	<0.001	<0.001

Table 3.11. Effects of fluquinconazole seed treatment on grain yield and quality, averaged over treatments applied in all previous years, on a take-all decline site at Rothamsted (CS/323)

Treatment	1999	2000	2001	2002
Grain yield (t ha^{-1})				
None	9.18	8.96	6.24	6.95
Fluquinconazole	9.82	9.14	6.67	7.32
SED [d.f.]	0.131[64]	0.116[58]	0.105[51]	0.159[46]
P	<0.001	0.11	<0.001	0.02
Thousand-grain weight (g)				
None	41.0	36.4	37.1	32.9
Fluquinconazole	41.7	36.7	37.8	35.0
SED [d.f.]	0.29[64]	0.33[58]	0.25[51]	0.55[46]
P	0.03	0.25	0.004	<0.001

Table 3.12. Effects on take-all index in June/July of fluquinconazole seed treatment applied to the current or preceding crops

	Treatment in year of measurement		Treatment in preceding year	
Year of measurement	None (take-all index)	Fluquincon-azole[a]	None (take-all index)	Fluquincon-azole[a]
Rothamsted (CS/476)				
1997 (1st wheat)	0	0	-	-
1998	36.6	-16.4*	34.2	-11.6
1999	69.0	-24.0***	60.2	-6.4
2000	76.8	-28.8***	59.7	+5.4
2001	17.6	-8.1***	11.7	+3.6*
2002	78.4	-26.9***	65.9	-1.9
East Winch				
1999 (2nd wheat)	53.4	-24.6***	-	-
2000	77.2	-14.4***	69.0	+1.9
2001	14.9	-5.7***	11.5	+1.1
2002	44.6	-23.9***	32.7	-0.2
Rothamsted (CS/508)				
1999 (2nd wheat)	24.2	-3.6	-	-
2000	67.7	-18.9***	59.8	-3.0
2001	49.8	-21.4***	35.3	+7.6
2002	68.5	-17.7***	53.6	+12.1***
Sacrewell				
1999 (2nd wheat)	43.6	-13.1***	-	-
2000	48.4	-20.5***	37.6	+1.7
2001	6.1	-1.6	4.9	+0.9
2002	33.0	-18.8***	23.0	+1.7
Rothamsted (CS/323; continuous wheat)				
1999	63.4	-22.7***	-	-
2000	37.8	-12.0***	29.0	+5.6**
2001	38.9	-16.3***	29.4	+2.7
2002	76.8	-22.9***	64.3	+7.8*

*, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$; ***, significant at $P \leq 0.001$.
[a]Difference from none.

Table 3.13. Effects of fluquinconazole seed treatment in 2000 on soil infectivity measured in June/July in the 2001 crop

Treatment	East Winch	Rothamsted (CS/508)	Sacrewell
Logit % roots with take-all in soil bioassay (back-transformed mean)			
None	-0.51(26.2)	0.14(56.3)	-0.47(27.7)
Fluquinconazole	-0.49(26.9)	0.37(67.2)	-0.23(38.4)
SED[53 d.f.]	0.060	0.078	0.080
P	0.75	0.004	0.004

Table 3.14. Effects on grain yield of fluquinconazole seed treatment applied to the current or preceding crops

	Treatment in year of measurement		Treatment in preceding year	
Year of measurement	None (t ha^{-1})	Fluquincon-azole[a]	None (t ha^{-1})	Fluquincon-azole[a]
Rothamsted (CS/476)				
1997 (1st wheat)	10.02	+0.29	-	-
1998	7.78	+0.46*	7.74	+0.53*
1999	5.77	+0.58	6.01	+0.09
2000	4.14	+0.77***	4.92	-0.78*
2001	7.35	+0.10	7.61	-0.41*
2002	3.61	+1.11*	4.20	-0.07
East Winch				
1999 (2nd wheat)	8.13	+0.77***	-	-
2000	4.94	+0.24	5.07	-0.01
2001	4.06	+0.38	4.09	+0.33
Rothamsted (CS/508)				
1999 (2nd wheat)	9.72	+0.02	-	-
2000	6.23	+0.38	6.55	-0.25
2001	5.24	+0.79**	5.93	-0.59*
2002	5.82	+0.82*	6.61	-0.76***
Sacrewell				
1999 (2nd wheat)	7.28	+0.22**	-	-
2000	4.77	+1.26***	5.50	-0.20
2001	3.00	+0.06	3.15	-0.23**
2002	4.74	+0.21**	4.82	+0.05
Rothamsted (CS/323; continuous wheat)				
1999	9.18	+0.64***	-	-
2000	8.96	+0.18	9.25	-0.40***
2001	6.24	+0.43***	6.53	-0.15
2002	6.95	+0.38*	7.16	-0.15

*, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$; ***, significant at $P \leq 0.001$.
[a]Difference from none.

Table 3.15. Eyespot in experiments and years in which there were significant effects of seed treatment, averaged over treatments applied in all preceding years

Treatment	Logit % stems affected (back-transformed mean)					
	Rothamsted (CS/476) 2000	Rothamsted (CS/508) 2000	Rothamsted (CS/508) 2001	Rothamsted (CS/508) 2002	Sacrewell 2001	Rothamsted (CS/323) 1999
Eyespot (all severity categories)						
None	-0.20(39.8)	-0.55(24.6)	-1.36(5.7)	-0.21(39.1)	0.02(50.5)	0.20(59.4)
Fluquinconazole	-0.12(55.6)	-0.32(34.0)	-0.99(11.5)	-0.07(46.3)	-0.21(39.3)	0.44(70.1)
SED [d.f.]	0.088[15]	0.073[57])	0.107[53]	0.083[15]	0.054[53]	0.070
P	0.003	0.003	0.001	0.096	<0.001	0.001
Moderate or severe eyespot						
None	-0.92(13.2)	-1.75(2.4)	-2.09(1.0)	-1.13(9.0)	-0.70(19.3)	-0.66(20.5)
Fluquinconazole	-0.57(23.7)	-1.35(5.9)	-1.79(2.2)	-0.98(11.9)	-0.90(13.7)	-0.55(24.5)
SED [d.f.]	0.098[15]	0.111[57]	0.094[53]	0.065[15]	0.067[53]	0.061
P	0.003	<0.001	0.003	0.034	0.004	0.07

Table 3.16. Estimated cost benefits of seed treatment, assuming a grain price of £55 t^{-1} and seed treatment cost of £132 t^{-1}, in Rothamsted experiment CS/508

	Annual value of harvest (£ ha^{-1})				Cumulative value of harvests (£ ha^{-1})			Cumulative cost benefit relative to no treatment (£ ha^{-1})			
Treatment sequence[a]	1999	2000	2001	2002	2000	2001	2002	1999	2000	2001	2002
0000	531	352	293	378	883	1176	1554	-	-	-	-
000F	531	352	293	370	883	1176	1546	-	-	-	-8
00F0	531	352	341	288	883	1224	1512	-	-	**+48**	-42
00FF	531	352	341	338	883	1224	1562	-	-	**+48**	**+8**
0F00	531	343	291	338	874	1165	1503	-	-9	-2	-51
0F0F	531	343	291	371	874	1165	1536	-	-9	-2	-18
0FF0	531	343	312	274	874	1186	1480	-	-9	**+19**	-74
0FFF	531	343	312	341	874	1186	1527	-	-9	**+19**	-27
F000	514	333	304	339	847	1151	1490	-17	-19	**+11**	-64
F00F	514	333	304	367	847	1151	1518	-17	-19	**+11**	-36
F0F0	514	333	333	295	847	1180	1475	-17	-19	**+40**	-79
F0FF	514	333	333	333	847	1180	1513	-17	-19	**+40**	-41
FF00	514	334	266	323	848	1114	1437	-17	-18	-27	-117
FF0F	514	334	266	348	848	1114	1462	-17	-18	-27	-92
FFF0	514	334	273	307	848	1121	1428	-17	-18	-20	-126
FFFF	514	334	273	300	848	1121	1421	-17	-18	-20	-133

[a]0, no seed treatment; F, fluquinconazole seed treatment. The four-character sequence shows non-treatment or treatment of the crops harvested in each of the four years, 1999 (second wheat) to 2002 (fifth wheat).

Table 4.1. Growth of isolates of the take-all fungus from repeatedly treated or non-treated plots on agar that was non-amended or amended with fluquinconazole at 0.01 or 0.1 mg l^{-1} (increase in colony diameter, in mm, after 4 days)

Treatment to	1999			2000			2001			2002		
plots	0	0.01	0.1	0	0.01	0.1	0	0.01	0.1	0	0.01	0.1
East Winch												
None	25.1	16.9	1.0	28.3	23.8	4.2	28.6	21.7	0.9	NT	NT	NT
Fluquincon.	25.9	17.7	1.3	29.5	26.0	4.7	27.6	19.9	1.0	NT	NT	NT
SED[15 d.f.]		0.83			0.99			1.00				
P		0.91			0.50			0.41				
Rothamsted (CS/508)												
None	27.7	23.3	0.1	34.2	29.8	4.1	25.4	20.9	1.7	31.1	19.6	0.1
Fluquincon.	32.4	26.7	0.5	34.1	29.9	4.2	26.9	21.7	0.8	31.4	21.1	0.3
SED[15 d.f.]		2.04			0.63			0.90			0.59	
P		0.33			0.96			0.19			0.29	
Sacrewell												
None	27.7	22.6	3.7	26.0	23.2	3.0	27.4	22.5	4.7	26.3	23.2	2.1
Fluquincon.	29.5	22.8	3.6	30.0	26.2	4.0	28.0	24.1	2.9	28.0	23.2	1.5
SED[15 d.f.]		1.03			2.04			1.96			0.74	
P		0.41			0.60			0.48			0.10	

SED and *P* values are for the interaction: plot treatment x fungicide concentration. See text for details of any single-factor effects.
NT, not tested.

Table 4.2. Diversity in communities of fungi on wheat roots from repeatedly treated or non-treated plots in experiment CS/508 at Rothamsted

Treatment to plots	1999	2000	2001	2002
Mean no. fungal genera/species per root piece				
None	5.39	4.16	3.41	4.13
Fluq.	5.23	4.70	3.39	4.31
SED[3 d.f.]	0.134	0.244	0.201	0.165
P	0.30	0.12	0.94	0.36
Mean no. fungal genera/species per plot				
None	25.3	26.0	26.0	29.0
Fluq.	25.5	27.3	26.0	29.3
SED[3 d.f.]	1.93	0.63	1.92	2.53
P	0.91	0.14	1.00	0.93
Logit % root pieces with Trichoderma *spp. (back-transformed mean)*				
None	-1.63 (3.2)	-1.18 (8.2)	-0.57 (23.7)	-0.08 (45.6)
Fluq.	-1.48 (4.5)	-0.76 (17.6)	-0.88 (14.1)	-0.29 (35.5)
SED[3 d.f.]	0.134	0.126	0.081	0.133
P	0.33	0.04	0.03	0.21
Logit % root pieces with Fusarium culmorum *(back-transformed mean)*				
None	-1.91 (1.6)	-1.09 (9.6)	-1.45 (5.2)	-1.08 (9.8)
Fluq.	-1.66 (3.0)	-1.13 (8.9)	-1.58 (3.6)	-1.20 (7.9)
SED[3 d.f.]	0.116	0.226	0.563	0.156
P	0.12	0.87	0.78	0.51

Table 5.1. Effects of annual applications of fluquinconazole seed treatment on take-all index (TAI, 0-100), soil infectivity and frequency of T-type components in populations of the take-all fungus (repeatedly treated or untreated plots only)

Treatment	East Winch			Rothamsted (CS/508)			Sacrewell		
	TAI	% diseased roots in soil bioassay[a]	% T2[a]	TAI	% diseased roots in soil bioassay[a]	% T2[a]	TAI	% diseased roots in soil bioassay[a]	% T2[a]
1999									
None	61.9	0.15 (56.7)	[100]	35.8	-0.10 (44.7)	-0.23 (38.4)	47.3	-0.15 (41.9)	0.99 (87.3)
Fluquincon.	31.3	0.15 (56.9)	[100]	18.2	-0.12 (43.6)	-0.35 (32.7)	26.3	-0.48 (27.4)	0.96 (86.7)
SED (3 d.f.)	14.79	0.114	-	6.89	0.300	0.041	14.71	0.138	0.501
P	0.13	0.98	-	0.08	0.95	0.06	0.25	0.10	0.96
2000									
None	83.0	0.11 (54.9)	0.86 (84.4)	69.6	0.46 (71.0)	-0.66 (20.6)	51.8	0.02 (50.4)	1.41 (93.8)
Fluquincon.	55.2	0.08 (53.7)	1.52 (94.9)	44.7	0.72 (80.3)	-0.35 (32.8)	31.4	-0.20 (39.6)	1.30 (92.6)
SED (3 d.f.)	6.76	0.158	0.377	11.09	0.130	0.226	5.43	0.156	0.180
P	0.03	0.89	0.18	0.11	0.14	0.262	0.03	0.25	0.60
2001									
None	17.1	-0.56 (24.3)	1.04 (88.5)	42.2	-0.04 (47.4)	-0.40 (30.5)	5.4	-0.46 (28.2)	1.35 (93.2)
Fluquincon.	8.3	-0.61 (22.3)	1.00 (87.5)	45.1	0.30 (64.1)	-0.24 (37.9)	7.6	-0.33 (33.7)	1.58 (95.5)
SED (3 d.f.)	4.19	0.135	0.418	19.40	0.186	0.420	1.94	0.164	0.320
P	0.13	0.70	0.92	0.89	0.16	0.73	0.33	0.49	0.52

[a]Shown as logit; back-transformed mean in parenthesis. Values in square brackets were not transformed to logits and not analysed statistically.

Table 5.2. Population structure of the take-all fungus, soil infectivity and take-all index in the following wheat crop in non-treated crops at the monitoring sites

Year of harvest	Position in wheat sequence	% T2	% diseased roots in soil bioassay	Take-all index (0-100)
Site B				
1998	2nd	21.9±6.15	48.1±1.59	86.5±2.86
1999	3rd	49.7±9.37	37.9±4.31	24.8±4.74
2000	4th	50.4±7.88	53.1±2.16	55.4±4.70
2001	5th	65.8±9.29	52.6±2.56	42.2±3.87
2002	6th	-	-	41.8±4.59
Site C				
1998	Break crop	[87.5][a]	0.5±0.25	-
1999	1st	44.6±11.19	20.8±4.89	8.5±3.23
2000	2nd	51.9±9.58	60.9±2.30	83.4±5.74
2001	3rd	46.4±7.46	55.4±3.83	49.8±4.79
2002	4th	-	-	95.2±1.66
Site L				
1998	1st	81.1±6.58	39.0±3.03	28.3±5.55
1999	2nd	71.0±4.82	42.1±2.47	93.5±2.12
2000	3rd	68.0±4.35	58.3±2.97	78.5±4.29
2001	4th	55.5±4.59	60.3±3.10	42.5±3.88
2002	5th	-	-	95.7±1.59
Site W				
1998	1st	45.3±9.38	13.3±2.69	*ca* 0
1999	2nd	49.0±7.91	34.7±4.89	63.3±9.21
2000	3rd	56.5±8.22	48.4±3.18	57.3±6.80
2001	4th	70.8±5.89	35.3±3.30	31.6±3.08
2002	5th	-	-	96.8±1.81

[a]Unreliable value, since based on very few isolates.

Table 5.3. Associations between T-type and other characteristics in populations of the take-all fungus in 1999

Site	Fungus T-type	Pigmentation score (0-5)	Morphology score (0-5)	Logit % sectoring (back-transformed mean)	Logit % dsRNA (back-transformed mean)
Rothamsted					
(CS/508)	T2	2.46	2.28	-1.25 (7.1)	-1.04 (10.7)
	T1	2.47	2.25	-1.51 (4.2)	-1.26 (7.0)
	T1c	-	-	-	-
SED (11 df)		0.202	0.130	0.270	0.198
P		0.99	0.79	0.29	0.29
Site B	T2	1.81	2.42	-0.83 (15.5)	0.63 (77.2)
	T1	2.92	2.56	-0.89 (14.1)	0.67 (78.7)
	T1c	-	-	-	-
SED (17 df)		0.255	0.196	0.183	0.204
P		<0.001	0.46	0.76	0.83
Site C	T2	2.05	2.67	-1.14 (8.9)	-0.39 (31.0)
	T1	3.02	2.87	-0.67 (20.2)	-0.09 (44.9)
	T1c	2.06	2.00	-0.94 (12.7)	-0.76 (17.5)
SED (15 df)		0.182	0.131	0.162	0.319
P		<0.001	<0.001	0.04	0.15
Site L	T2	2.17	2.24	-1.02 (11.0)	-0.40 (30.5)
	T1	2.53	2.46	-0.72 (18.8)	-0.49 (26.6)
	T1c	1.94	2.04	-0.64 (21.4)	0.37 (67.0)
SED (20 df)		0.226	0.147	0.166	0.251
P		0.05	0.03	0.07	0.005
Site W	T2	2.52	2.48	-1.06 (10.3)	-0.04 (47.4)
	T1	2.74	2.33	-0.84 (15.1)	0.23 (60.8)
	T1c	3.00	2.83	-0.78 (16.9)	0.53 (73.9)
SED (20 df)		0.335	0.287	0.192	0.252
P		0.38	0.23	0.336	0.10

-, no isolates found.

Table 5.4. Associations between T-type and other characteristics in populations of the take-all fungus in 2000

Site	Fungus T-type	Pigmentation score (0-5)	Morphology score (0-5)	Logit % sectoring (back-transformed mean)	Logit % dsRNA (back-transformed mean)
Rothamsted					
(CS/508)	T2	2.02	2.25	-1.21 (7.7)	-0.86 (14.6)
	T1	2.47	2.16	-1.23 (7.4)	-0.34 (33.3)
	T1c	-	-	-	-
SED (11 df)		0.117	0.086	0.238	0.126
P		0.003	0.32	0.94	0.001
Site B	T2	2.21	2.55	-1.06 (10.2)	0.37 ()
	T1	2.62	2.61	-0.82 (15.7)	0.42 ()
	T1c	2.07	2.22	-0.75 (17.9)	0.12 ()
SED (22 df)		0.233	0.199	0.206	0.201
P		0.07	0.13	0.29	0.31
Site C	T2	2.36	2.38	-0.94 (12.8)	-0.28 (35.7)
	T1	2.45	2.50	-0.72 (18.8)	-0.14 (42.7)
	T1c	2.25	2.27	-0.86 (14.6)	-0.06 (46.3)
SED (21 df)		0.169	0.153	0.181	0.326
P		0.52	0.32	0.47	0.79
Site L	T2	2.18	2.34	-0.71 (19.1)	-0.74 (18.1)
	T1	2.94	2.66	-0.57 (23.9)	-0.35 (32.6)
	T1c	3.01	2.37	-0.43 (29.2)	-0.83 (15.4)
SED (23 df)		0.212	0.127	0.133	0.242
P		<0.001	0.04	0.14	0.13
Site W	T2	2.34	2.39	-0.97 (12.1)	0.65 (78.0)
	T1	2.30	2.24	-0.70 (19.2)	-0.24 (37.5)
	T1c	2.28	2.17	-0.85 (15.0)	-0.18 (40.8)
SED (23 df)		0.184	0.153	0.128	0.348
P		0.95	0.35	0.14	0.03

-, no isolates found.

Table 5.5. Associations between T-type and other characteristics in populations of the take-all fungus in 2001

Site	Fungus T-type	Pigmentation score (0-5)	Morphology score (0-5)	Logit % sectoring (back-transformed mean)	Logit % dsRNA (back-transformed mean)
Rothamsted					
(CS/508)	T2	3.04	2.66	-0.87 (14.5)	-1.04 (10.6)
	T1	2.83	2.56	-0.29 (35.5)	-0.77 (17.1)
	T1c	-	-	-	-
SED (11 df)		0.268	0.190	0.279	0.204
P		0.50	0.60	0.06	0.20
Site B	T2	2.22	2.27	-0.68 (19.8)	-0.19 (40.1)
	T1	2.94	2.37	-0.54 (25.0)	0.01 (49.9)
	T1c	2.45	2.29	-0.50 (26.3)	0.16 (57.6)
SED (20 df)		0.267	0.266	0.225	0.322
P		0.04	0.90	0.70	0.60
Site C	T2	2.95	2.97	-0.52 (25.7)	-0.38 (31.2)
	T1	2.79	2.56	-0.47 (27.7)	-0.43 (29.5)
	T1c	2.00	2.25	-0.78 (16.8)	0.27 (62.7)
SED (23 df)		0.204	0.211	0.207	0.295
P		<0.001	0.009	0.30	0.05
Site L	T2	2.34	2.42	-0.84 (15.1)	-0.70 (19.3)
	T1	2.52	2.61	-0.71 (18.9)	-0.78 (16.8)
	T1c	2.00	2.48	-0.81 (15.9)	-0.46 (28.0)
SED (25 df)		0.185	0.162	0.176	0.272
P		0.03	0.50	0.70	0.50
Site W	T2	2.88	2.46	-1.01 (11.2)	-0.38 (31.6)
	T1	2.27	2.20	-0.45 (28.4)	-0.47 (27.5)
	T1c	2.00	2.21	-0.42 (29.8)	0.29 (63.5)
SED (20 df)		0.278	0.158	0.257	0.276
P		0.02	0.20	0.06	0.02

-, no isolates found.

Table 5.6. Associations between sub-populations of Ggt identified by RFLP type, determined using the probing method (T-typing), and those identified using a PCR-based method (see text).

ITS4/ 5 *Hpa* II type	RFLP type		
	T1	T1c	T2
A-type	458	10	71
B-type	5	0	413

Fig. 3.1. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, Rothamsted (CS/476).

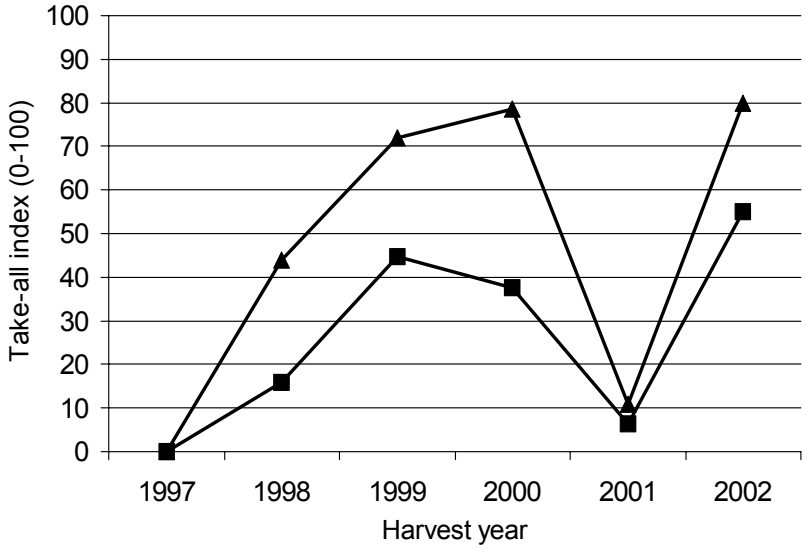


Fig. 3.2. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, East Winch.

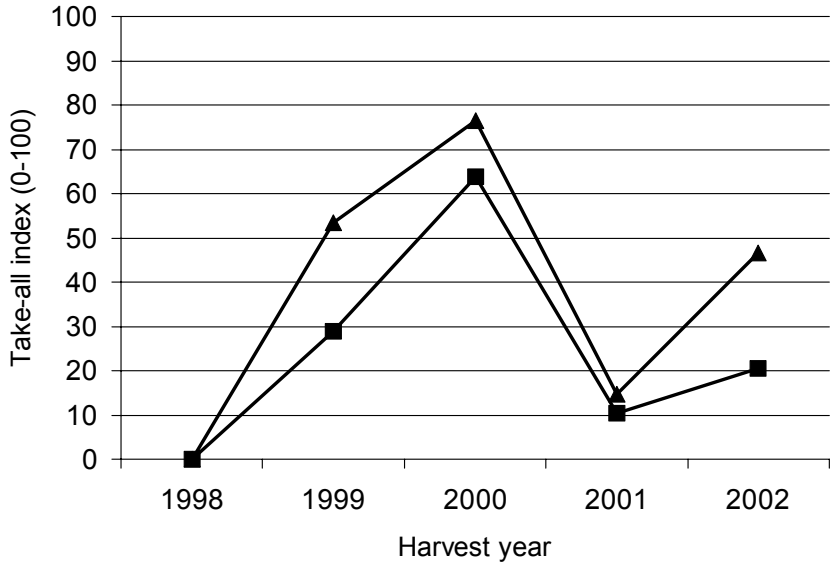


Fig. 3.3. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, Rothamsted (CS/508).

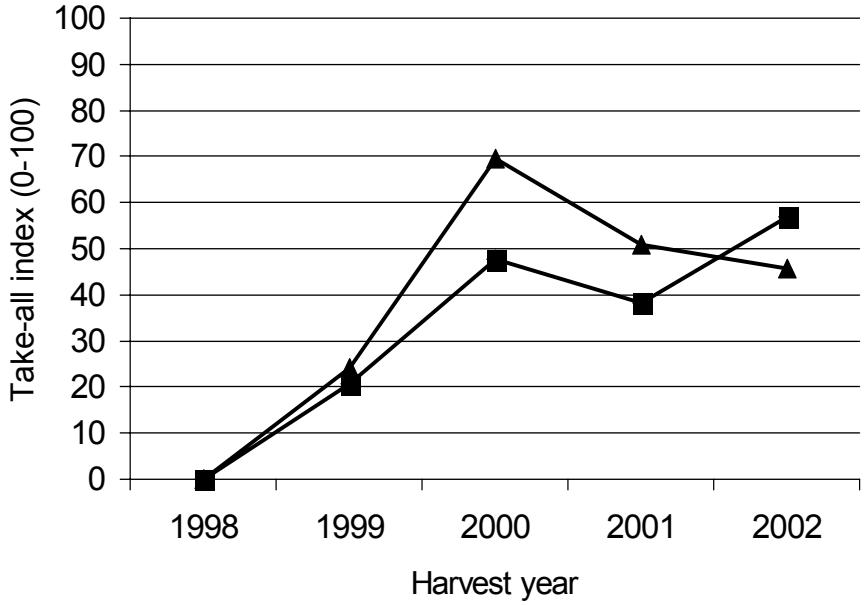


Fig. 3.4. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, Sacrewell.

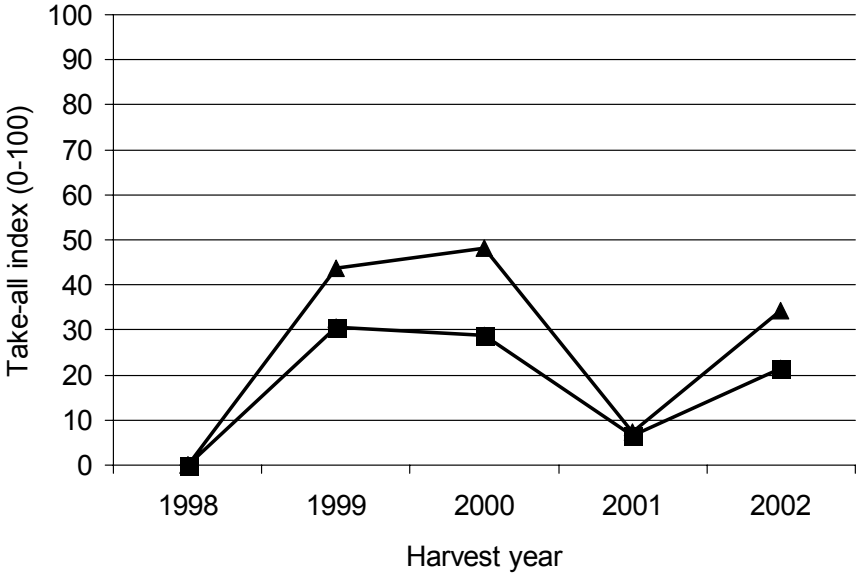


Fig. 3.5. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of applying a single treatment in each year (-◆), Rothamsted (CS/476).

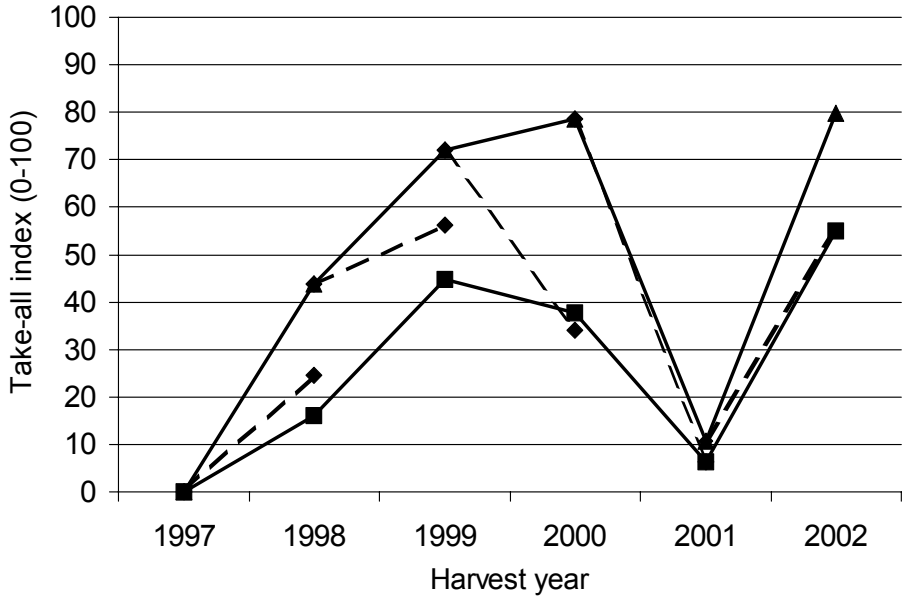


Fig. 3.6. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of applying a single treatment in each year (-◆), East Winch.

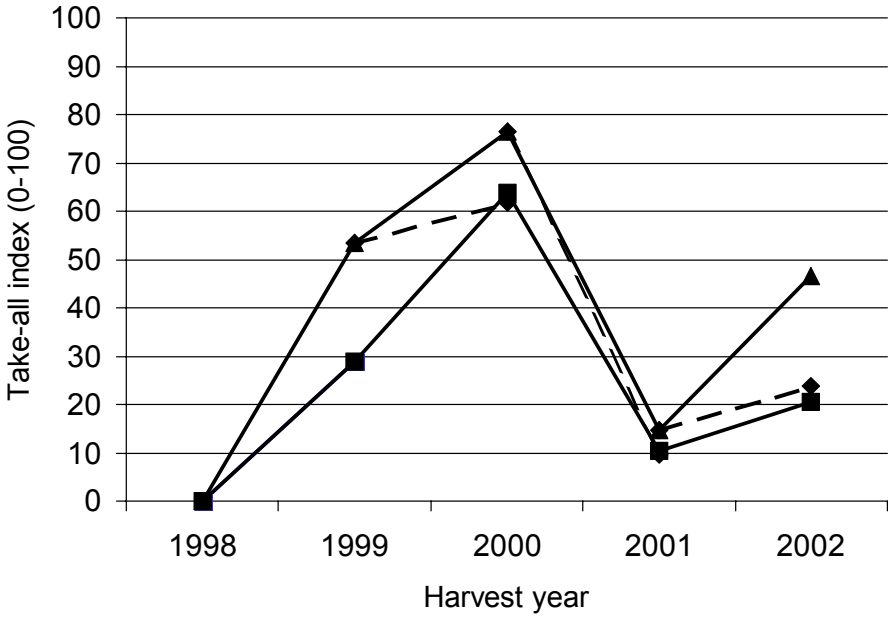


Fig. 3.7. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of applying a single treatment in each year (-◆), Rothamsted CS/508.

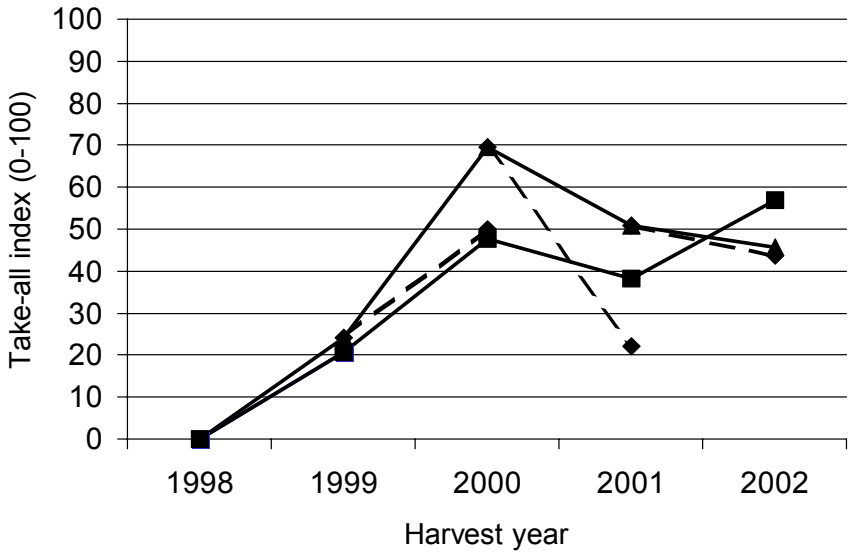


Fig. 3.8. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of applying a single treatment in each year (-◆), Sacrewell.

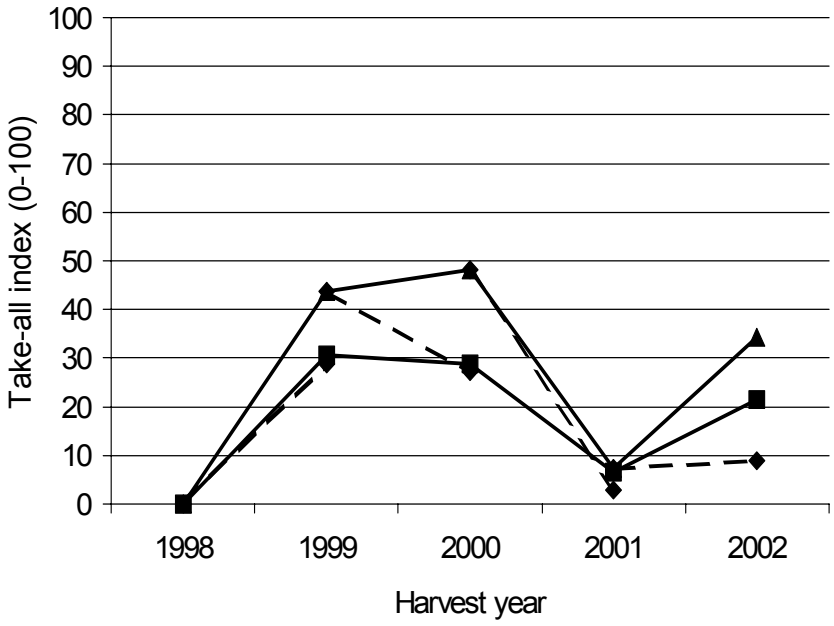


Fig. 3.9. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of withholding treatment in each year (-◆), Rothamsted (CS/476).

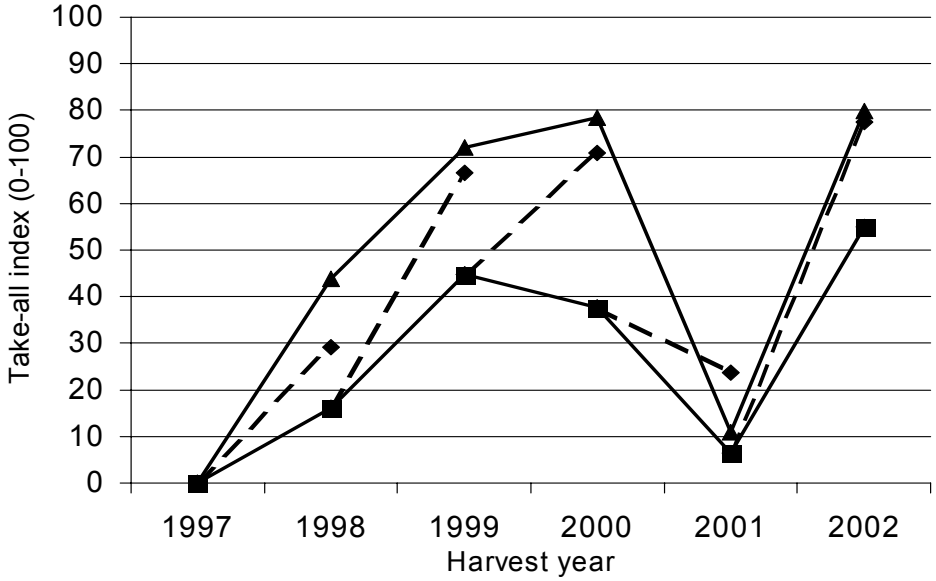


Fig. 3.10. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of withholding treatment in each year (-◆), East Winch.

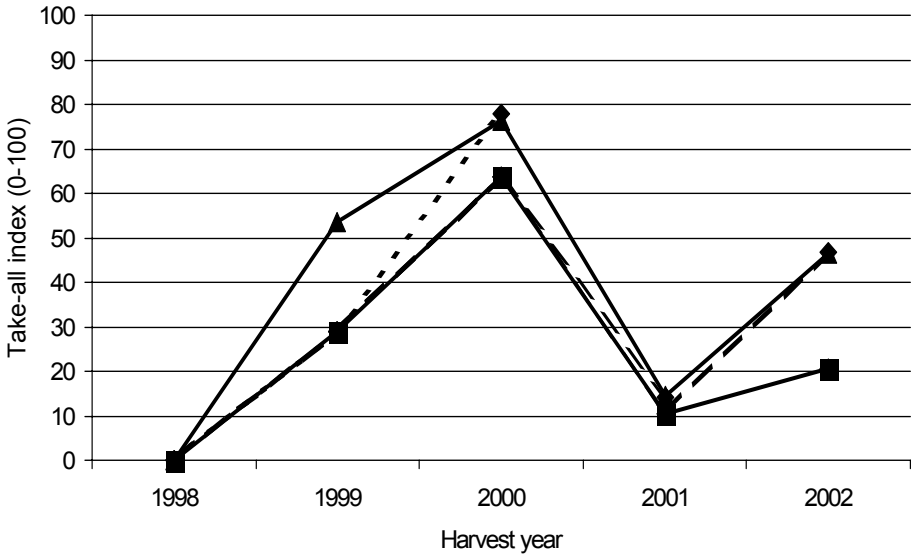


Fig. 3.11. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of withholding treatment in each year (-◆), Rothamsted (CS/508).

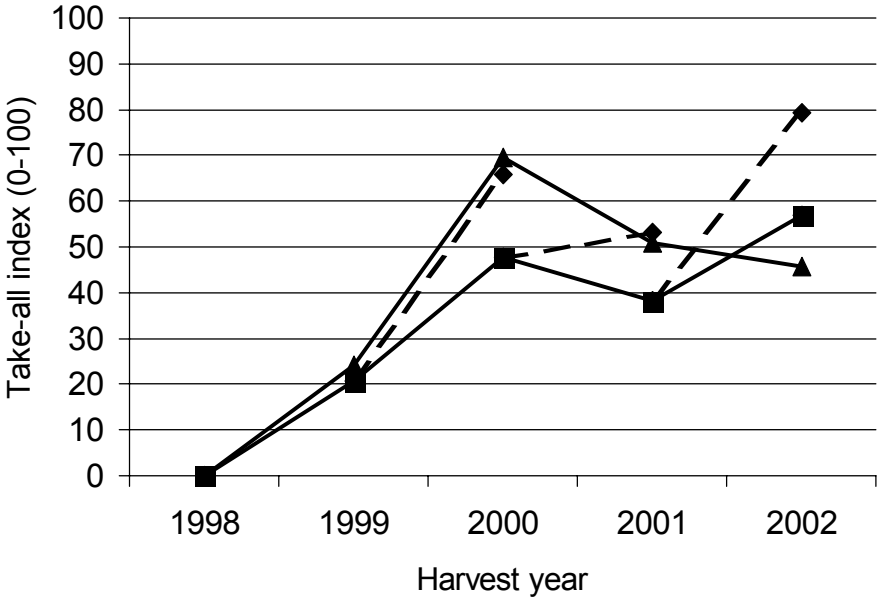


Fig. 3.12. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of withholding treatment in each year (-◆), Sacrewell.

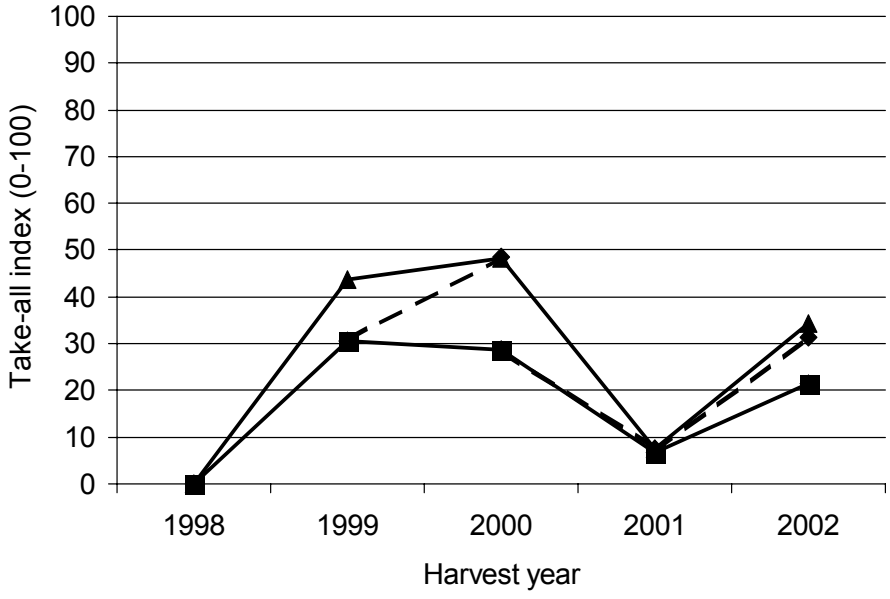


Fig. 3.13. Take-all epidemics in plots sown with untreated (♦) or treated (■)seed in all years, and effects of sowing treated seed only in the second wheat (-◆), Rothamsted (CS/476).

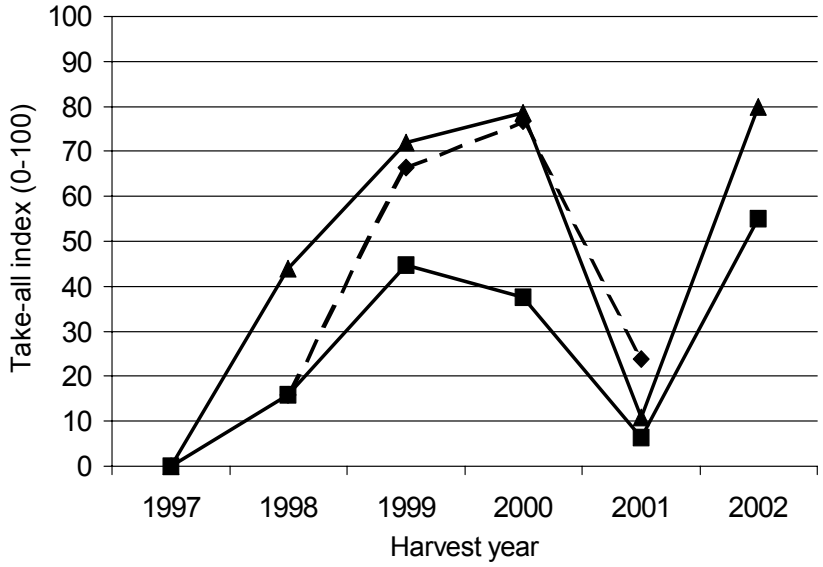


Fig. 3.14. Take-all epidemics in plots sown with untreated (♦) or treated (■)seed in all years, and effects of sowing treated seed only in the second wheat (-◆), East Winch.

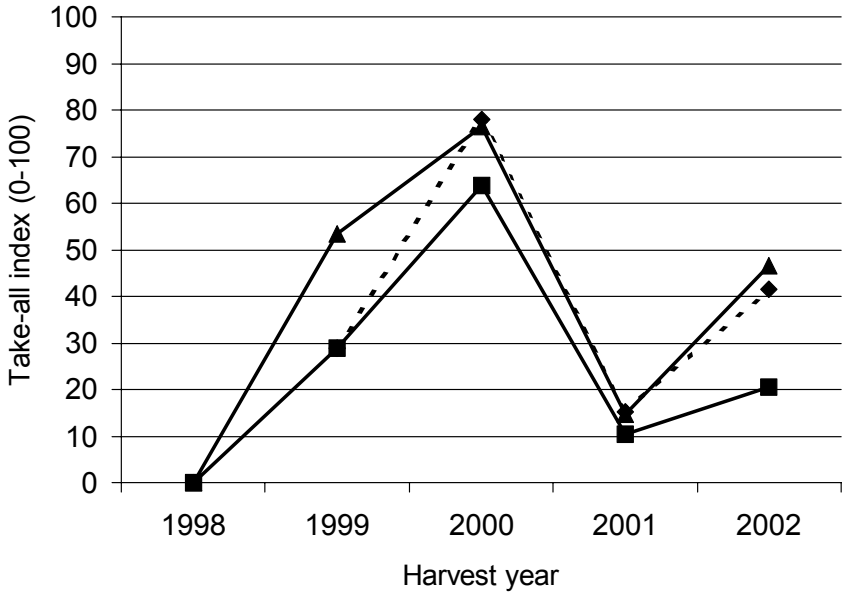


Fig. 3.15. Take-all epidemics in plots sown with untreated (♦) or treated (■)seed in all years, and effects of sowing treated seed only in the second wheat (-◆), Rothamsted (CS/508).

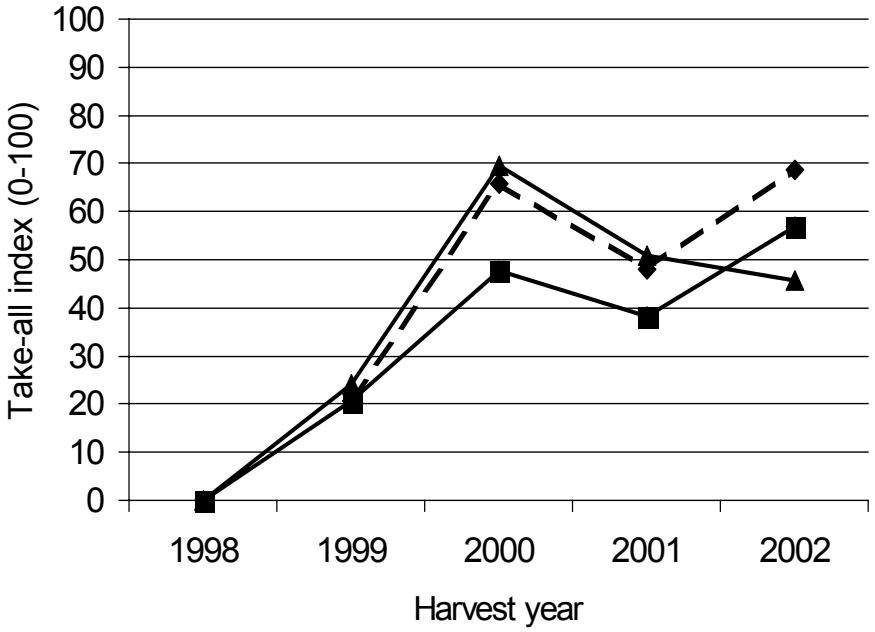


Fig. 3.16. Take-all epidemics in plots sown with untreated (♦) or treated (■) seed in all years, and effects of sowing treated seed only in the second wheat (-◆), Sacrewell.

Fig. 5.2. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops in fluquinconazole-treated plots at East Winch.

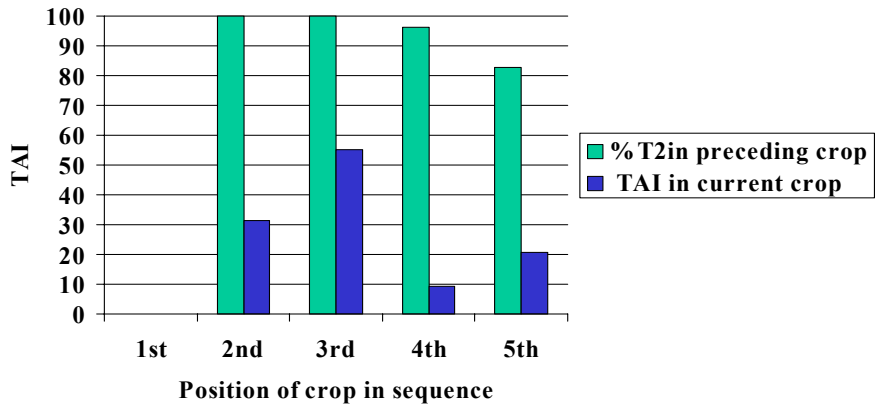


Fig. 5.3. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops in non-treated plots at Rothamsted (CS/508).

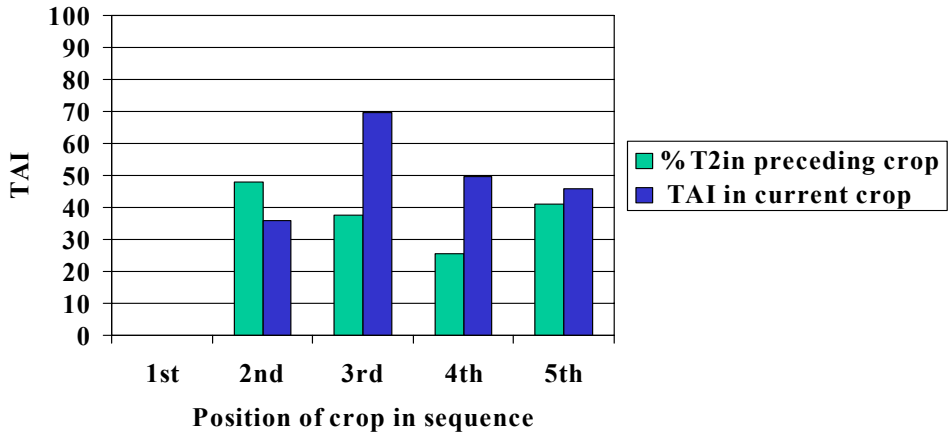


Fig. 5.4. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops in fluquinconazole-treated plots at Rothamsted (CS/508).

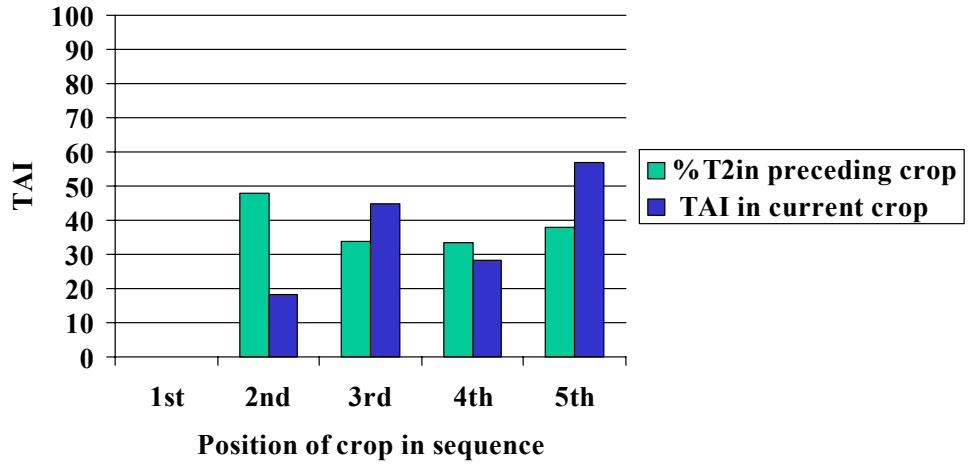


Fig. 5.5. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops in non-treated plots at Sacrewell.

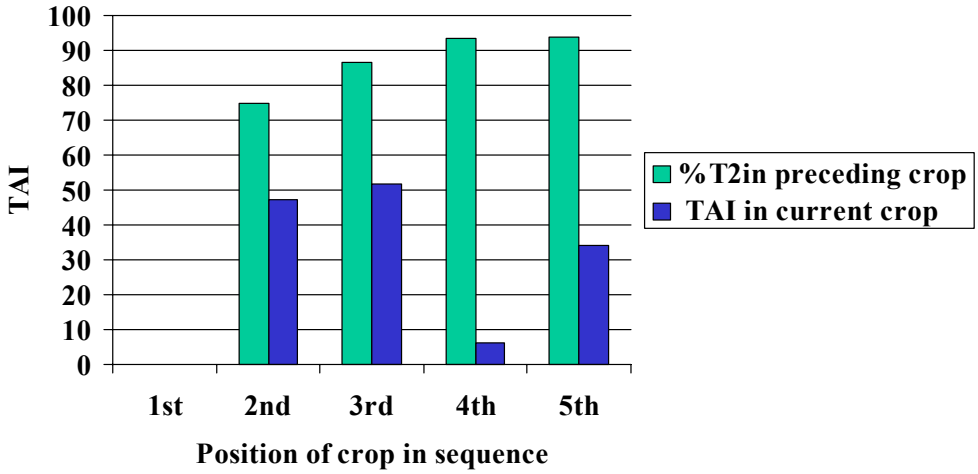


Fig. 5.6. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops in fluquinconazole-treated plots at Sacrewell.

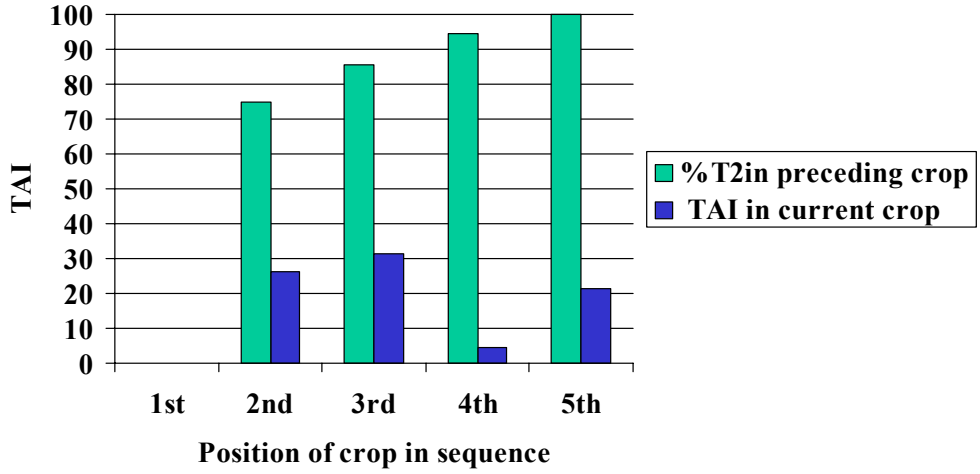


Fig. 5.7. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops at monitoring site B.

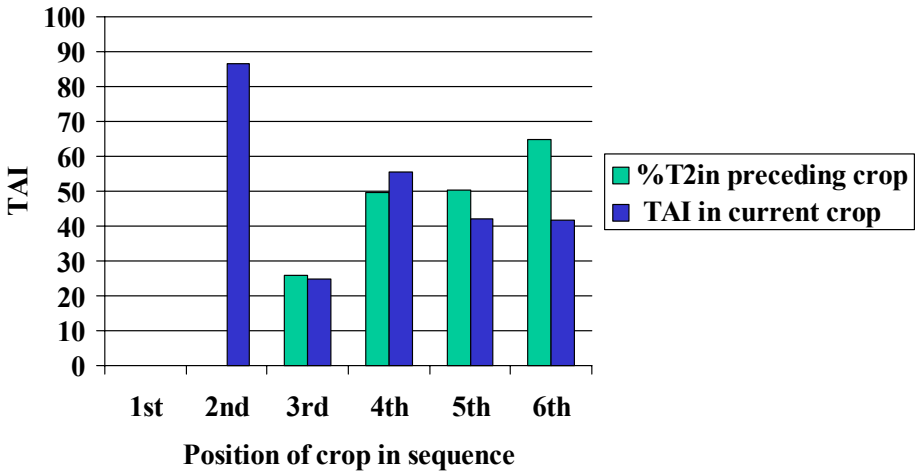


Fig. 5.8. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops at monitoring site C.

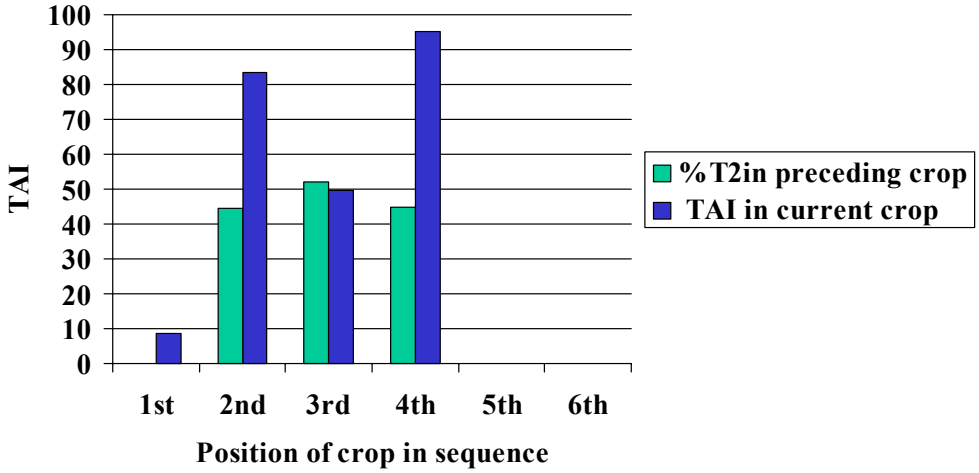


Fig. 5.9. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops at monitoring site L.

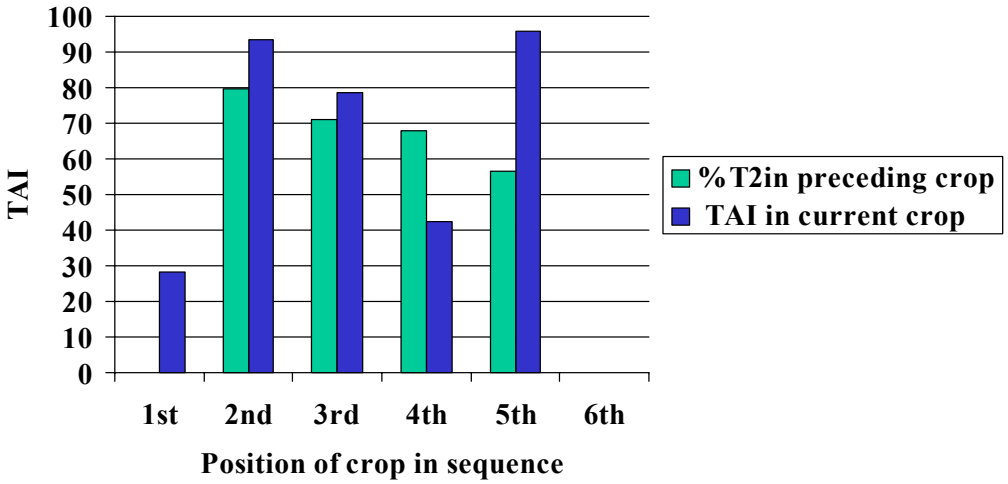


Fig. 5.10. Take-all index (TAI, 0-100) in successive wheat crops and population structure (%T2) of the take-all fungus in the preceding crops at monitoring site W.

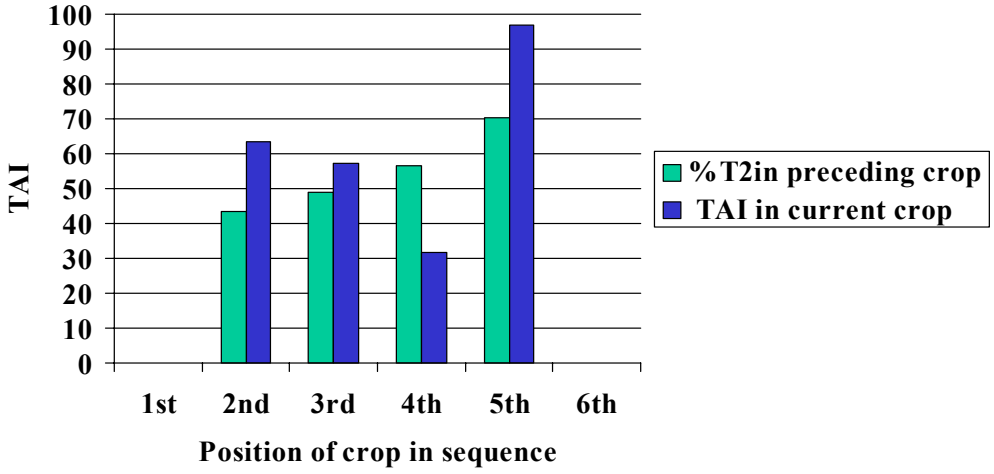
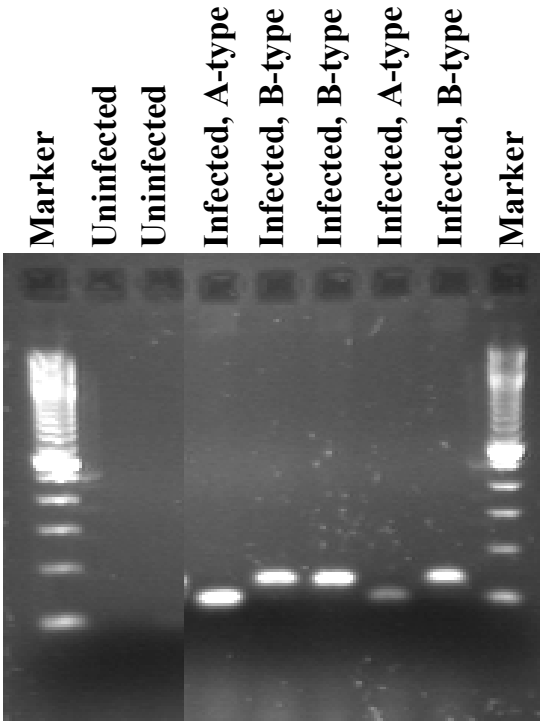


Fig. 5.11. Identification of A- and B-type isolates of Ggt using a Ggt-specific PCR assay and DNA prepared from Ggt-infected wheat roots.



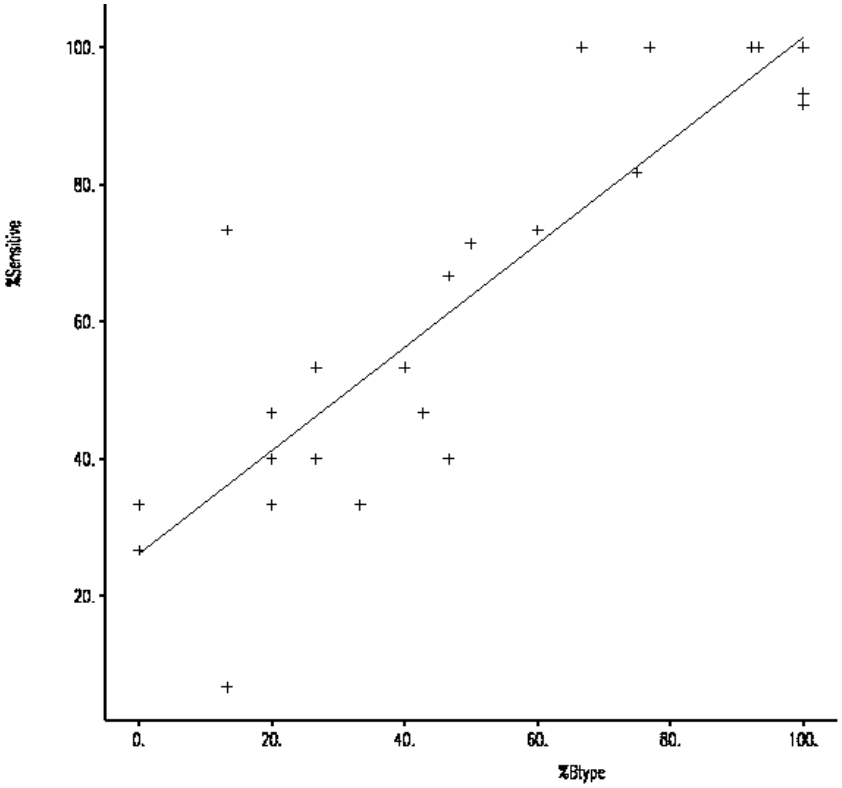


Fig. 5.12. Regression of percentage isolates sensitive to silthiofam on percentage classified as B-type in populations of the take-all fungus from individual plots. Regression equation: $y = 0.753x + 26.11$, 76.5% variance accounted for, $F = 79.09$, 5 d.f., $P<0.001$.