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# Assessing take-all risk in second wheats using the Predicta B test

by

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## Contents

# Abstract

Soil samples were taken after the first wheat harvest in 2007 from three experiment sites at two locations. Joint replicated soil seedling bioassays and take-all DNA quantification measurement were done on a total of 40 soil samples. A strong positive correlation was found between the take-all infectivity of the soil, as measured by a soil seedling bioassay, and the take-all DNA in the soil in two of the three sites. These two sites had very different soil types. At the third site there was also a positive correlation between the two variables but this was weaker.

Two of the three sites were sown to a second winter wheat in October 2007 and the severity of take-all was assessed in July 2008 on those plots where the take-all DNA and the take-all infectivity of the soil had been measured. On both occasions, there was a strong positive correlation between the infectivity of the soil, measured prior to sowing the crop, and the percentage of plants in the subsequent crop showing moderate or severe disease symptoms. Take-all DNA in the soil, measured prior to sowing the crop, also showed a strong positive correlation with the percentage of plants showing moderate or severe symptoms of the disease in one experiment but the positive correlation was weaker in the second experiment.

# Summary

Take-all, caused by the Ascomycete fungus *Gaeumannomyces graminis* var. *tritici*, is a root disease which can occur on wheat, barley, triticale and rye to varying degrees. Severe infection causes a loss in yield and affects grain quality. A recent study at Rothamsted has shown this root disease may have an environmental impact because where severe patches occur more soil mineral N was detected which is available for leaching.

The risk of severe take-all occurring is largely dependent on the amount of inoculum in the soil at the time of sowing. At Rothamsted, detecting this inoculum has been achieved by a soil bioassay, using fully susceptible wheat plants as bait. The amount of infection in the bioassay i.e. the percentage of roots infected gives a measure of the take-all infectivity of the soil and often relates to the severity of the disease in the following susceptible crop. That is, the more roots infected in the bioassay the greater the chance of severe disease occurring in the subsequently sown wheat crop. This method of risk assessment is useful as a research tool. However, this method is not commercially viable because it is both time consuming, labour intensive and also needs controlled environmental conditions (Rothamsted Research Association Newsletter, Issue 27 June 2008).

A direct method for assessing take-all inoculum in the soil has been developed in Australia over the last 9 years and has recently been successfully tested in New Zealand for 3 years. In this direct method, called PreDicta B (www.planthealthaustralia.com.au), the pathogen DNA is detected using real-time PCR. Although wheat-cropping conditions and take-all behaviour are very different in Australia from those in the UK, this method may potentially be useful in assessing risk to second wheats in the UK because: 1) climatic conditions in New Zealand are more akin to the UK; and, 2) take-all epidemics have been developing more quickly over the last 10-15 years, so that amounts of soil inoculum after first wheats are now equivalent to what was previously expected after second wheats. As a consequence inoculum would be more uniformly distributed in a field, although sampling procedures still need careful consideration. In addition, taking advantage of the field trials established within the Defra funded Wheat Genetic Improvement Network (WGIN) core project (www.WGIN.org.uk) for other purposes in Herts, Cambs and

Herefordshire we have identified consistent differences post-harvest in take-all levels in the soil between wheat genotypes when grown as first wheats (Rothamsted Research Association Newsletter, Issue 27 June 2008).

The aim of this project was to compare and evaluate the molecular based PCR method for detecting take-all DNA in the soil with the take-all infectivity of the soil, as measured by a seedling bioassay, on the subsequent development of take-all in the following crop. The project had the following specific objectives:
(a) Identify areas (plots) from two pre-existing multifactorial experiments, where inoculum was expected to develop differentially, for soil sampling.
(b) Assess take-all infectivity of the soils by soil core bioassay soon after harvest.
(c) Assess take-all inoculum in soil samples by real time PCR. Soil samples to be taken at the same time as those above.
(d) Assess take-all in the following wheat crop in all plots sampled above.

The two existing field experiments at Rothamsted had contrasting crop sequences and included a one or two year ley of ryegrass, cocksfoot, barren brome or blackgrass, one or two year fallow and continuous wheat.

These sequences were likely to build up different amounts take-all inoculum when winter wheat was introduced to the whole experiment in autumn 2006. Stubble and soil samples were taken after harvest in August 2007. Six random samples, each approximately 10cm deep, were taken from each plot. Soils were crumbled to an even texture, large stones being removed, and a 500g sub-sample taken for DNA analysis. Five pots were filled with the remaining soil (300g/pot) and used for the wheat seedling bioassay.

Recent work at Rothamsted has shown that wheat genotypes can differ in their ability to build up the take-all fungus when grown as a first crop. An opportunity also arose to sample a winter wheat variety trial in Suffolk where differences in take-all inoculum was likely to occur. Soil samples and processing followed the same procedure as above.

## Key results

- In two out of the three experiments there was a strong positive correlation between the percentage of roots infected in the bioassay and the take-all DNA

detected in the soil. In the third experiment the correlation was weaker but positive.

- The two experiments giving the strong positive correlations were at different locations and on different soil types.
- In the two experiments at Rothamsted, where the severity of take-all was assessed in the following wheat crop, there was a positive correlation between the percentage of plants infected in the bioassay and the percentage of plants with moderate or severe symptoms of take-all.
- At the Rothamsted site, where a strong positive correlation existed between the percentage of roots infected in the bioassay and the take-all DNA detected in the soil there was an equally strong positive relationship between the take-all DNA in the soil and the percentage of plants with moderate or severe symptoms of take-all in the following wheat crop.
- At the second Rothamsted site where a weaker positive correlation had been detected between the soil bioassay results and the take-all DNA detected in the soil, there was also a positive but weaker correlation detected between the take-all DNA in the soil and the percentage of plants with moderate or severe symptoms of take-all in the following wheat crop.
- Future modifications to the preparation of soil samples prior to shipment to Australia for DNA testing are thought likely to increase further the robustness of this rapid test.

## Conclusions and implications

This project confirmed that the bioassay method for detecting the take-all infectivity of the soil is a good indicator of the potential risk of severe disease occurring in a following crop. The positive relationship between the take-all DNA in the soil assessed by the PreDicta B test and the percentage of roots infected in the bioassay in two out of the three sets of data is encouraging. Also this study indicates that the PreDicta B test worked well on two different soil types, namely, loam over clay, Ashley series (Suffolk) and flinty clay loam, Batcombe series (Rothamsted). Further evaluation will be required on small plots as well as on a field scale before this method could be used more widely.

The molecular technique has the distinct advantage in that it both quantifies and predicts take-all inoculum levels in the soil. The PreDicta B test is easier and less time

consuming and therefore the results were available quicker, even though the samples had to be sent to Australia for testing. The PreDicta B test would allow a more accurate field-by-field risk assessment for take-all. In turn, this may reduce the number of crops at risk from severe take-all by informing the farmer / farm agronomist earlier so that appropriate changes to the rotation could be made when high take-all inoculum levels are detected. The wheat crop is already the most profitable crop in the UK rotation. If wheat prices continue to steadily rise farmers will move towards trying to grow wheat in closer cropping sequences where the risks will be greater from take-all.

Less take-all in second and subsequent wheat crops would result in these crops being grown profitably. Farmers would be more confident that applications of pesticides and fertilizers to second wheat crops will return a high yield and good quality grain. Improved grain quality would be beneficial to the millers and baking industry who would have a greater choice of suitable grain. There may also be significant environmental benefits because water courses surrounding fields in second and third wheat crops may be at a lower risk of diffuse pollution from unused fertiliser applications.

# Technical detail

## Introduction

Take-all disease is usually negligible in first wheat crops grown after 'clean' break-crops (i.e. in the absence of susceptible grass weeds or volunteers), but second wheat crops can be severely affected. Assessment of the amount of take-all in a crop indicates the damage being done to that crop, but will not indicate the risk to the following crop. This can be achieved by assessing the amount of inoculum of the fungal pathogen in the soil under the previous crop, at least in the early stages of an epidemic, before take-all decline sets in. We have demonstrated this using a soil bioassay. However, this is labour-intensive, needs controlled-environment conditions for plant growth and is, therefore, not commercially viable.

A direct method for assessing take-all inoculum, called PreDicta B, where the pathogen DNA is detected using real-time PCR, has been developed in Australia and recently been tested in New Zealand. The prospect of a quicker, easier test for take-all inoculum would provide farmers with a more informed take-all risk assessment in the intercropping period and thereby avoidance of severely infected crops.

The main aim of this project is to compare and evaluate the molecular based PCR method for detecting take-all DNA in the soil with the take-all infectivity of the soil, as measured by a seedling bioassay, on the subsequent development of take-all in the following crop.

## Methods and materials

Two field experiments at Rothamsted provided wheat sequences which ranged from first wheat after a one or two year break to continuous wheat. These were expected to generate different amounts of take-all inoculum after the 2007 harvest.

### Main purpose of pre-existing experiments

A previous experiment at Rothamsted had shown that different short term grass leys can affect development of take-all and take-all suppression in wheat grown

subsequently (Gutteridge *et al.,* 2006). Although some perennial grasses are poor at supporting and transmitting take-all, they can allow extremely rapid build-up of take-all subsequently. It is likely that these soils do not encourage antagonists of the take-all fungus. Annual grasses (weeds rather than sown set-aside or ley grasses) which tend to transmit take-all better and, to a varying extent, partially negate the take-all suppressiveness that can begin to develop even under a single wheat crop.
Two field experiments were designed to investigate the nature of suppression in more detail.

The first experiment (Rothamsted 1), located on a large relatively flat field, consisted of 23 treatments x four replicates in a random block design, and followed a second wheat crop. In the first preparatory year, plots of ryegrass, cocksfoot, blackgrass and barren brome were established with and without nitrogen in spring. All other plots were either sown to wheat or not (fallow). In the second preparatory year, plots of ryegrass and cocksfoot were either ploughed and sown to wheat or left (2 year ley). The annual grass plots were sown to wheat. Further grass plots were established for each of the four species after either wheat or fallow. In addition, each grass species was sown with wheat after a wheat crop in year 1. Other treatments were a continuous wheat and two year fallow. All plots (92) were ploughed in autumn 2006 and sown to winter wheat cv. Hereward. In Appendix 1, the full plot history of this experiment is given and the 14 treatments selected for the current study are indicated.

The second experiment (Rothamsted 2) situated in the same field but at a distance of approximately 70 meters to the East and on a slight slope, consisted of 8 treatments x 4 replicates in a random block design, also followed a second wheat. Plots of ryegrass, tall fescue and rye brome were grown either as pure stands or with wheat. Other treatments were wheat and fallow. The site was ploughed in autumn 2006 and sown to winter wheat cv. Hereward. The full cropping history and treatment selection are given in Appendix 2.

Recent work at Rothamsted within the WGIN programme has shown that winter wheat varieties can differ in their ability to build up the take-all fungus in the soil when grown as a first wheat crop. A first winter wheat variety trial in Suffolk, where take-all inoculum was likely to develop differentially was also included in the study

(Experiment 3, see appendix 3 for details). The experiment was a first winter wheat crop grown after sugar beet 2005 and Linseed in 2006.

The soil type of the Rothamsted fields were flinty clay loam (Batcombe series), whereas Experiment 3 in Suffolk was on loam over clay (Ashley series).

Soil and stubble samples were taken after harvest from six random points to a depth of approximately 10cm from selected treatments from experiments 1 and 2 (see tables 1 and 2) and from all the varieties in the trial in Suffolk (Experiment 3). Soils were stored at all times in a cold room at 4°c except when being prepared. Soils were crumbled to an even texture and roots from the stubble cut into approximately 1cm pieces. The soil and root pieces were mixed thoroughly and returned to the cold room.

## Soil bioassay and DNA preparation

Moist coarse sand (50cc) was put into 11cm tall plastic drinking cups, previously drilled with four drainage holes, and then 300g of the prepared soil added. Ten wheat seeds cv. Hereward (not treated with fungicides) were placed on the soil surface in each pot and covered with horticultural grit. There were five replicates of each treatment arranged in a randomised block design. Pots were then kept in a growth room with a 16 hour day and 15/10$^{o}$C day / night temperatures. Pots were watered regularly and, after five weeks, roots were washed free of soil and examined for take-all lesions. Total numbers of plants and roots (main axes) and numbers infected were recorded (Slope *et al.,* 1979).

A sub-sample of 500g was taken from each soil and dried in an oven at 40°c for 36 hrs. The soil was allowed to cool before being transferred to a labelled bag and put into a deep freeze at -20°c, following the protocol for export provided by SARDI, Australia. A total of 18 samples were selected from Rothamsted 1 see Table 3, 12 from Rothamsted 2 see Table 4 and 10 from Experiment 3 in Suffolk.

## Crop sampling

After soil and stubble sampling the sites containing the Rothamsted experiments 1 and 2 were ploughed on September 28$^{th}$ 2007 and sown to winter wheat cv. Hereward

on October 8th 2007. The ploughed furrows were in the opposite direction to the previous year to minimise soil movement. As a further precaution the outer two rows on either side of the plots were treated as discard rows and not sampled. The take-all severity of this following winter wheat crop was used to determine the relationship between (a) take-all infectivity of the soil, as measured by the wheat seedling bioassay, and (b) the take-all DNA of the soil, determined after harvest of the previous crop. Plant samples were only taken from treatments where the take-all infectivity of the soil had been previously determined. On July 16th 2008 ten 20cm lengths of row were dug from each plot at random points along two, approximately parallel, zig-zag transects from experiments 1 and 2. The root systems were washed and allowed to dry. These were re-wetted by soaking in water before being assessed. Roots were examined in a white tray containing water and take-all assessed on a 0-5 scale as: absent (0); slight, affecting 1-10% of the root system (1); slight, affecting 11-25% of the root system (2); moderate, affecting 26-50% of the root system (3); moderate, affecting 51-75% of the root system (4); severe, affecting more than 75% of the root system (5). A take-all index on a 0-100 scale was calculated by adding the percentage of plants in each category multiplied by its score value and dividing the total by 5. This procedure differs slightly from others in use but takes account of conditions in which take-all develops in UK crops (Bateman *et al*., 2004; Gutteridge *et al*., 2003). Percentages of plants in the moderate and severe categories, with which yield losses are often most closely associated (Bateman *et al*., 1990), were also determined and analysed.

## Results

The level of take-all inoculum in the soil, as measured by the percentage of roots infected in the bioassay, was determined before samples were sent to SARDI in Australia for DNA analysis. Therefore, a selection of soils was made from each experiment which covered the range of inoculum concentrations found in the bioassay.

In Rothamsted experiment 1 (18 sent samples) and experiment 3 (10 sent samples) located in Suffolk there was a strong positive relationship between the percentage of roots infected in the bioassay and the amount of take-all DNA detected (Figures 1 and 3). However, at the 2nd site Rothamsted 2 (12 sent samples) the correlation was only

modestly positive (Figure 2A). In experiments 1 and 2 at Rothamsted the overall level of disease which developed in the soil bioassay was between 45 and 90% and this was higher than from experiment 3 in Suffolk where the range was 30 to 70% infection. Likewise the take-all DNA levels detected by the PreDicta B test spanned a higher and wider range from both Rothamsted sites, than from the site in Suffolk. Upon close inspection of the data generated for experiment 2, two data points were clear outlier situated well above the predicted relationship. Therefore, these two data points where the take-all DNA value was considerably greater than the level of infection obtained in the soil bioassay were removed and the correlation recalculated. This improved the strength of the overall correlation.

## Figure 1 Rothamsted 1 Experiment 1

**The relationship between the percentage of roots infected in the bioassay and the take-all DNA in the soil in experiment 1**

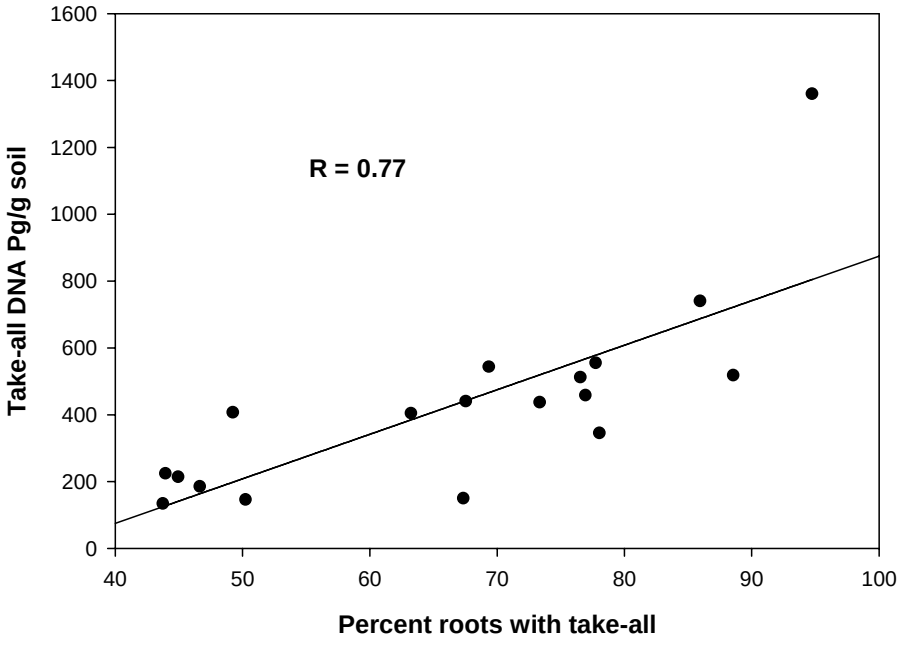


**Figure 2 A. Rothamsted 2 Experiment 2 – all data included**

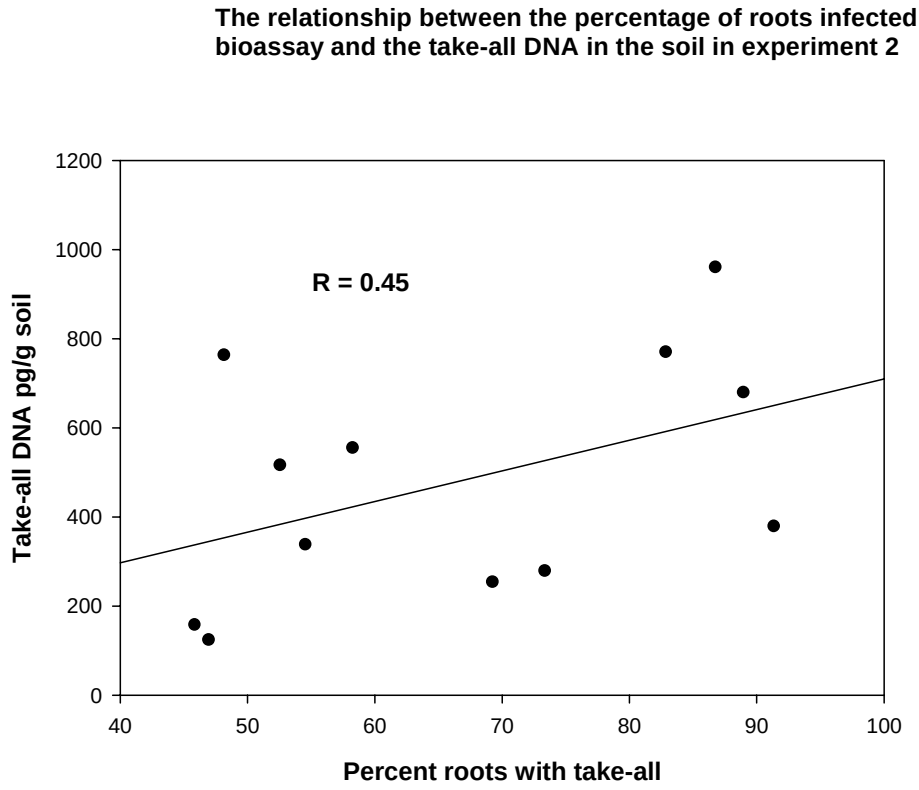
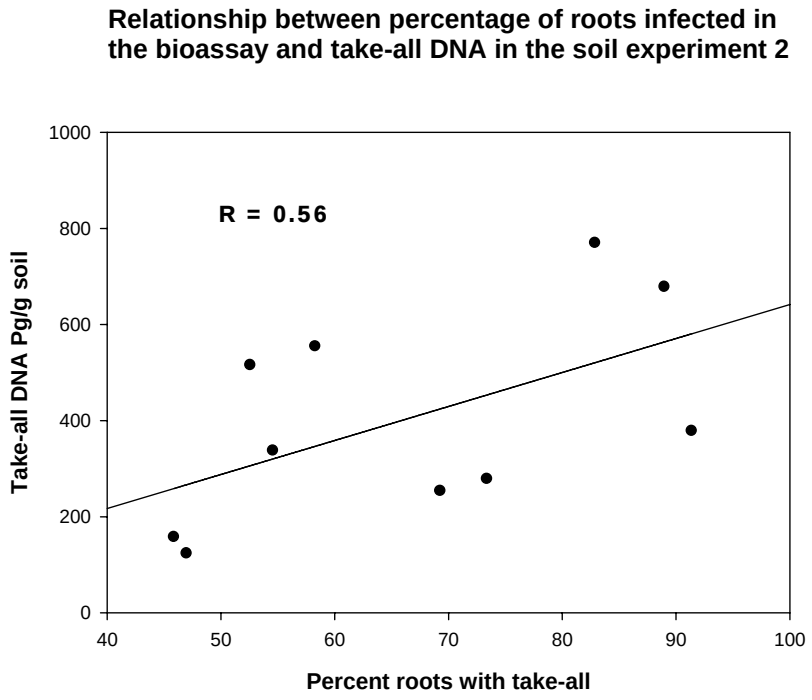
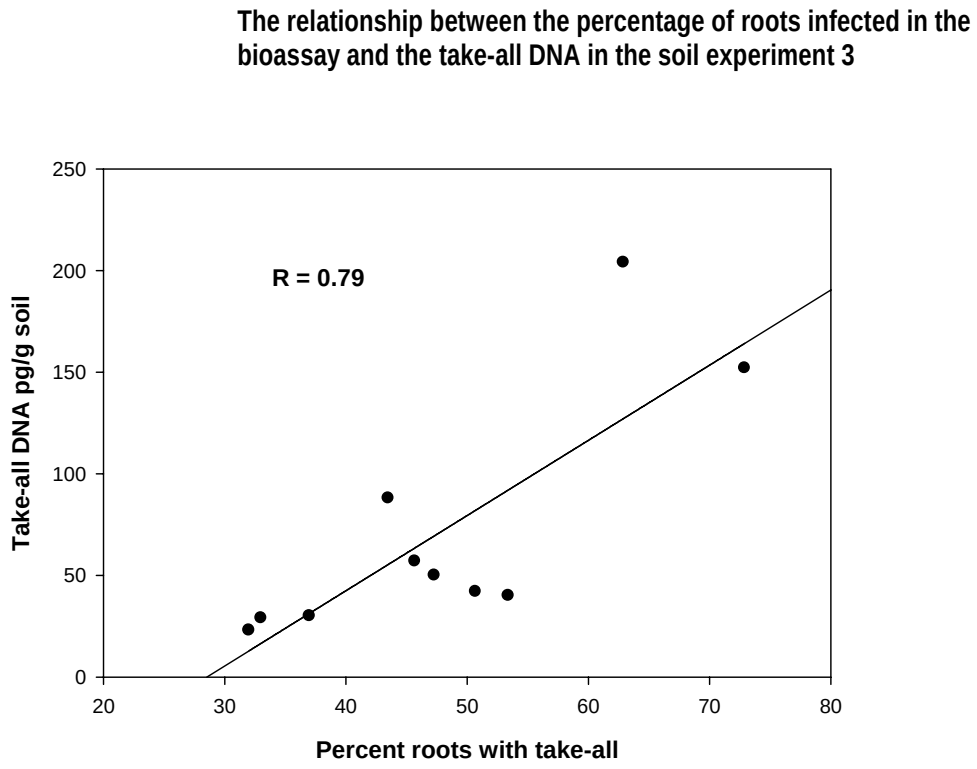



**Figure 2B Rothamsted 2 Experiment 2 – recalculated after removal of two data points (see text for details)**



## Figure 3 Experiment 3 (Suffolk)




Data for the severity of take-all in the following crop is only available from the two Rothamsted sites. There was a positive relationship at both sites between the bioassay and the percentage of plants with moderate or severe take-all (Figures 4 and 5). However, only in Rothamsted 1 did the take-all DNA correlate well with the disease in the following crop (Figure 6), and this was where a positive relationship with the bioassay also occurred (Figure 1). In the Rothamsted 2 experiment where the relationship between the bioassay and the take-all DNA was modest, the correlation between the take-all DNA and severity of take-all in the following crop was also only modest.

## Figure 4 Rothamsted 1 Experiment 1

**The relationship between percentage of roots infected in the autumn bioassay and the disease in the following crop in experiment 1**

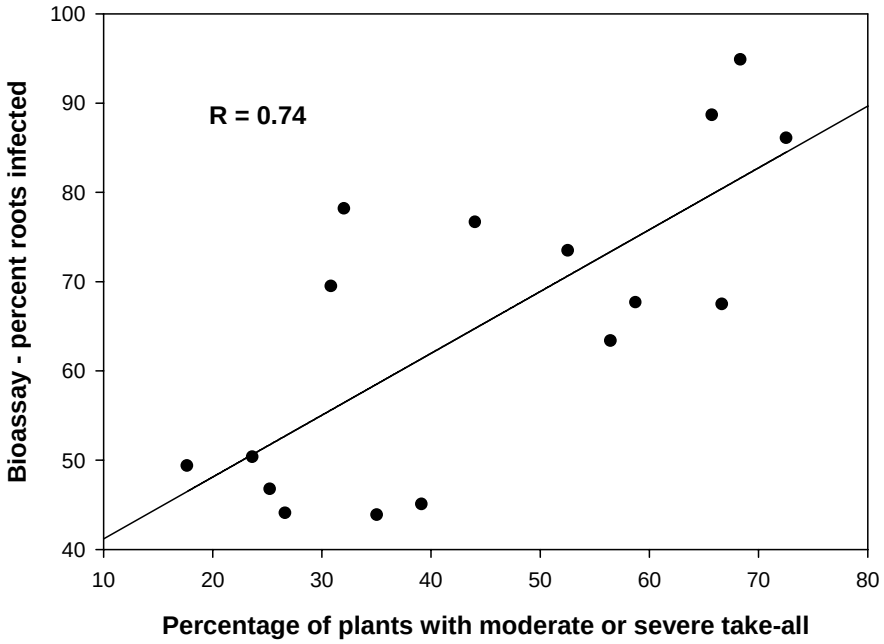


**Figure 5 Rothamsted 2 Experiment 2**

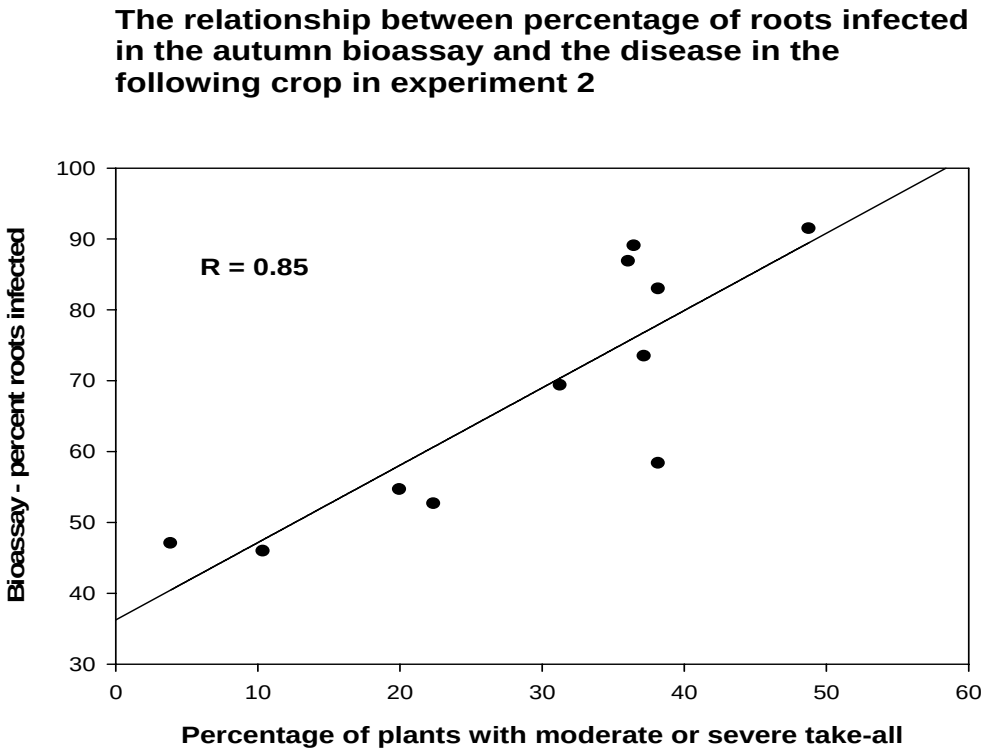



**Figure 6 Rothamsted 1 Experiment 1**

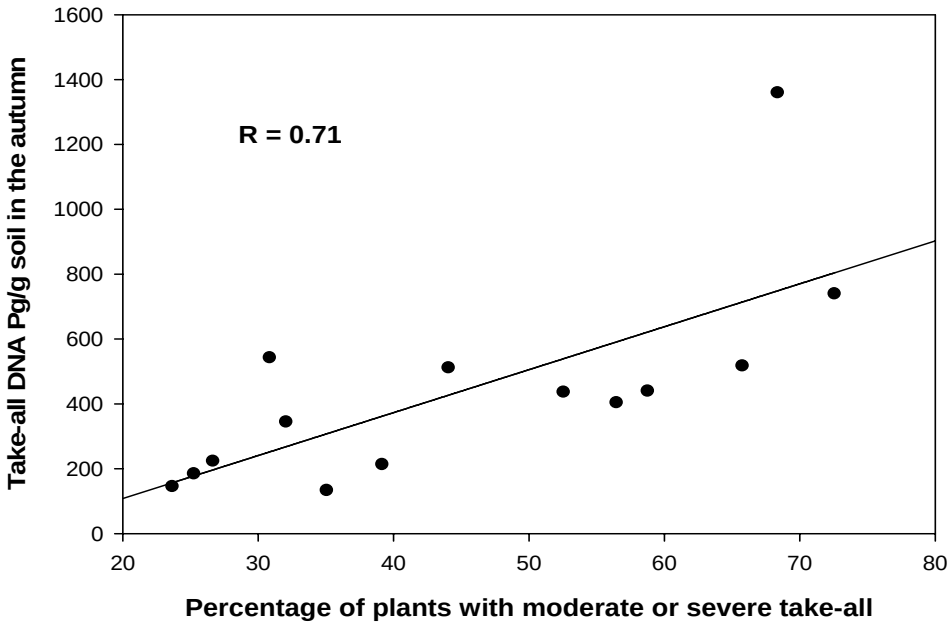
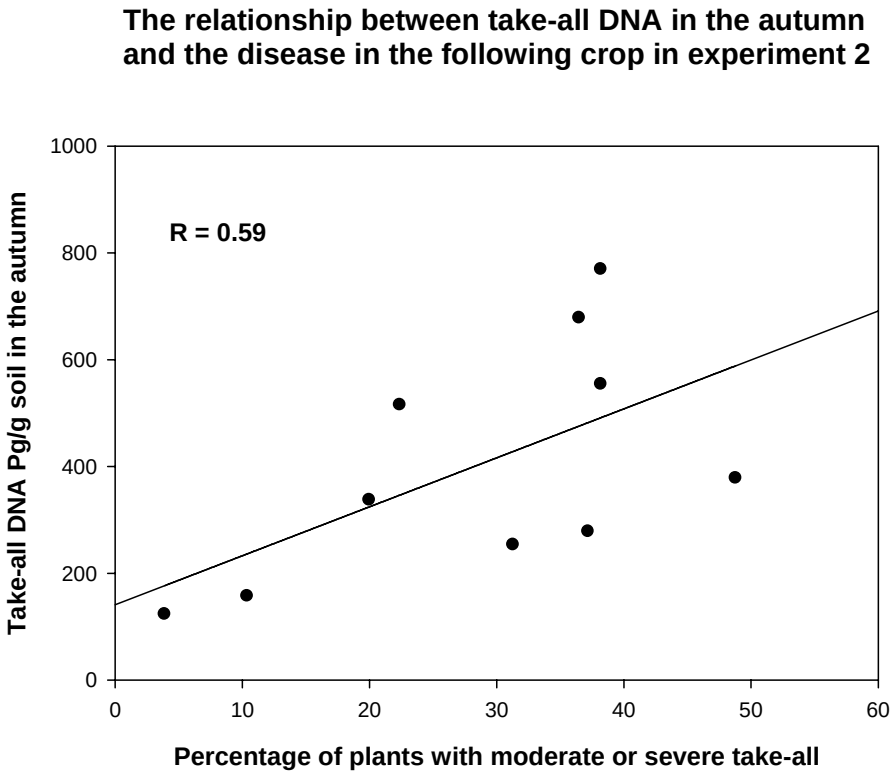


**Figure 7 Rothamsted 2 Experiment 2**




## Discussion

The risk of severe take-all occurring in a susceptible crop is dependent on the amount of inoculum present at the time of sowing. A soil core bioassay, taken after harvest and using wheat seedlings as bait plants, has been used at Rothamsted for many years to assess the take-all infectivity of the soil. This often relates to the severity of the disease in a following susceptible crop. This method is not commercially viable due to time and labour constraints. A molecular technique, based on real time PCR, for detecting take-all DNA in the soil has been developed by SARDI in Australia and this project has evaluated its potential use in the UK.

Data from the three experiments showed that take-all DNA detected in the soil correlated well with the percentage of roots infected in the bioassay in two of the experiments but was only modest in the third.

Take-all was assessed on plants taken from a following wheat crop from two of the experiments used in this study. The percentage of plants in the moderate and severe categories often relates more closely with yield loss and, hence, the damage done to that crop. A correlation between the percentage of plants in these two categories and the percent roots infected in a bioassay the previous autumn was strongly positive on both occasions. There was a strong positive correlation between take-all DNA detected in the soil the previous autumn and percentage of plants in the moderate and severe categories but only on one occasion. This was in the experiment where there was a relationship between the take-all DNA and the bioassay was high (Experiment 1). In experiment 2 the association between take-all DNA detected in the soil the previous autumn and the percentage of plants in the moderate and severe categories was weaker but positive. Therefore the potential for this molecular assessment of take-all inoculum in the soil to work in the UK is promising.

The data from experiment 2 where the correlation between DNA content in the soil and soil infectivity was only modest was very interesting. Close inspection of the twelve data points, revealed two as obvious outliers, where the DNA content detected was high but the take-all infectivity was far lower. This would suggest that in these two soils there had been a significant amount of take-all fungus present which was either dead or was still alive but now unable to infect wheat roots even under ideal conditions. An examination of the cropping history of each plot also failed to reveal an obvious explanation. Even after removal of the two outlier data points there was still considerable scatter amongst the remainder which suggested that other unknown reason(s) reduced the reliability of the PreDicta B test. Another possible explanation may have been the inter-contamination of the soil samples during the drying process. In order to meet the export requirement to Australia the soils had to be dried at 40°c for a minimum of 24 hours. The drying process followed the standard procedure for drying soils at Rothamsted, where soils are put into open cardboard trays and placed on the shelves in the oven. The oven had a circulating airflow to maintain an even temperature and it is possible that any root debris on the surface, as it dried out and became lighter, could be transferred to other trays. This possible source of contamination was not foreseen and the position of the trays had not been recorded. In order to eliminate this possible source of contamination soils dried for the take-all DNA assessments will in future be done in self contained units.

This project has shown that the molecular technique for detecting take-all DNA in the soil could offer UK farmers and farm advisors a more accurate field-by-field risk assessment for take-all. This may reduce the number of crops at risk from severe take-all by changing the rotation when high inoculum levels are detected. The wheat crop is already the most profitable crop in the UK rotation. As wheat prices continue to steadily rise farmers will move towards trying to grow wheat in closer cropping sequences where the risks will be greater from take-all.

Further work is required to confirm the predictive value of the molecular method in detailed plot work, on a field scale, on different soil types and after growing a range of wheat cultivars and other crop species. In the case of soil type, preliminary evidence suggest that the PreDicta B test and the sampling method deployed could work as well on loam over clay soil in Suffolk as on the flinty clay loam soil at Rothamsted. These two sites also had very different overall take-all soil inoculum levels and the method clearly worked equally well in both situations.

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# Appendix

## Experiment 1

The experiment was on a flinty clay loam soil on Great Knott 11 field on the Rothamsted Experimental Farm and followed winter wheat in 2003 and 2004. Preparatory crops (Table 1) were sown in September 2004 in plots 10m x 3m with a 3m discard (sown to wheat) between plots. The experiment was coded R/CS/625 and consisted of 23 treatments and four replicates arranged in a randomised block design. There were two preparatory years and then the site was sown to winter wheat cv. Hereward in the following two years.

## Experiment 2

The experiment was on a flinty clay loam soil on Great Knott 11 field on the Rothamsted Experimental Farm and followed winter wheat in 2004 and 2005. Preparatory crops (Table 2) were sown in September 2005 in plots 10m x 3m with a 3m discard (sown to wheat) between plots. The experiment was coded R/CS/626 and consisted of 8 treatments and four replicates arranged in a randomised block design. After the preparatory year the site was sown to winter wheat cv. Hereward in the following two years.

## Experiment 3

The experiment was on a loam over clay soil (Ashley series) near Woolpit in Suffolk and followed sugar beet in 2005 and linseed in 2006. The experiment was sown in October 2006 and consisted of 13 different winter wheat varieties sown in long strips across the field. Each strip was divided into twelve to accommodate different fungicide treatments for the control of other diseases.

Table 1 Experiment 1 Treatments. Samples were taken from the treatment numbers highlighted

| Treatment number | Preparatory crop 2005 | Preparatory crop 2006 |
|---|---|---|
| 1 | Ryegrass + N2 | Ryegrass + N2 |
| 2 | Ryegrass + N1 | Ryegrass |
| 3 | Ryegrass + N1 | Wheat |
| 4 | Wheat | Ryegrass + wheat |
| 5 | Wheat | Ryegrass + N2 |
| 6 | Wheat | Ryegrass |
| 7 | Blackgrass | Wheat |
| 8 | Wheat | Blackgrass |
| 9 | Fallow | Blackgrass |
| 10 | Wheat | Blackgrass + wheat |
| 11 | Wheat | Barren brome + wheat |
| 12 | Wheat | Barren brome |
| 13 | Fallow | Barren brome |
| 14 | Barren brome | Wheat |
| 15 | Wheat | Cocksfoot + Wheat |
| 16 | Wheat | Cocksfoot + N2 |
| 17 | Wheat | Cocksfoot |
| 18 | Cocksfoot + N2 | Cocksfoot + N2 |
| 19 | Cocksfoot | Wheat |
| 20 | Cocksfoot + N1 | Cocksfoot |
| 21 | Fallow | Wheat |
| 22 | Fallow | Fallow |
| 23 | Wheat | Wheat |

Table 2 Experiment 2 Treatments. Samples were taken from the treatment numbers highlighted

| Treatment number | Preparatory crop 2006 |
|---|---|
| 1 | Wheat |
| 2 | Fallow |
| 3 | Rye-brome |
| 4 | Wheat + Rye-brome |
| 5 | Ryegrass |
| 6 | Wheat + Ryegrass |
| 7 | Tall fescue |
| 8 | Wheat + Tall fescue |

Table 3 The percentage of roots infected in the soil bioassay, taken after harvest in 2007, from experiment 1 at Rothamsted. All plots shown and those highlighted were selected for GGT DNA determination.

| Rothamsted 1 Experiment 1 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Plot | % roots infected | Plot | % roots infected | Plot | % roots infected | Plot | % roots Infected |
| 2 | 63.3 | 24 | 84.9 | 47 | 78.2 | 71 | 80.6 |
| 3 | 67.4 | 25 | 86.5 | 50 | 87.8 | 72 | 90.6 |
| 4 | 70.3 | 27 | 81.1 | 52 | 46.7 | 73 | 72.9 |
| 5 | 81.0 | 31 | 43.9 | 53 | 73.8 | 74 | 75.1 |
| 8 | 49.5 | 32 | 80.0 | 54 | 75.0 | 75 | 44.0 |
| 9 | 74.0 | 33 | 67.6 | 56 | 87.8 | 76 | 76.7 |
| 10 | 45.0 | 34 | 76.6 | 57 | 77.8 | 77 | 83.5 |
| 11 | 79.9 | 35 | 76.1 | 58 | 84.0 | 79 | 78.1 |
| 15 | 43.8 | 37 | 87.6 | 60 | 69.4 | 80 | 72.3 |
| 16 | 67.7 | 38 | 86.0 | 62 | 70.4 | 83 | 79.8 |
| 20 | 79.4 | 40 | 88.6 | 63 | 77.0 | 85 | 85.1 |
| 21 | 73.3 | 42 | 79.0 | 65 | 70.0 | 88 | 84.8 |
| 22 | 94.8 | 43 | 49.3 | 66 | 73.4 | 91 | 81.5 |
| 23 | 59.1 | 46 | 50.3 | 68 | 66.7 | 92 | 80.1 |

Table 4 The percentage of roots infected in the soil bioassay, taken after harvest in 2007, from experiment 2 at Rothamsted. All plots shown and those highlighted were selected for GGT DNA determination.

| Rothamsted 2 Experiment 2 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Plot | % roots infected | Plot | % roots infected | Plot | % roots infected | Plot | % roots infected |
| 2 | 52.6 | 10 | 69.3 | 18 | 89.0 | 26 | 84.5 |
| 3 | 76.7 | 11 | 52.9 | 21 | 82.9 | 27 | 58.3 |
| 5 | 45.9 | 13 | 73.4 | 22 | 51.0 | 28 | 86.8 |
| 7 | 47.0 | 14 | 56.0 | 23 | 91.4 | 31 | 48.2 |
| 8 | 78.9 | 16 | 47.3 | 24 | 88.5 | 32 | 54.6 |

Table 5 Summary of take-all DNA and percent roots infected in the bioassay from experiments 1, 2 and 3

| Experiment 1 | | | Experiment 2 | | | Experiment 3 | |
|---|---|---|---|---|---|---|---|
| Plot No | % roots infected | GGT DNA Pg/g soil | Plot No | % roots infected | GGT DNA Pg/g soil | % roots Infected | GGT DNA Pg/g soil |
| 2 | 63.3 | 402 | 2 | 52.6 | 515 | 62.9 | 204 |
| 3 | 67.4 | 148 | 5 | 45.9 | 157 | 72.9 | 152 |
| 10 | 45.0 | 212 | 7 | 47.0 | 123 | 45.7 | 57 |
| 15 | 43.8 | 132 | 10 | 69.3 | 253 | 50.7 | 42 |
| 22 | 94.8 | 1358 | 13 | 73.4 | 278 | 37.0 | 30 |
| 33 | 67.6 | 438 | 18 | 89.0 | 678 | 53.4 | 40 |
| 34 | 76.6 | 510 | 21 | 82.9 | 769 | 33.0 | 29 |
| 38 | 86.0 | 728 | 23 | 91.4 | 378 | 32.0 | 23 |
| 40 | 88.6 | 516 | 27 | 58.3 | 554 | 47.3 | 50 |
| 43 | 49.3 | 405 | 28 | 86.8 | 959 | 43.5 | 88 |
| 46 | 50.3 | 144 | 31 | 48.2 | 762 | | |
| 52 | 46.7 | 183 | 32 | 54.6 | 337 | | |
| 56 | 77.8 | 553 | | | | | |
| 60 | 69.4 | 541 | | | | | |
| 63 | 77.0 | 456 | | | | | |
| 66 | 73.4 | 435 | | | | | |
| 75 | 44.0 | 222 | | | | | |
| 79 | 78.1 | 343 | | | | | |
| | | | | | | | |
| Mean | 64.1 | 429 | | 66.6 | 480 | 47.8 | 72 |