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# Developing a rationale to integrate take-all control measures, reduce disease impact and maximise wheat margins

By

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**Contents**

**Abstract**

- This project undertook an epidemiological assessment of existing take-all data provided by ADAS (UK) and INRA (France) for consecutive epidemics of take-all in a sequence of winter wheat crops. From the analysis of these data we outlined an epidemiological model for take-all decline.

- Analysis of the ADAS data provided insight into changes in the magnitude of infection and disease suppression of take-all during a sequence of wheat crops. First wheat crops were characterised by low levels of primary (infectious crop debris to root) and secondary (infectious root to healthy root) infection, and there was no detectable disease suppression. This meant that at the time of crop harvest, disease was still progressing rapidly, but from a low starting point. For second, third and forth wheat crops, a significant increase in primary infection, secondary infection and disease suppression was detected. This was followed in subsequent crops by a reduction to intermediate levels of secondary infection and disease suppression in fifth and seventh crops. Silthiofam seed treatment marginally, but not significantly, delayed the onset of take-all decline.

- Spatial analysis of the INRA data detected average disease patches measuring 0.5 – 1.0 m in diameter. These patches can aggregate into the larger patches of disease which growers are familiar with. Patches did not increase in size during the course of the epidemic but a significant increase in the severity of disease was detected, which makes patches more noticeable visually. We conclude that the spatial structure of an epidemic is predetermined at the root level by primary infection, reflecting changes in inoculum that are affected by cultivation and management practice during the inter-cropping period.

- These analyses were used to compile a theory for the spatio-temporal dynamics of take-all decline from which the outline of an epidemiological model was developed. The model includes terms for host dynamics (root production), pathogen dynamics (primary infection, secondary infection, inoculum production and inoculum decay) and antagonist dynamics during the within-crop and inter-crop periods accounting for epidemics over a sequence of successive wheat crops. Initial simulations show clearly that a rise and fall in disease severity, characteristic of take-all decline, can only be obtained when the antagonist population reduces the rate of disease transmission. This new analysis can be used to explore combinations of potential control measures to identify those which are sufficiently promising to justify testing, and help define experimental designs, important variates and sampling procedures to improve efficiency of research.

**Summary**

***Introduction***

There is no single control method which is consistently highly effective in preventing losses caused by take-all. As a result, the economics of non-first wheat crops are marginal at current wheat prices. A long-term objective of take-all research is therefore, to identify which control methods, or combinations of methods, are likely to be most practical, effective and economically beneficial.

The biology of take-all means that there are a large number of factors (sowing date, soil type, soil moisture, previous cropping, etc.) which affect disease development and yield loss, and hence the appropriate course of treatment. The situation is further complicated because take-all epidemics build and decline across seasons. This has made progress with take-all research slow. Choosing the best treatment combinations to test has been based largely on subjective judgement. Also, treatments can only be tested across a small range of the possible field conditions, making it difficult to apply the results of the research to the wide range of conditions experienced in practice.

Recent advances that combine data from controlled environment and field experimentation with mathematical modelling (1) are now providing a much better understanding of the epidemiology of take-all. This approach could now be used to develop a framework to objectively identify beneficial treatment combinations for testing, and to better interpret results from field trials.

The current model incorporates phases of primary infection of seminal roots (from particulate inoculum surviving in soil from a previously infected crop), and secondary infection as disease spreads from root to root (Fig. 1). It is important to understand the relative contribution of primary and secondary infection in take-all epidemics, and to know which of these processes is being controlled by particular treatments. Treatment combinations which are likely to be complementary might then be selected for testing in an objective way and the results of trials interpreted for a wider range of conditions.

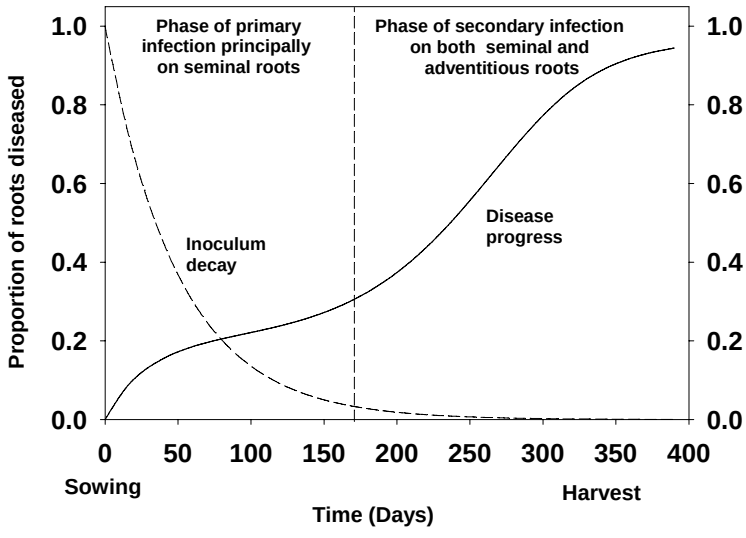


**Fig. 1. Schematic summary of principal phases of primary and secondary infection in epidemics of take-all disease in the field. The decay of particulate inoculum for primary infection derived from the previous susceptible crop is shown (dashed line).**

High yields depend on the production of a healthy root system. Nutrient and water requirements are supplied in the first instance, by the seminal roots for crop establishment and then progressively, by the crown roots for further crop growth and grain filling. Because the particulate inoculum decays quickly in the absence of host roots (due to the poor saprotrophic ability of the pathogen) primary infection is largely restricted to the seminal roots. In contrast, secondary infection is stimulated and dominated by the production of crown roots. Optimisation of the use and integration of cultural, chemical and biological forms of control with partial variety tolerance or resistance and take-all decline depends on understanding which epidemiological processes are affected.

Take-all decline is a well recognised phenomenon yet little is known concerning the fundamental aspects of its epidemiology. Take-all epidemics evolve from the dynamic interactions between pathogen, host and their environment over successive wheat crops. It follows then that the extent of primary infection in one crop depends on the extent of secondary infection in the previous crop and survival of inoculum between crops (3) . Take-all decline is commonly perceived to be the result of a build up in the antagonist population which may modify primary infection, secondary infection or the survival of inoculum but it is not yet known which epidemiological process is affected.

Recent studies involving mathematical modelling of epidemiological data from previous HGCA projects have shown that Latitude seed treatment (silthiofam) affects primary infection of the seminal roots, but has no detectable or direct effect on secondary infection (4). This means that control of disease may be ephemeral and disappear long before the end of the epidemic. Preliminary analysis of data from a recent HGCA funded rotation experiment indicates that take-all decline seems to be linked with differences during the secondary phase of the epidemic (20), but that this can not be confirmed until a more formal analysis has been undertaken, supplemented by additional data from other (INRA) take-all trials.

If the apparent timing of the take-all decline effect can be confirmed it may explain how take-all decline appears to develop even where Latitude (which affects primary infection) is used. But more importantly it may explain how other factors such as sowing date, seed rate, N timing, variety choice and Amistar use can be combined.

In this analysis, we use an epidemiological model to ask (i) How do the processes of primary infection and secondary infection change during the growth of a sequence of consecutive wheat crops and the development of take-all decline? (ii) Are changes in primary and secondary infection affected by previous applications of silthiofam?

The two main features that dictate the invasion of a soil-borne plant pathogen during consecutive epidemics in a series of crops, such as that for take-all, concern (i) the extent of local disease amplification and (ii) the distance over which the pathogen can disperse. Likewise, for integrating measures for control, we might consider complementary treatments that reduce both amplification and dispersal of disease or inoculum. The epidemiological analysis above highlights the

potential for significant amplification of disease via secondary infection yet, whilst other studies suggest that *G. graminis* can spread several centimeters in soil (1), it is not clear if this amplification is associated with significant dispersal of the pathogen. Recent data made available by INRA-Rennes provided the opportunity to analyse the spatial evolution of take-all in second wheat crops and to distinguish between disease amplification and dispersal. In particular the work addresses the following questions: (i) Is an epidemic of take-all described by a characteristic patch size? (ii) Does the characteristic *size* of a patch change during the course of the epidemic? (iii) Does the *intensity* of patchiness change during an epidemic?

***Materials and methods***

Temporal epidemiological data

Data quantifying changes in the number of susceptible and infected roots over time were obtained by assessment of epidemics of take-all in field experimentation performed at ADAS, Rosemaund in Herefordshire (19, 20). The experimental site was on a deep, moisture retentive silty clay loam soil of the Bromyard series representing moderate to high take-all risk. The experiment was initiated in 1997 and involved growth of consecutive wheat crops so that, in 2002, between one and six consecutive wheat crops had been grown either with or without seed treatment using silthiofam. In 2003, to test for residual effects of silthiofam, plots were re-sown with winter wheat but plots previously treated with silthiofam were left untreated providing between one and seven consecutive wheat crops that had been grown either with or without previous seed treatment using silthiofam (20). Oilseed rape was grown in all plot areas prior to continuous wheat cropping. Each treatment was replicated four times, fully randomised within blocks, with a plot size of 3.5m by 24m. In order to minimise soil and inoculum movement between plots over the life of the study the experimental area was ploughed across the direction of the plots and secondary cultivations done in the same direction as the plots. The wheat variety, cv. Equinox was used in all years. All rotational positions were given the same level of inputs which were according to good local practice. Latitude was applied at a rate of 25g silthiofam per 100 kg seed. Autumn plant populations were assessed after full emergence but prior to tillering by counting plant number in five 1m lengths of row per plot. The numbers of diseased and disease-free roots were assessed on five occasions in 2001-2002 and six occasions in 2002-2003 on a replicated sub-set of plots chosen to represent each rotational position present in that year. Each take-all assessment was done on a sample of 20-25 plants/plot taken from one 12m length of the plot. Grain yield was assessed by taking a 2.25 x 10m combine swath from the end of the plot which had not been previously destructively sampled. Grain yields were corrected for moisture and are expressed at 85% dm. Grain moisture and specific weight were assessed using a Dickey-John GAC moisture meter. Thousand grain weight was assessed by counting accurately using a numigral seed counter and weighing approximately 500 seeds per plot.

Spatial epidemiological data

Naturally occurring epidemics of take-all were monitored over two years, 2003 and 2004 in INRA experimental fields near Rennes (48°01'N, 1°43'W) in western France. Second wheat crops were sown with cultivar "Cap Horn" at a density of 300 seeds.$m^{-2}$. Seeds were treated with silthiofam 25g a.i..100$kg^{-1}$ seed (Latitude®). Nitrogen fertilizer was applied twice, as ammonium solution (34% N) at a total rate of 120kg $ha^{-1}$. Weeds, foliar and stem diseases and aphid populations were maintained below economic threshold levels, as recommended in ‘‘les Avertissements Agricoles du Service de la Protection des Végétaux’’ (Anon. (2003)).

To generate a range of disease levels, three different management practices were introduced during the fallow period between harvest of the first wheat crop and the sowing of the second wheat crop: host (wheat) or non-host (mustard) cover crops were sown or the soil was kept bare; these treatments are designated W (wheat), M (mustard) and BS (bare soil). After a stubble break and a crosskill roller, the cover crops were direct-seeded. The cover crops were sown after harvesting of the first wheat 77 and 46 days before sowing the second wheat crop in 2003 and 2004 respectively.

In order to generate different disease levels and associated differences in disease aggregation, two cultivation methods were used when sowing the second wheat: ploughing and power harrowing followed by drilling (thereafter referred to as P or ploughed plots) and conservation tillage accompanied by a broadcast sowing with a direct drill (referred to as CT or tilled plots).

The experimental design consisted of six plots (two soil cultivation methods x three cover crops) arranged as a split-plot with cultivation method as the main treatment; a 5m x 5m observation grid was delimited in each plot and divided into one hundred 50cm x 50cm quadrats. Three assessments were performed each year at mid stem elongation, booting and grain milk stage. For each assessment, three plants were randomly chosen in each quadrat and removed from the field with particular care not to trample the surrounding plants. The roots were washed, the numbers of diseased and healthy roots were counted on each plant and the percentage of the root system showing black stellar discolouration was evaluated for each plant on a 10 by 10 scale. This allowed computing three variables: plant incidence (ratio of the number of diseased plants on the number of observed plants), root incidence (ratio of the number of diseased roots on the total number of roots) and severity (proportion of diseased root system). These variables were evaluated separately on the seminal and crown root systems (which can be combined to obtain results on the whole root system).

***Results and Discussion***

The aim of this project was to undertake an epidemiological assessment of existing disease data provided from previous HGCA-funded field experiments (UK) and supported by data from INRA (France) for consecutive epidemics of take-all in a sequence of winter wheat crops. From the analysis of this data we have outlined an epidemiological model for take-all decline. The report of this work forms three sections (i) An epidemiological analysis of take-all decline (ii) An analysis of the spatial

structure and patch dynamics of take-all in a second wheat crop and (iii) Synthesis of the above into a mathematical framework for take-all decline.

Epidemiological analysis of the UK data provided useful insight into changes in the magnitude of primary infection, secondary infection and disease suppression of take-all during a sequence of wheat crops. First wheat crops were characterised by low levels of primary and secondary infection and, with no detectable disease suppression. This meant that at the time of crop harvest, disease was still progressing rapidly. For second, third and forth wheat crops, a significant increase in primary infection, secondary infection and disease suppression was detected. This was followed in subsequent crops by a reduction to intermediate levels of secondary infection and disease suppression in fifth and seventh crops. Silthiofam marginally, but not significantly, delayed the onset of take-all decline.

Spatial analysis of the INRA data extended our knowledge concerning the patch dynamics of take-all in second wheat crops. Hierarchical spatial analysis detected average disease patches measuring 0.5 – 1.0m in diameter. Importantly, patches did not increase in size during the course of the epidemic but a significant increase in the severity of disease was detected. We conclude that the spatial structure of an epidemic is predetermined at the root level by primary infection, reflecting changes in inoculum dynamics that occur as a result of cultivation and management practice during the inter-cropping period. This structure is reinforced during the course of the epidemic by secondary, root to root infection.

These analyses were used to compile a theory for the spatio-temporal dynamics of take-all decline from which the outline of an epidemiological model was developed. The model includes terms for host dynamics (root production), pathogen dynamics (primary infection, secondary infection, inoculum production and inoculum decay) and antagonist dynamics during the within-crop and inter-crop periods accounting for epidemics over a sequence of successive wheat crops. Initial simulations show clearly that a rise and fall in disease severity, characteristic of take-all decline, can only be obtained when the antagonist population produces a negative effect on the rate of disease transmission.

The modelling work carried out provides a framework to understand how the agronomic and management factors which are known to impact on final take-all severity impact on the epidemic and the development of take-all decline across the rotation. With this understanding it will possible to understand how the agronomic and management inputs are likely to interact and how they should be combined both within a single cropping year and across a run of continuous cereals to minimise the impact of take-all. The range of management factors that could be included in this are wide ranging including cultivation method and date, planting date, variety/cereal species choice, seed treatments, foliar fungicides, seed rate and nutritional practice.

**Technical detail**

***Epidemiological analysis of take-all decline***

*Modelling*

For each plant, change in the number of susceptible, $S$, and diseased, $I$, roots over time, $t$, (where $t$ = degree-days > $4^0$C) was described by a compartmental model.

$$\begin{aligned}
&\text{Susceptible roots} && \frac{\mathrm{d}S}{\mathrm{d}t} = bS\frac{(\kappa - N)}{\kappa} - (r_p X + r_s I)S \ , \\
&\text{Diseased roots} && \frac{\mathrm{d}I}{\mathrm{d}t} = (r_p X + r_s I)S \ , \\
&\text{Particulate inoculum} && \frac{\mathrm{d}X}{\mathrm{d}t} = -r_d X \ . \\
&\text{Secondary infection} && \frac{\mathrm{d}r_s}{\mathrm{d}t} = -r_{ds} r_s
\end{aligned} \qquad (1)$$

We assume that the production of total roots, $N$, increases logistically in number over time with a rate $b$ to a maximum, $\kappa$, where $N = S + I$. Disease is initiated by primary infection with a rate, $r_p$, from particulate inoculum, $X$, that decays exponentially with time at a rate, $r_d$. Parameters for root production, $N$ and $b$, were estimated first and independently for each epidemic. No differences were found between wheat crops and these parameters were fixed for further parameter estimation. The precise time at which epidemics began is not well defined by this data. However previous experimentation (4) suggests that field epidemics are initiated approximately twenty days after sowing. Disease spreads by secondary infection from diseased to susceptible roots with a rate $r_s$. Preliminary inspection of the data suggest that (i) first sampling occurred midway into the primary phase of the epidemic (ii) most epidemics ceased at levels well below 100% of diseased roots suggesting some suppression of the rate of disease progress during the course of the epidemic. Consequently, model (1) was fitted assuming all epidemics began at 200 degree days and with a fixed decay, $r_d$, in inoculum, $X$, that led to less than five percent of particulate inoculum remaining at 800 degree days. Sub-maximal final disease levels were made possible by introducing a parameter, $r_{ds}$, that describes a gradual decay in the rate of secondary infection over time. Hence, three parameters, were estimated on final fitting, $r_p$, $r_s$ and $r_{ds}$.

*Results*

Model (1) provided a good description of disease data for all epidemics (Fig. 2). However, for certain epidemics, the rate of primary infection could not be determined from the data and was fixed at the value of the previous crop (Table. 1).

| Treatment | Wheat crop | $r_p$ Average | $r_p$ 95% Lower | $r_p$ 95% Upper | $r_s$ Average | $r_s$ 95% Lower | $r_s$ 95% Upper | $r_{sd}$ Average | $r_{sd}$ 95% Lower | $r_{sd}$ 95% Upper |
|---|---|---|---|---|---|---|---|---|---|---|
| - silthiofam | WW1 | 0.00023 | 0.000025 | 0.0019 | 0.00017 | 0.00003 | 0.00110 | 0.0006 | 0.00011 | 0.0027 |
| | WW2 | 0.00048 | 0.000072 | 0.0033 | 0.00065 | 0.00022 | 0.00190 | 0.0015 | 0.00097 | 0.0023 |
| | WW3 | 0.00048* | - | - | 0.00055 | 0.00035 | 0.00087 | 0.0015 | 0.00110 | 0.0020 |
| | WW4 | 0.00048* | - | - | 0.00065 | 0.00044 | 0.00095 | 0.0016 | 0.00130 | 0.0020 |
| | WW5 | 0.00038 | 0.000073 | 0.0019 | 0.00042 | 0.00014 | 0.00126 | 0.0012 | 0.00073 | 0.0020 |
| | WW7 | 0.00052 | 0.000177 | 0.0015 | 0.00030 | 0.00012 | 0.00071 | 0.0010 | 0.00063 | 0.0016 |
| + silthiofam | WW1 | 0.00031 | 0.000058 | 0.0020 | 0.00019 | 0.00003 | 0.00104 | 0.0007 | 0.00021 | 0.0024 |
| | WW2 | 0.00048* | - | - | 0.00070 | 0.00051 | 0.00094 | 0.0015 | 0.00127 | 0.0018 |
| | WW3 | 0.00048* | - | - | 0.00067 | 0.00041 | 0.00107 | 0.0015 | 0.00111 | 0.0020 |
| | WW4 | 0.00048* | - | - | 0.00063 | 0.00043 | 0.00093 | 0.0016 | 0.00126 | 0.0020 |
| | WW5 | 0.00047 | 0.000132 | 0.00167 | 0.00038 | 0.00015 | 0.00095 | 0.0011 | 0.00075 | 0.0018 |
| | WW7 | 0.00052 | 0.000162 | 0.00169 | 0.00030 | 0.00012 | 0.00073 | 0.0009 | 0.00058 | 0.0016 |

* Parameter not estimated

**Table 1. Parameter estimates for the rates of primary infection, $r_p$, secondary infection, $r_s$, and decay in the rate of secondary infection $r_{ds}$ together with lower and upper confidence limits obtained from fitting model (1) to disease data for take-all epidemics at different stages of rotation in a sequence of consecutive wheat crops.**

For wheat crops grown with no previous silthiofam treatment, change in the number of infected roots increased sigmoidally over time from initial levels of between 0.18 and 0.78 diseased roots after 484 degree days to between 9.7 and 16.0 diseased roots after 2975 degree days (Fig. 2a). During the same period the numbers of susceptible, healthy roots increased from between 5.93 and 6.62 roots to a maximum of between 16.2 and 29.9 after 1953 degree days and then remained relatively static or declined to between 17.04 and 21.40 after 2975 degree days (Fig. 2c).

For wheat crops grown following silthiofam treatment to previous wheat crops, change in the number of infected roots increased sigmoidally over time from initial levels of between 0.4 and 1.5 diseased roots after 484 degree days to between 8.9 and 15.6 diseased roots after 2975 degree days (Fig. 2b). During the same period the numbers of susceptible, healthy roots increased from between 5.0 and 7.4 roots to a maximum of between 18.1 and 30.9 after 1953 degree days and then declined to between 14.3 and 23.8 after 2975 degree days (Fig. 2d).

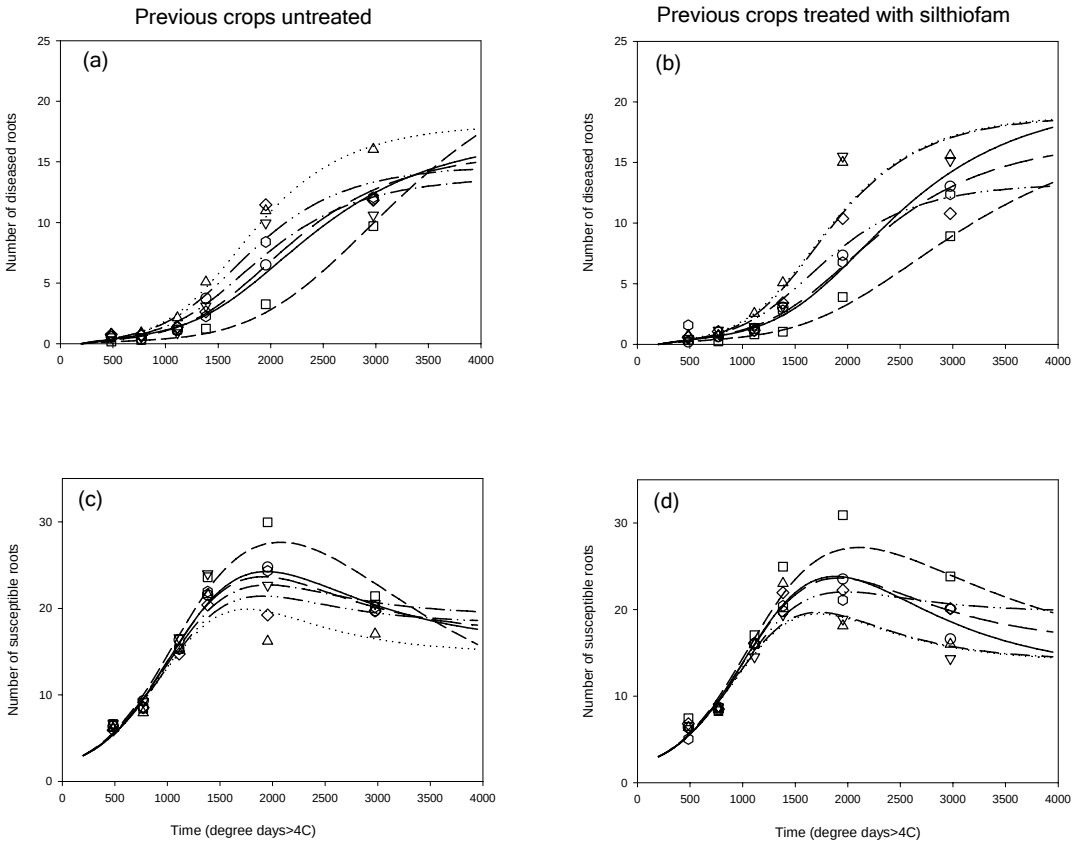


**Fig. 2. Change in the number of diseased (a, b) and susceptible (c, d) roots over time for wheat crops treated (a, c) or untreated (b, d) with silthiofam.**

Model 1 was fitted to disease data to describe the trends in disease progress for the different wheat crops for which the severity of disease was recorded as the proportion of roots diseased. All epidemics displayed the characteristic monotonic-sigmoidal shaped disease progress curves consistent with consecutive phases of primary and secondary infection respectively. In the absence of previous silthiofam treatments, the proportion of disease roots increased from between 0.026 and 0.114 after 484 degree days to between 0.312 and 0.485 diseased roots after 2975 degree days (Fig. 3a). When previous wheat crops had been treated with silthiofam, the proportion of disease roots increased from between 0.027 and 0.106 after 484 degree days to between 0.273 and 0.521 diseased roots after 2975 degree days (Fig. 3b).

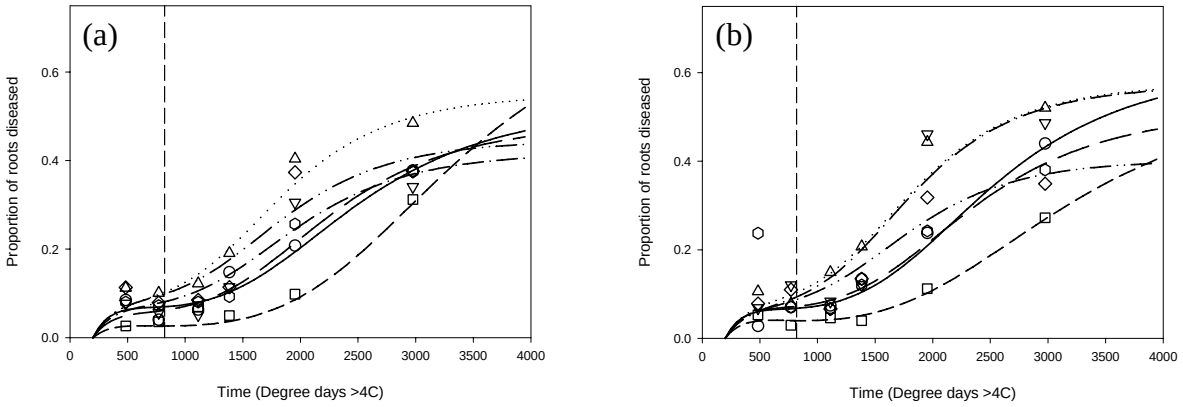


**Fig. 3. Change in the proportion of diseased roots over time for wheat crops treated (a) or untreated (b) with silthiofam.**

For crops with no history of previous silthiofam treatment, first wheat crops were characterised by low levels of disease severity throughout both the primary and secondary phases of the epidemic (Fig. 3a). In contrast, second wheat crops were characterised by high severity of disease during both primary and secondary phases of the epidemic. Higher-order wheat crops showed intermediate levels of disease severity during the primary and secondary phases of each epidemic. A notable feature of the first wheat crop suggested from the fitting of model (1) was the continuing trend in disease progression despite lower levels of disease at the time of the final assessment (just prior to harvest). In contrast, epidemics for all other crops were well into the asymptotic portion of the curve and displayed a distinct levelling-off. Also notable was the ranking of disease severity (suggested by model fits) at approximately 1400 degree days and corresponding to mid-stem elongation when disease is most highly correlated with crop loss, were in the order 1$^{st}$, 7$^{th}$, 5$^{th}$, 3$^{rd}$, 4$^{th}$, 2$^{nd}$ wheat (Fig. 3a).

Similar results were obtained for crops following crops treated with silthiofam. First wheat crops were again characterised by low levels of disease severity throughout both the primary and secondary phases of the epidemic (Fig. 3b) and second wheat crops by high severity of disease during both phases. Higher-order wheat crops showed intermediate levels of disease severity during the primary and secondary phases of each epidemic. The distinction between a 1$^{st}$ wheat crop and the remaining crops with respect to its asymptotic behaviour was again evident but less clear. In particular, disease severity in the 3$^{rd}$ wheat crop was equally bad during the secondary phase of the epidemic as disease severity in the second wheat crop. According to the fit of model (1), disease levels at around 1400 degree days were in almost exactly the same order as those crops with no history of silthiofam treatment and were ranked 1$^{st}$, 7$^{th}$, 5$^{th}$, 4$^{th}$, 3$^{rd}$, and 2$^{nd}$ wheat from low to high disease severity respectively.

Parameters representing the rate of primary infection, $r_p$, secondary infection, $r_s$ and disease suppression, $r_{ds}$ were estimated by fitting model (1) to disease data. The rate of primary infection increased monotonically over time, increasing from first to second crops then remaining static. (Fig.4.).

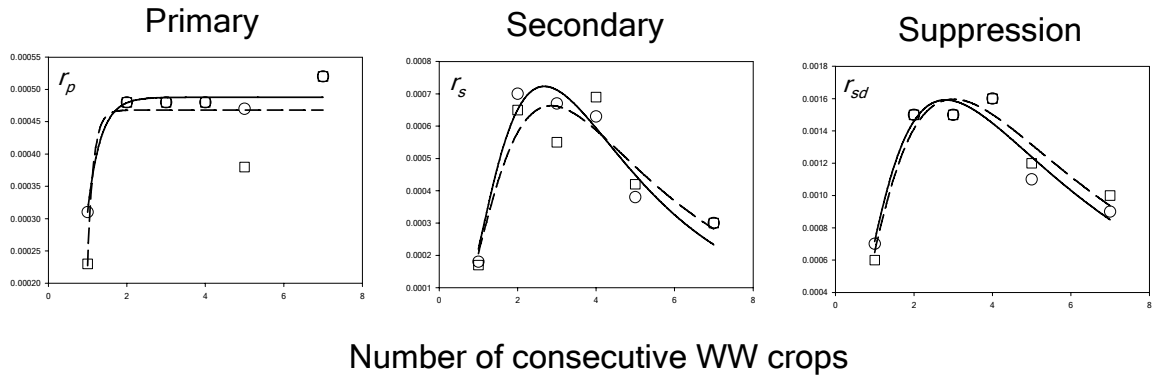


**Fig. 4. Change in parameter estimates for the rate of primary infection, $r_p$, secondary infection, $r_s$, and suppression of secondary infection, $r_{sd}$, with numbers of consecutive wheat crops with (circles, solid line) or without (squares, broken line) a previous history of silthiofam treatment.**

Parameters describing the initial rate of secondary infection and suppression in the rate of secondary infection increased to a maximum in 3rd or 4th wheat crops and then decayed again. For epidemics for wheat crops grown following a history of silthiofam use in previous crops, the estimated rates of primary infection followed a similar trend but were marginally lower than for crops with no history of treatment and for 1st to 4th wheats, increased rates of secondary infection (Fig. 4). Change in levels of suppression of secondary infection was not affected by prior use of silthiofam.

*Discussion*

The objective of this work was to provide an epidemiological analysis of existing disease data that described the disease dynamics of take-all in consecutive crops of winter wheat and the long-term effects caused by previous treatments with silthiofam. Although not collected specifically for use in model fitting, this data was of adequate temporal resolution to allow significant progress in the interpretation and understanding of take-all decline and followed the broad patterns of disease characteristic of take-all decline (12) .

Disease progress could not be described using a model with terms for primary infection, secondary infection, root growth and inoculum decay alone (1). With these terms only, the model describes disease progression that is asymptotic towards 100%. With the exception of the epidemics in first wheat crops, visual observation of disease data suggests a levelling-off in the rate of disease

progress well below this value (Fig. 3). To account for this shape, we introduced a term for the dynamical suppression in the rate of secondary infection, $r_{sd}$, that provided a significantly improved fit to the data. This progressive reduction in the rate of secondary infection may conceivably be due to changes in the susceptibility of the host (14) or to changes in the soil environment (e.g. drying of the upper soil horizons, in which the pathogen is normally most active), but the low estimated values for this parameter in first wheat crops suggests that the mechanism responsible develops during the cropping sequence. The most likely candidate for the decay in the rate of secondary infection is a build-up of antagonists, already well cited for its part in take-all decline. (5-8, 11, 13, 16-18, 21). Whilst, for the benefit of model simplification, we attributed the mechanism for disease suppression to the rate of secondary infection, we cannot be certain (without further experimentation) that the suppressive effects of antagonists do not act during the primary infection phase, the effect of which would be subsumed within the rates of primary infection, $r_p$ and inoculum decay $r_d$.

Fitting model 1 to disease data detected clear trends in parameters associated with primary infection, secondary infection and disease suppression for epidemics over the course of seven consecutive wheat crops (Fig. 4). Low rates of primary infection in first wheat crops are consistent with low levels of particulate inoculum whilst low initial rates of secondary infection may reflect a delay in the onset of root to root spread, a consequence of reduced and delayed primary infection. That little evidence of disease suppression could be detected in 1$^{st}$ wheat crops is consistent with the view that take-all decline is a consequence of the build up of antagonists over a sequence of wheat crops. In particular, the continuing upward trend in disease progression at the time of harvest of a 1$^{st}$ wheat crop (and suggested by extrapolation of fitted curves) may account for higher than expected primary infection in 2$^{nd}$ wheat crops. This accounts, in turn, for the early onset, and high initial rates of secondary infection detected in 2$^{nd}$ wheat crops. That we detected a significant increase in the rate at which secondary infection is suppressed in 2$^{nd}$ wheat crops suggests that, at this stage in the cropping sequence, the antagonist population has achieved the levels of multiplication and dissemination that allow it to react effectively to the initiation of take-all lesions. It would be interesting to know, at this point, if the overall suppression in the rate of secondary infection is due to a reduction in probability of lesion initiation (reduction in root susceptibility) of a disease lesion or to its extensification after initiation (reduction in infectivity). The behaviour of the antagonist population as either generalists, able to grow on roots exudates and to reduce the susceptibility of a disease-free root, or specialists, requiring interaction with the pathogen and able only to reduce the infectivity of an already infected root, has important implications for the persistence and management of the antagonist population in long-term wheat cropping. For subsequent wheat crops, no further change in the rate of primary infection was detected. In this analysis, the rate of primary infection reflects both the quantity and infectivity of particulate inoculum. These may well be affected by the behaviour of the antagonist population between wheat crops which means that the high levels of inoculum produced during the growth of a 2$^{nd}$, 3$^{rd}$ or 4$^{th}$ wheat crop may be subject to colonisation by antagonists and hence less

capable of infecting the next wheat crop. Earlier work suggests that take-all decline does not result from loss of virulence in the population of *G. graminis* (6). Finally, a reduction in disease elicits a concomitant reduction in suppression leading towards equilibrium between disease and its suppression by the antagonist population.

The treatment of previous wheat crops with silthiofam had only a marginal effect on these processes and did not prevent take-all decline. We detected small increases in the rate of primary infection over all wheat crops and small displacements in curves describing initial rates of secondary infection and disease suppression. A plausible explanation is that the control of disease afforded by silthiofam is at the expense of an associated build up in levels of disease suppression imposed by the antagonist population. Removal of the silthiofam treatment may provide an opportunity for take-all to spread, in the first instance, in the presence of a reduced antagonist population. The antagonist population would then be expected to compensate for increased disease in due course. On balance, however, we conclude that silthiofam does not to have any major effect on the development of take-all decline.

***Spatial characterization of a take-all epidemic***

*Spatial analysis*.

A simple index of dispersion, D, was calculated as the ratio of observed to predicted (binomial) variance for disease incidence amongst quadrats of different sizes where values of $D > 1$ represent an aggregated distribution, $D = 1$, a random distribution and $D < 1$, a dispersed distribution.

*Results*

*Diseased plants*. Disease at the plant scale was generally well dispersed in both years, particularly for plots that had been ploughed (Fig. 5) leading to a low index of dispersion. No significant changes in either the characteristic patch size or the degree of patchiness were detected during the course of the epidemic and the effects of inter-crop management were inconsistent.

*Diseased roots.* Disease amongst roots was clearly aggregated (Fig. 6) with a characteristic patch size of between two and four quadrats (0.5 – 1.0 m diameter). The characteristic patch size did not change during the course of the epidemic but we detected a significant increase in the intensity of patches for all treatments with a trend towards more aggregation for tilled plots.

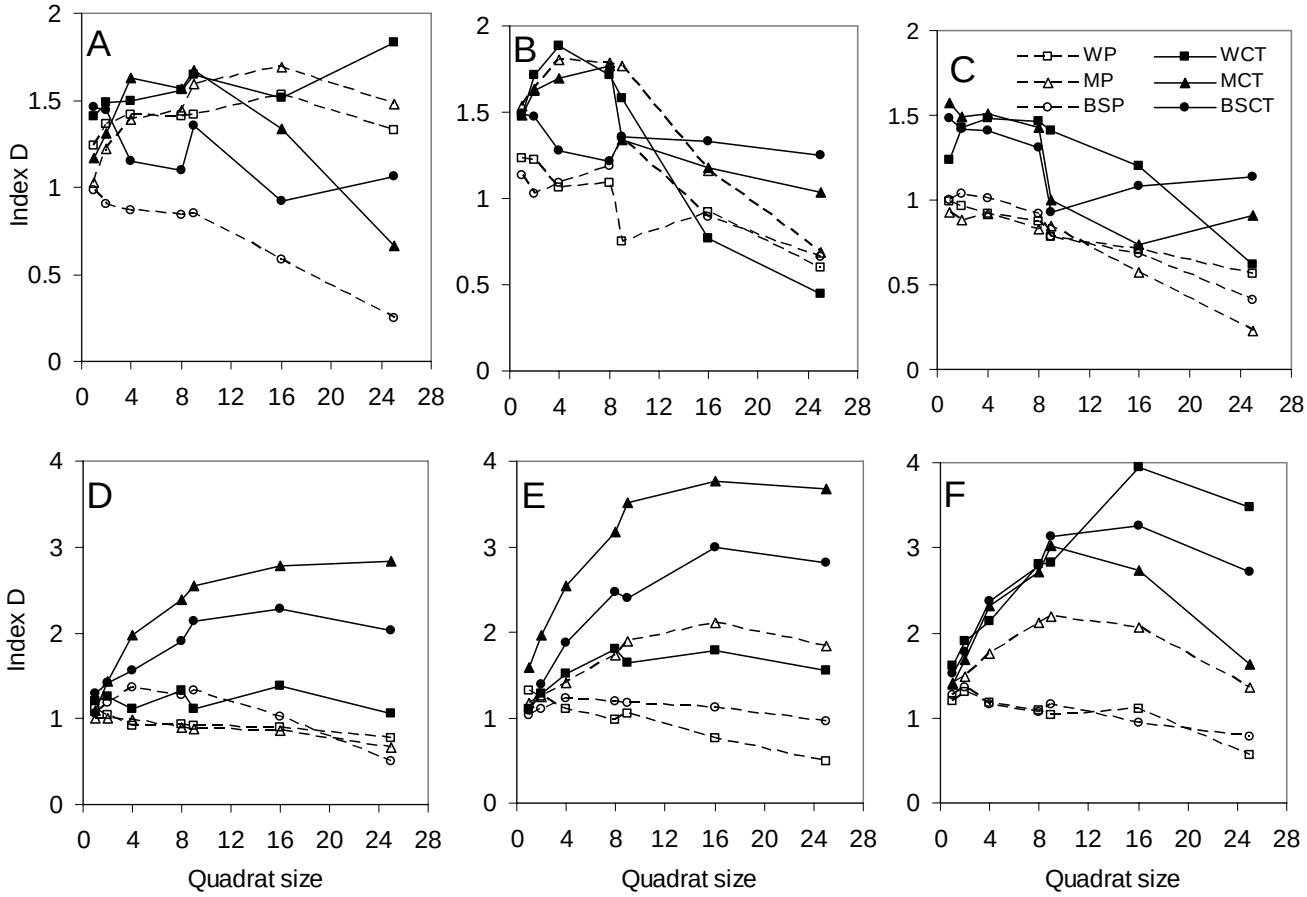


**Fig. 5. Relationship between D index of aggregation and quadrat size (expressed as number of 50 x 50 cm quadrats) for natural wheat take-all epidemics – plant disease incidence observed on crown roots. A, B and C, 2003 epidemic, D, E and F, 2004 epidemic. A and D, at stem elongation, B and E at booting, C and F, at grain milk growth stage. WP: wheat cover crop and ploughing, WCT: wheat cover crop and conservation tillage, MP: mustard cover crop and ploughing, MCT: mustard cover crop and conservation tillage, BSP: bare soil cover crop and ploughing, BSCT: bare soil cover crop and conservation tillage.**

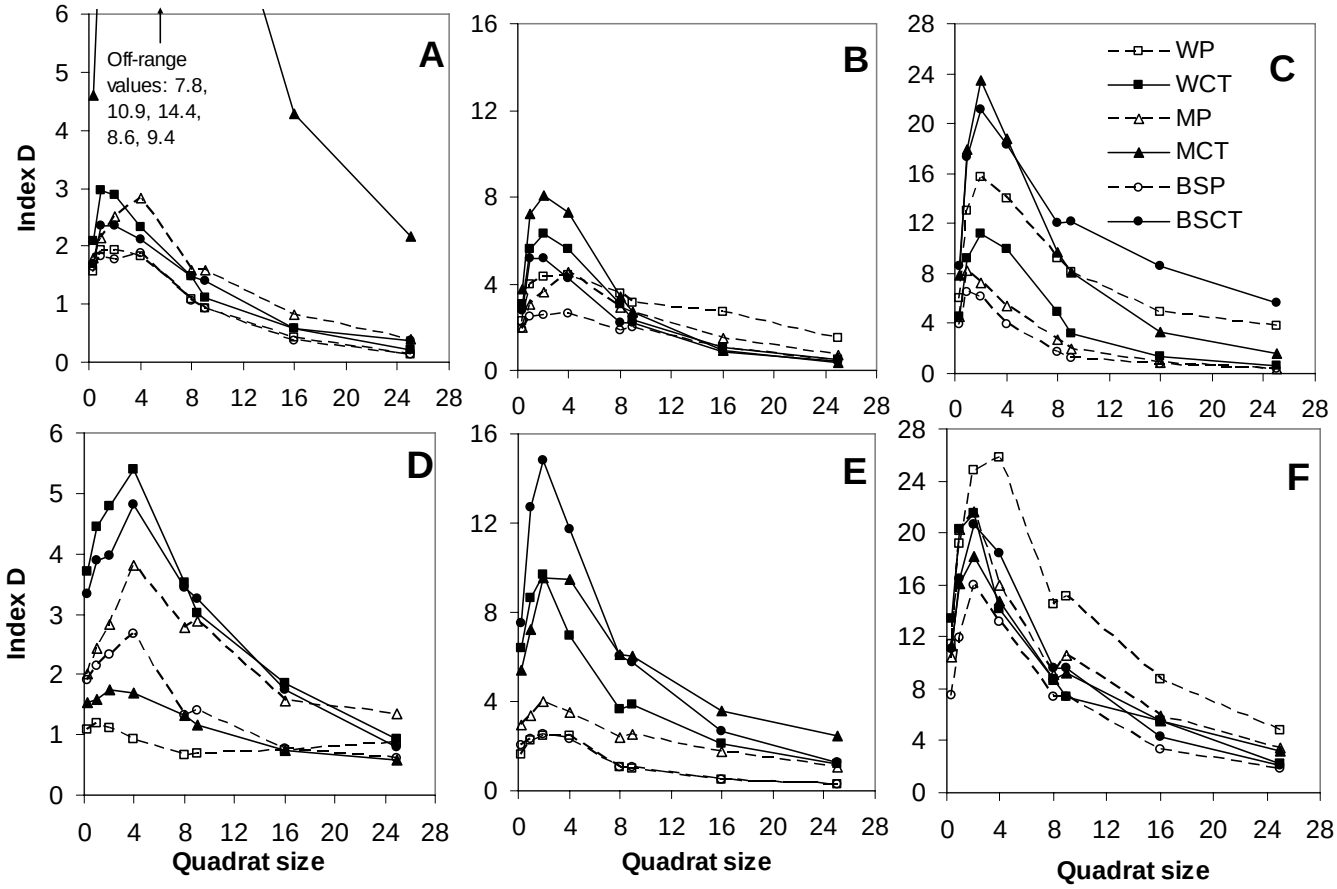


**Fig. 6: Relationship between index of aggregation, D, and quadrat size (expressed as number of 50x50 cm quadrats) for natural wheat take-all epidemics – crown root disease incidence. A, B and C, 2003 epidemic, D, E and F, 2004 epidemic. A and D, at stem elongation, B and E, at booting, C and F, at grain milk growth stage. WP: wheat ploughing, WCT: wheat conservation tillage, MP: mustard ploughing, MCT: mustard conservation tillage, BSP: bare soil ploughing, BSCT: bare soil conservation tillage.**

*Discussion*

The experimentation here has demonstrated a clear absence of focal expansion during the secondary infection phase of a take-all epidemic. The small distances in growth that have been quantified in placement experiments elsewhere (1,9,10), were not of a detectable magnitude in our field experimentation. In contrast, we detected high levels of disease amplification during the secondary phase of the epidemic as disease spreads from root to root. These results are consistent with a spatial structure and average patch size that is determined in the first instance, by inter-crop management and the survival of inoculum. The effects of these factors on the distribution of initial inoculum are expressed during the primary infection phase. This is followed by the amplification of disease as the pathogen spreads locally from root to root by secondary infection.

That the inter-crop period and associated cultivations are largely responsible for dispersal of inoculum and spatial epidemic structure leads us to conjecture a theoretical epidemiological model for the development of disease in consecutive wheat crops and for take-all decline (Fig. 7).

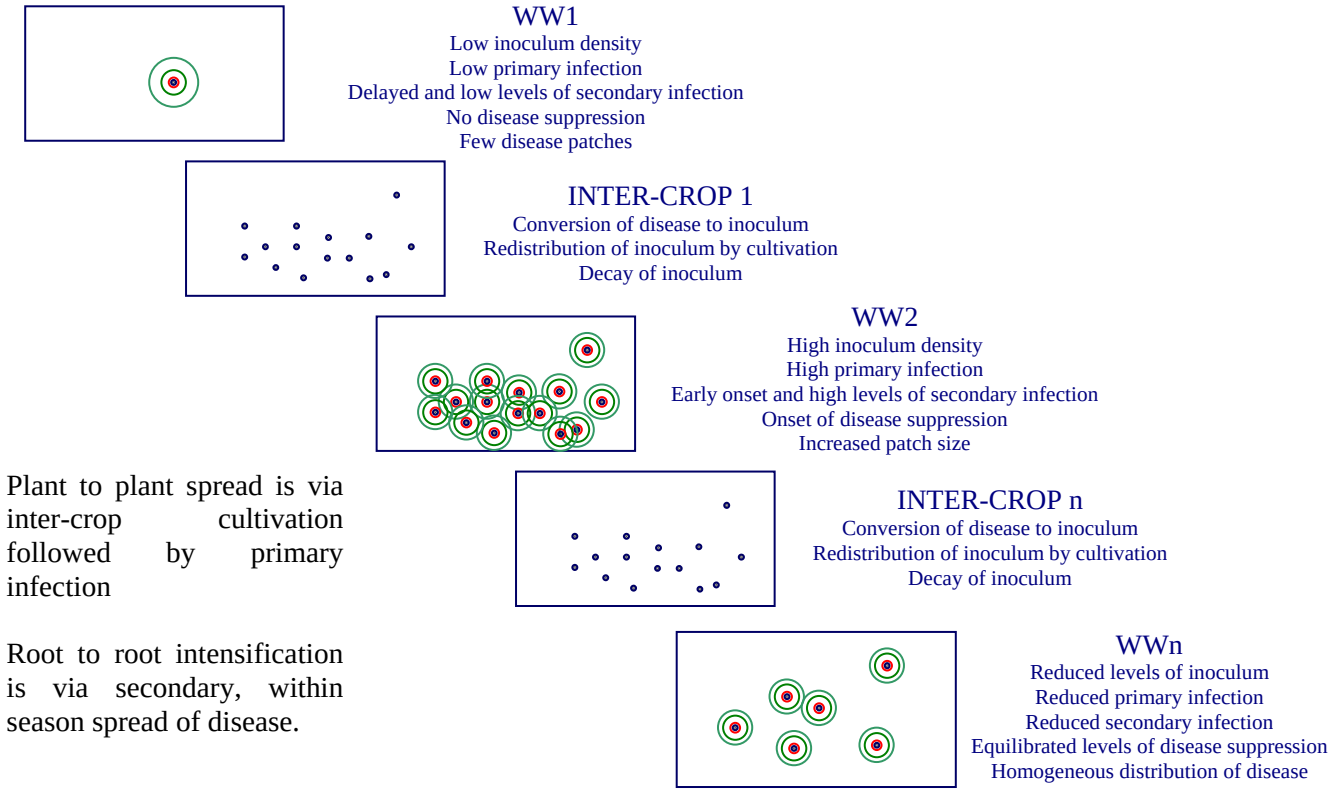


**Fig. 7: Schematic representation for the epidemiological theory of take-all decline (disease and inoculum dynamics) during a sequence of winter wheat crops.**

WW1: Particulate soil inoculum is at very low levels, probably randomly dispersed and leading to low levels of primary infection and low levels of secondary infection. Importantly, there is little influence of disease suppression from specific antagonists and the trend in disease dynamics is steeply upwards. This allows for the continued production of infested root material that can be converted to inoculum by saprotrophic colonization during the inter-crop period.

INTER-CROP 1: Localised patches of inoculum (infested roots) are spread by cultivation to create an average patch size of between 0.5 and 1.0 m in diameter. The average patch size and density of inoculum may depend on the type of cultivation and inter-crop management that affects colonization and survival in root debris.

WW2: Higher levels of inoculum lead to increased rates of primary infection for which the spatial structure reflects the distribution of initial inoculum. Higher rates of primary infection lead to a higher initial rate of secondary infection. However, this is accompanied by a rapid build-up of antagonists and a notable suppression of disease.

INTER-CROP 2: Patches of inoculum (infested roots) are spread by cultivation to create an average patch size larger than 0.5 and 1.0 m in diameter. The average patch size and density of inoculum may depend on the type of cultivation and inter-crop management that affects colonization and survival in root debris.

WW3+: Higher levels of inoculum lead to increased rates of primary infection for which the spatial structure reflects the distribution of initial inoculum. Higher rates of primary infection lead to a higher initial rate of secondary infection. However, high initial levels of antagonists lead to early suppression of disease and the possible reduction in the overall severity of disease.

WW and INTERCROP n: The system enters a period of dynamical equilibrium, higher levels of initial inoculum, primary infection and secondary infection being balanced by suppressive activity of antagonists.

***An epidemiological model for take-all decline***

*Introduction*

To date, no mechanistic modelling framework exists for describing take-all epidemics over a sequence of consecutive wheat crops that links within-crop dynamics and inter-crop dynamics of disease and that takes into account the dynamics of an antagonist population and its dependency on the dynamics of the pathogen. In this section we extend model (1) to account for (i) the dynamics and effects of an antagonist population and (ii) an inter-cropping period to describe disease dynamics over a sequence of wheat crops. In particular, we demonstrate, by simulation, the contribution of the antagonist population to the build-up and suppression of disease during take-all decline.

*Model derivation*

Within-crop model

Model (1) was extended to include a general antagonist population, $A$, to give:

$$\begin{aligned}
&\text{Susceptible roots} && \frac{dS}{dt} = b\left(\kappa - (S+I)\right) - \left(\frac{r_p X}{1+vA} + \frac{r_s I}{1+wA}\right) S \\
&\text{Infected roots} && \frac{\mathrm{d}I}{\mathrm{d}t} = \left(\frac{r_p X}{1+vA} + \frac{r_s I}{1+wA}\right) S - eI - gIA \\
&\text{Inoculum} && \frac{dX}{dt} = hI - mX - nXA \\
&\text{Antagonists} && \frac{dA}{dt} = \left(pS + qI + rX\right) A - sA - uA^2
\end{aligned} \qquad (2)$$

The meanings of the individual terms of the model are considered in two sections, corresponding to parameters associated with either the underlying pathogen-host model and those concerned with the antagonists, $A$.

*Parameters relevant to the underlying pathogen-host model (ie. $A = 0$ in model (2)).* The creation of new susceptible roots is governed by monomolecular dynamics, with growth rate, $b$, and carrying capacity, $\kappa$. Both primary and secondary infection cause susceptible roots to become infected and the rates of both are proportional to the number of susceptible roots available for infection. Primary infection is proportional to the amount of inoculum in the soil and proceeds at rate, $r_p$, whereas secondary infection is proportional to the number of roots already infected and proceeds at rate $r_s$. Infected roots die at a characteristic rate, $e$, which corresponds to a combination of their natural death and decay and the loss of infected tissue implied by the production of new inoculum during the season. This conversion of infected roots into inoculum is modelled as being proportional to the amount of infected tissue and as occurring at rate, $h$. Lastly, inoculum decays at a characteristic rate $m$.

*Parameters associated with the antagonists.* These naturally split into two classes, those concerned with the population dynamics of the antagonist and those related to the effect of the antagonist population upon the epidemic. The antagonist is modelled as bulking up on any (or all) of:

- susceptible roots, at rate $p$;
- infected roots, at rate $q$;
- soil-borne inoculum, at rate $r$.

In all three cases the rate of increase of the antagonist population is jointly proportional to the rate, the amount of relevant tissue ($S$, $I$ or $X$) and the density of antagonists already present. The population is subject to natural death at some characteristic rate, $s$, and a density dependent effect with associated parameter, $u$.

The model allows for two mechanisms by which antagonists may affect the pathogen; by reducing the rates of infection or by reducing the infectious period. The effect of the antagonist population on the rates of primary and secondary infection is modelled by the hyperbolic term controlled by the parameters $v$ and $w$ respectively. The parameter, $g$, corresponds to the removal of infected tissue by antagonists leading to a reduction in the infectious period associated with secondary infection, with $n$ playing an analogous role for particulate inoculum and so leading to a reduction in the infectious period associated with primary infection.

The generic nature of this model allows us to test different hypothesis within a single model framework. In particular, concerning the specific or non-specific bulking-up of antagonists

Inter-crop model

Between crops, the overall dynamical processes are reduced, corresponding to there being no host tissue in the system. The number of both susceptible and infected roots is fixed at 0, with the governing equations for the inoculum and antagonists given by:

$$\begin{aligned} &\text{Inoculum} && \frac{dX}{dt} = -mX - nXA \\ &\text{Antagonists} && \frac{dA}{dt} = rXA - sA - uA^2 \end{aligned} \qquad (3)$$

*Linking the within-crop model (2) and the inter-crop model (3)*

*Transition at the end of the within-crop period.* To link the end of within-crop behaviour to the start of the inter-crop period, the population densities at the end of the growing season are denoted by $(S_{end}, I_{end}, X_{end}, A_{end})$ and the values at the start of the between season period are denoted $(S_{start}, I_{start}, X_{start}, A_{start})$. The mapping is given by $S_{start} = 0$, $I_{start} = 0$, $X_{start} = X_{end} + \frac{I_{end}}{\Theta}$ and $A_{start} = A_{end}$ corresponding to the removal of all susceptibles and removal of infected roots with conversation of some proportion $\Theta$ to new soil borne inoculum during the mixing associated with cultivation. The inoculum already present in the soil is unaffected, and the number of antagonists similarly unchanged.

*Transition at the start of a new within-crop period*. If the population densities at the end of the between season period are denoted $(S_{end}, I_{end}, X_{end}, A_{end})$ and the values at the beginning of the next growing season are $(S_{start}, I_{start}, X_{start}, A_{start})$, and denoting the initial number of susceptible roots by $S_0$, the mapping is given by $S_{start} = S_0$, $I_{start} = 0$, $X_{start} = X_{end}$ and $A_{start} = A_{end}$ corresponding to the sowing of a small initially entirely uninfected root population, leaving the amount of inoculum and antagonist population entirely unchanged.

The multi-season model (models 2 and 3) was used to simulate changes in numbers of susceptible and infected roots (shown below as the proportion of diseased roots) and the density of antagonists over time for a series of seven consecutive wheat crops. The model was parameterized from previous experimentation and, for the antagonist population in particular, using intuitive biological considerations (Table 1). The within-crop period and inter-crop period were 300 and 65 days respectively. In particular we tested the hypothesis that the antagonist population must have a

negative effect on disease transmission (the rate of secondary infection) to reproduce qualitative consistency with the characteristic rise and fall in disease severity during take-all decline.

| Parameter | Meaning | Value |
|---|---|---|
| $b$ | Monomolecular growth rate | 0.016 |
| $k$ | Monomolecular carrying capacity | 40 |
| $r_p$ | Rate of primary infection | 0.045 |
| $r_s$ | Rate of secondary infection | 0.0008 |
| e | Rate of decay of infected tissue | 0.003 |
| g | Antagonist mediated increase in decay rate of infecteds | 0 |
| h | Rate of creation of inoculum from infected tissue | 0.0001 |
| m | Rate of decay of inoculum | 0.04 |
| n | Antagonist mediated increase in decay rate of inoculum | 0 |
| p | Rate of antagonist production on susceptible roots | 0.0004 |
| q | Rate of antagonist production on infected roots | 0.0004 |
| r | Rate of antagonist production on inoculum | 0.0004 |
| s | Rate of decay of antagonists | 0.0005 |
| u | Rate of additional density dependent decay of antagonists | 0.0008 |
| v | Effect of antagonists on rate of primary infection | 0 |
| w | Effect of antagonists on rate of secondary infection | 0 or 0.125 |
| $S_0$ | Initial number of susceptibles at the start of a season | 0.1 |
| $I_0$ | Initial number of infecteds at the start of a season | 0 |
| $X_0$ | Initial density of inoculum at the start of the *first* season | 0.01 |
| $A_0$ | Initial population of antagonists start of the *first* season | 0.01 |
| $\Theta$ | Proportion of infected roots becoming new inoculum | 5 |

**Table 2: Parameters used in the multi-season model for describing the dynamics of take-all during consecutive within-crop and inter-crop periods for a sequence of wheat crops. The effect of the antagonist population is introduced as a reduction in the rate of secondary infection according to the term $1+wA$ where $w = 0.125$.**

*Results of initial simulations*

The results of two simulations are presented here (Fig. 8). In the former all the parameters controlling the suppressive effects of the antagonist population are set to zero (Fig. 8, black line), whereas in the

latter, $w$ is given a non-zero value (Table 1) corresponding to the antagonist reducing the rate of secondary infection (Fig. 8, red line).

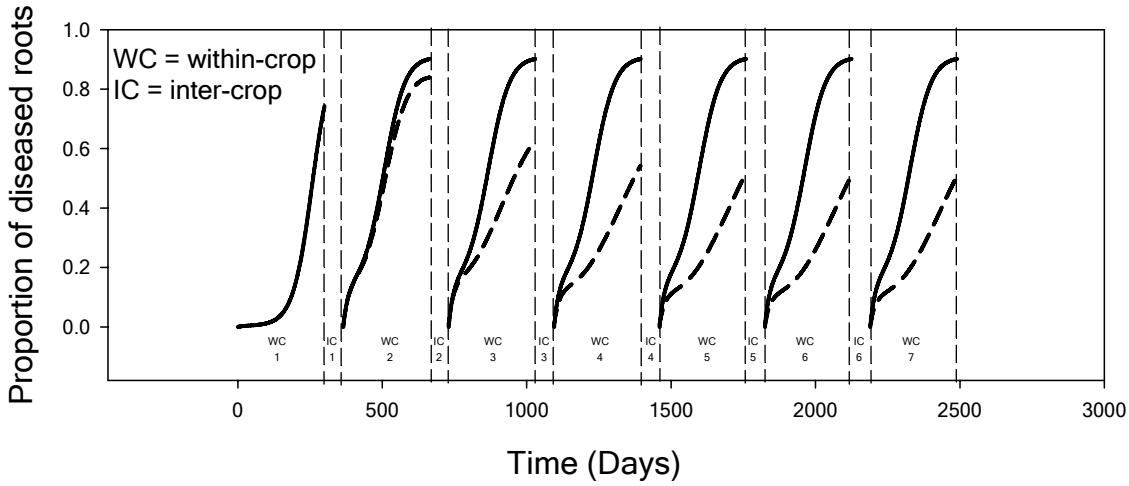


**Fig. 8. Simulation of change in the proportion of diseased roots over time for a series of seven successive wheat crops in the absence (solid) or presence (broken) of antagonistic activity. An increase followed by a reduction in disease severity leading to the well known phenomenon of take-all decline could only be obtained when the build-up of antagonists reduced the rate of disease transmission.**

Consistent with consecutive phases of primary and secondary infection, all epidemics showed an initial rise to a plateau followed by a second increase in the rate of disease transmission. In the absence of antagonism, disease severity during the primary phase was relatively low in first wheat crops but increased rapidly during the secondary phase, leading to consistently high disease severity during both phases in second and subsequent crops (Fig. 8). During the same period, the antagonist population followed cycles of increasing relative density during the within-crop periods and decay during the inter-cropping periods (Fig. 9). Overall, the relative density of antagonists increased from low levels in first and second wheat crops to achieve maximum densities in third and subsequent wheat crops.

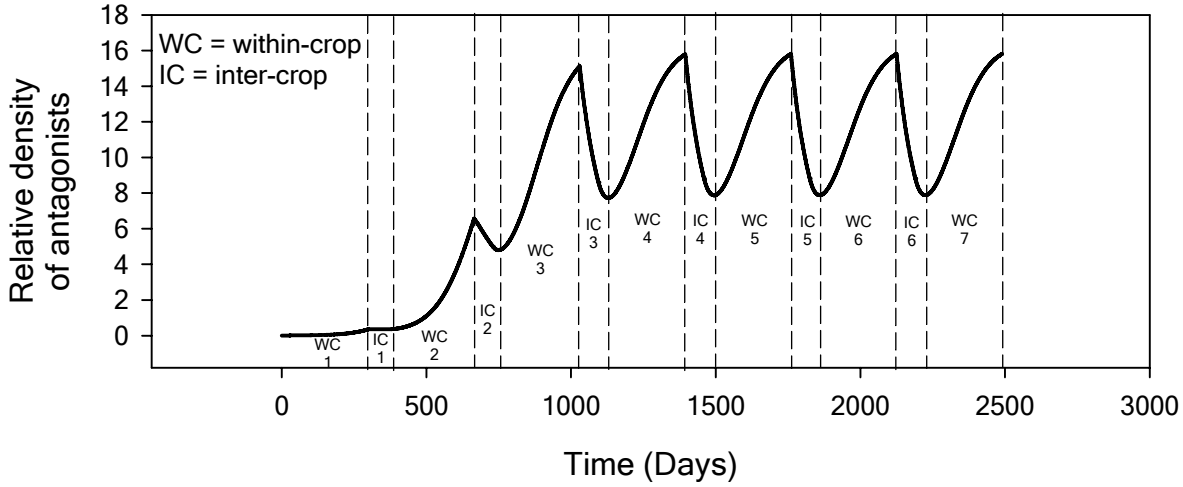


**Fig. 9. Simulation of change in the relative density of the antagonist population in response to changing disease levels during a sequence of wheat crops.**

In the presence of antagonism, epidemics followed an identical course in first wheat crops as those grown in the absence of antagonism. However, by reducing the rate of secondary infection, the increasing levels of the antagonist led to a peak in disease in the second wheat crop, followed by a reduction in disease severity in subsequent crops.

*Discussion*

This project has used existing data from ADAS and INRA to analyse the dynamics of take-all in a sequence of consecutive wheat crops and the spatio-temporal evolution of disease patches during the within-crop period of a second wheat crop. The results of these analysis have been integrated into a conceptual model for take-all decline culminating in the derivation of a mathematical model that can be extended to provide a coherent platform for integrating control measures for the better disease management of, in particular, non first wheat crops. We consider below the next, most important steps, in the development of this framework.

*Model development.* The key to the rigorous development of an epidemiological model is a detailed understanding of the behaviour of its underlying processes. These are best identified using a combination of controlled environment and field experimentation together with mathematical modelling. Whilst these processes have been well documented for the within-crop dynamics of the host and the pathogen (1), relatively little is known concerning the saprotrophic dynamics of spread and survival of *G. graminis* during the inter-crop period and less still of the effects of the antagonist population on the epidemiological processes of a take-all epidemic.

*Accounting for variability*. In its present form, the multi-season model is deterministic which means that, for a given set of parameters and initial conditions, the model will always simulate the same outcome. We acknowledge two principal forms of variability in the take-all system, demographic variability and environmental variability. Demographic variability can be considered the result of local, chance events, that lead to different outcomes of epidemic behaviour from otherwise identical conditions. In this situation we consider that the parameter values of the model do not change but have certain variability associated with them. We also acknowledge the larger scale, environmental variability associated with differences in soil types or differences in climate from one year to the next. This source of variability can be considered as leading to differences in the values of parameters from site to site and from year to year. By incorporating a wide range of wheat crop sequences both within and across years, combined with replicate samples from each plot, the ADAS data in particular, holds the potential to disentangle some of these sources of variability. Further analysis of this data would allow us to predict, not simply average levels of disease across all wheat crops, but the *risk* of disease as we pass through sequences of "good" and "bad" take-all years.

*Model simplification.* The within-season model, model (2), presented here includes more than twenty parameters. One of the main features of the management of the take-all system will concern criteria for invasion and persistence of both the pathogen and the antagonist population. Together with estimates of system variability, this will provide predictions of the long-term sustainability of disease management protocols. By eliminating unnecessary parameters or combining sets of related parameters, predictions of within-crop invasion and long-term persistence can be derived mathematically and tested experimentally.

*Screening control treatments*. A wide range of control opportunities exist for take-all, all of which can produce small reductions in the spread of disease. Conventional approaches to study effectiveness of disease control have relied on empirical experiments, often with few replicates and a single observation of disease over time. This strategy is valuable in screening treatments but the absence of epidemiological understanding makes it almost impossible to consider a plausible strategy for combining treatments and improving the control of disease. The modelling framework presented here, combined with appropriate experimentation, will allow us to screen existing treatments against parameters of the multi-season model and investigate how treatments might best be combined to control disease during sequence of wheat crops. For example, the effects of silthiofam to control primary infection have been well documented yet strategies for how best to deploy and combine the chemical with alternative control measures remain empirically derived. Large-scale plot experimentation is expensive. By linking directly and strategically to key parameters of the multi-season model, epidemiological micro-plots or microcosm bio-assays, such as the pathozone bio-assay (15), offer the opportunity for rapid and efficient screening of, for example, existing or novel forms of variety tolerance or resistance (2).

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