Take-all disease: New insights into an important wheat root pathogen

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Highlights

- The ancestral wheat species *T. monococcum* has been shown as a potential source of resistance genes against take-all. In addition, modern wheat cultivars show variation in their ability to build up inoculum, indicating that this trait is under genetic control. Different wheat cultivars can be used to manipulate the level of inoculum in the field and therefore the disease levels in subsequent years.
- Recent discoveries on the avenacin synthesis pathway from oats, provide potential for engineering this pathway into wheat to provide high level resistance to take-all.
- The soil microbiome influences the three phases of disease development, and each phase can be modulated by host genotype.
- Host Induced Gene Silencing (HIGS) has been successfully used in wheat to silence a pathogen effector gene during root infection, showing its potential for functional validation of pathogen genes.

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Keywords

Gaeumannomyces tritici, Triticum aestivum, *Magnaporthaceae*, genetic resistance, microbiome, molecular interactions

Abstract

Take-all disease, caused by the fungal root pathogen *Gaeumannomyces tritici,* is considered to be the most important root disease of wheat worldwide. Here we review the advances in take-all research over the last 15 years, focusing on the identification of new sources of genetic resistance in wheat relatives and the role of the microbiome in disease development. We also highlight recent breakthroughs in the molecular interactions between *G. tritici* and wheat, including genome and transcriptome analyses. These new findings will aid the development of novel control strategies against take-all disease. In light of this growing understanding, the *G. tritici*-wheat interaction could provide a model study system for root-infecting fungal pathogens of cereals.

Take-all disease, an important root disease of cereals

Roots are essential organs with many important physiological roles, including plant anchorage, water and nutrient uptake. Roots are constantly in contact with the soil microbiome, containing both beneficial and pathogenic organisms; these rhizosphere (see Glossary) interactions can have a strong impact on plant health and on the

environment [1]. Root diseases routinely cause significant reduction in yield and product quality [2]. Due to climate change, root health is expected to worsen in areas where autumns and winters will become milder and wetter, which are conditions that favour fungal diseases that can threaten food production [3].

Wheat (*Triticum* spp.) is one of the most important staple crops, being widely produced and increasingly consumed globally [4]. Therefore, wheat losses by various pests and pathogens are of considerable concern. Take-all disease of wheat is caused by the soil-borne fungal pathogen *Gaeumannomyces tritici* (Gt), a member of the *Magnaporthaceae* family formerly known as *Gaeumannomyces graminis var. tritici,* and is the most damaging root disease of wheat worldwide [5]. The fungus also infects other cereals including barley (*Hordeum vulgare*), rye (*Secale cereale*) and triticale (*Triticosecale*). Unlike other fungal pathogens able to infect roots such as *Fusarium*, *Rhizoctonia*, *Verticillium,* and *Pythium* species, which have a broad host range and can also infect and damage different plant tissues, *G. tritici* is only able to infect roots and its host range is limited to cereals. This disease causes significant financial losses by reducing wheat yield and grain quality; both direct and indirect consequences of damage to wheat roots are described in Figure 1. No resistant wheat cultivars to take-all are available, and chemical control is still limited. Therefore, control measures are largely restricted to crop rotation, and new control strategies are urgently needed.

Here we review the advances in take-all research over the last 15 years. This important wheat disease has been understudied between 2005 and 2015 due to the difficulty to do genetic studies and the usual gene function studies with the fungus. Despite these difficulties, significant advances have been achieved in recent years. New sources of genetic resistance from ancestral wheat species and other take-all resistant cereal crops have shown great potential to protect wheat roots. We also highlight the role of the soil microbiome in the disease outcome. The availability of a sequenced genome of the fungal pathogen (*Gaeumannomyces tritici*) has allowed transcriptomic studies to provide a better understanding of the genes involved in fungal infection and the wheat response to these infections. Host-Induced Gene Silencing (HIGS) has been successfully applied to silence *G. tritici* genes during plant infection, providing a good alternative to validate the function of candidate pathogen virulence genes. These advances set this pathosystem as a promising model for understanding

fungal root pathogens adapted towards cereals, but further work is necessary to translate these findings into cereal crop protection strategies (see Outstanding Questions Box).

Disease cycle, root infection process, and control methods

During the intercrop period the fungus survives saprophytically as mycelium in crop debris present in the soil, and can also be found on cereal volunteers and grassy weeds. Primary infection starts when the roots of young seedlings contact crop debris harboring mycelium, then dark runner hyphae grow on the root surface to produce multiple infections along the root [6]. Hyaline hyphae branch from the runner hyphae and produce simple hyphopodia to penetrate the root epidermis, then invade the root cortex and finally colonise and destroy the vascular tissue, hindering water and nutrient uptake [7] (Figure 2).

Secondary infections occur via root-to-root contact, with severely infected plants often occurring in patches. The most characteristic field symptoms of take-all disease are the whiteheads, caused by premature ripening, and blackened stem bases. After harvest, the fungus survives saprophytically in the crop debris and a new cycle begins. In addition, the fungus can reproduce sexually, forming perithecia containing unitunicate asci and ascospores on stem bases and stubble. The fungus is commonly referred to as homothallic, due to its capacity for self-fertilisation. However, experiments in laboratory conditions reveal that outcrossing is also possible [8] (Figure 3A). Asexual spores are also produced under *in vitro* conditions [9]. The fungus can produce both phialidic conidia, which can germinate *in vitro,* and microconidia, which have not been shown to germinate. The role of the asexual spores in nature and their contribution to the disease is unclear. Take-all disease levels are usually low in the first wheat crop in a rotation, but take-all fungal inoculum builds up in the soil nearby the roots (take-all inoculum build-up (TAB)). In the ensuing 2-4 years disease levels increase which may be followed by disease decline (take-all decline (TAD)) (Figure 3B).

Take-all disease is widely distributed throughout the temperate wheat growing regions around the globe, and it has also been reported at high altitude in subtropical and even

tropical areas [10]. Surprisingly, there are no reports about the global incidence and yield losses caused by take-all disease. In the UK it has been estimated that half of the UK wheat crops are affected by take-all annually, which causes between 5 to 20% annual yield losses. However, more than 50% of the crop can be lost in years of high disease severity [11]. The disease is more important under wet soil conditions, but the fungus can also produce disease in dry areas. The later disease is known as “dryland” take-all, and is an important disease of wheat both in Australia and in the U.S. Pacific. Contrary to “wetland” take-all, the characteristic disease patches are not observed in “dryland” conditions, as the lower humidity of the upper soil layer restricts fungal growth. These conditions prevent the growth of the fungus from plant to plant, and every infected plant is the result of a primary infection. Also, the pathogen symptoms are limited to the roots because the pathogen is unable to grow into the crown and culm base as observed in wet conditions [12].

Control measures predominantly consist of a crop rotation with non-cereals, to reduce fungal inoculum levels in soil. Chemical control methods are limited to two fungicides that can be used as a seed coat: fluqinconazole and silthiofam. However, these chemicals are not completely effective because not all fungal isolates are fungicide sensitive [5]. A new fungicide, 4-chlorocinnamaldehyde thiosemicarbazide (PMDD), has been recently proposed as a promising fungicide to control wheat root diseases, including take-all disease [13]. However, seed treatments are only effective during the seedling phase, so the pathogen can still attack the roots throughout the growing season, thus, seed treatments often perform inconsistently. In addition, a recent study suggests that the high use of silthiofam in wheat fields of China may have caused evolution of resistance to silthiofam, resulting in a reduction of the control efficiency of this fungicide [14].

New taxonomic classification

Gaeumannomyces is a genus belonging to the family Magnaporthaceae [15]. This family includes other species that also cause devastating diseases on cereals and grasses, including the rice leaf and panicle blast pathogen, *Magnaporthe oryzae*, and the summer patch fungus of turfgrasses, *Magnaporthe poae* [16]. Interestingly, *M.*

oryzae can also infect wheat roots under laboratory conditions [17], and the root infection process by this fungus resembles the developmental processes typical of root infecting fungi [18].

Traditionally, ascospore size, hyphopodial morphology and host preference were used to discriminate between species and varieties within Gaeumannomyces, the four previously recognised main varieties being *Gaeumannomyces graminis* var *graminis* (Ggg), *Gaeumannomyces graminis* var *avenae* (Gga), *Gaeumannomyces graminis* var *tritici* (Ggt) and *Gaeumannomyces graminis* var *maydis* (Ggm).

More recently a phylogenetic study combining multi-locus phylogenetics, based on partial gene sequences of ITS, LSU, TEF1 and RPB1, as well as morphology data, has led to the reclassification of this genus [19]. This study classified 19 species within Gaeumannomyces, 12 being newly recognised as species within the genus. Within these new species, *Gaeumannomyces tritici* and *Gaeumannomyces avenae* (previously Ggt and Gga) clustered outside of Ggg clade and were proposed as new species (Table 1). Ggg was previously the most genetically diverse clade, but has now been proposed to be split, forming 14 cryptic species: *G. arxii, G. australiensis, G. californicus, G. ellisiorum, G. floridanus, G. fusiformis, G. glycinicola, G. graminicola, G. graminis, G. hyphopodioides, G. oryzicola, G. oryzinus, G. setariicola* and *G. walker* [19].

Closely related non-pathogenic endophytic fungi in the Magnaporthaceae family such as *G. hyphopodioides,* present during the build-up and disease outbreak phases, have previously been shown to inhibit take-all disease [20]. Therefore, promoting natural populations of these species using soil-based crop genetic management strategies may provide an effective biocontrol solution. Gh has been proposed to restrict the development of Gt in cereal and grass roots by inducing host resistance [21]. These closely related fungi occur naturally in grasses and could be introduced through grass leys in the year preceding the wheat crop [22].

Within *G. tritici* several methods continue to be used to distinguish isolates. Two distinct genetic groups have been consistently identified and have been referred to as T1/T2 [23], A/B [5], A1/A2 [24], G1/G2 [25] and N/R (based on ability of isolates to

infect rye) [26]. Interestingly, a strong correspondence has been found between the diagnostic methods. T2, B, G1, and R isolates correspond to each other based on phylogenetic analysis conducted using DNA sequences of two regions, and a universal molecular descriptor of isolates has been suggested [27]. Some associations between isolate distribution and disease severity have been made [28,29], however these associations are not always consistently seen under field conditions. Potentially these two types represent two cryptic species, as the same genetic differences have been reported over many years, with both types being present in the same fields, suggesting that gene flow rarely occurs between them. In addition, heterokaryon formation between different strains is very rare in this fungus, and most strains tested from a single site are vegetatively incompatible [30], which suggests that genetic exchange between strains must be limited to sexual reproduction in this species.

Further characterisation of isolates can be made through their different sensitivity to silthiofam [29], which can be combined with typing (A/B) to differentiate between isolates and study population structure.

Sources of genetic resistance in wheat relatives

Identifying and utilising sources of genetic resistance to take-all disease would be an ideal management strategy, being easy for farmers to apply, affordable and sustainable. Currently, there are no available sources of genetic resistance in hexaploid wheat, and therefore the search for resistance sources has widened through the primary, secondary and tertiary gene pools of wheat.

Take-all resistance has been identified in *Haynaldia villosa* (2n = 14, VV), a cross-pollinating, annual species belonging to Triticeae. TH3 is an amphiploid generated from a cross between *T. durum* and *H. villosa*, which retains resistance to take-all. Field experimentation on an F_1 generation derived from a cross between TH3 and wheat also maintained some of this resistance [31]. Furthermore, cytological and genomic *in situ* hybridisation (GISH) analysis revealed a monotelosome from *H. villosa* in one resistant line. This was located on chromosome 3V and could be a promising avenue for future experimentation.

Screening of the diploid einkorn wheat, *Triticum monococcum* (A^mA^m), under field conditions has revealed seven accessions that demonstrated moderate to strong take-all resistance [32]. *T. monococcum* is closely related to the AA genome progenitor of both tetraploid durum wheat and hexaploid wheat, *Triticum urartu* [33], but has not been used widely in wheat breeding [34]. Analysis of the whole genome diversity of *T. monococcum* using DArT genotyping did not find a relationship to take-all susceptibility, suggesting multiple or more complex sources of resistance. Two accessions, MDR031 and MDR046, had the highest and most consistent take-all resistance over 5 years of field trialling [32]. The genetic and mechanistic basis of this resistance is not known. This species has previously been used to introgress genetic loci conferring resistance to powdery mildew and leaf rust [35,36]

Psathyrostachys huashania Keng (2n = 14, NsNs) is a wild wheat relative that also demonstrates high levels of resistance to take-all, and has successfully been introgressed into a wheat background [37]. A wheat-*P.huashania* substitution line has identified chromosome 2N as having resistance properties through molecular analysis, and this source could be used to improve wheat breeding for resistance to multiple diseases.

Rye is a species closely related to wheat and is considered to be highly take-all resistant. Triticale has intermediate take-all resistance. Tissue-based resistance from this source may be explained by the production of hydroxamic acids [38], however, rye may also have the capacity to produce new roots in response to pathogen contact as a form of disease escape [39]. The inheritance of rye resistance to take-all was studied in the 1960s by using addition lines in which rye chromosomes were separately introgressed into wheat [40]. Although no improved resistance was found by any individual chromosomal addition, the addition lines with chromosome I or VII showed less damage by the fungus, which suggests that multiple genes located in different chromosomes may be involved in the rye resistance to take-all. The vastly improved cereal genomic resources available nowadays will facilitate to the future identification of the genetic basis of resistance to take-all in rye.

Oats (*Avena sativa*) are described as immune to *G. tritici*. This is because of the production of antifungal triterpene compounds called avenacins from the oat roots.

There are four main avenacins produced: A-1, A-2, B-1 and B-2. The main avenacin, A-1, has a branched sugar chain at the C-3 position, conferring antifungal activity [41]. Steps in the synthesis pathway of avenacins have been revealed [42–46], with the most recently discovered enzyme being the cytochrome P450, AsCYP72A475 [47]. Further understanding of this pathway may allow its engineering into wheat, thereby providing a new source of genetic resistance. However, oats are susceptible to *G. avenae*, which can infect by detoxifying avenacin. Therefore, resistance durability could be questioned if deployment leads to possible selection for avenacin insensitive *G. tritici* isolates, and/or a general increase in abundance of *G. avenae* wheat infecting isolates.

Although no source of genetic root resistance is currently available in hexaploid wheats, a genetic trait has been identified in which hexaploid bread wheat cultivars differ in their ability to build-up take-all inoculum in the soil in their first year of rotation [48]. This low take-all build up (lowTAB) trait (Figure 3C) influences disease severity and productivity in the second year of growing wheat [49]. An increase in grain yield of 2.4 tonnes/ha was reported in a high take-all disease pressure year. This novel genetic trait has been explored in 71 modern UK elite wheat cultivars in a first wheat situation across multiple field sites and seasons [49]. Variations in the level of take-all inoculum in soil cores were observed across the cultivars and trial sites, which suggests that other environmental, microbial and/or agronomic factors also influence take-all build-up. Although the genetic mechanism of this finding is not known, lowTAB can still be exploited by farmers, making short wheat rotations more profitable.

Take-all disease and the soil microbiome

The soil microbiome plays an important role in plant growth, plant health and stress tolerance, including pathogen control [50]. The microbial composition of the soil is highly dynamic both spatially and temporally and responds to changes in soil conditions. Microbial communities have been shown to correlate with changes in land use, soil type, soil moisture, nutrient composition and plant diversity [51–54]. The root system architecture can also impact on the rhizosphere and the root microbiome [55], and plants can modulate the root microbiome via root secreted exudates containing plant derived compounds and signaling molecules that influence the microbial

assemblages in the rhizosphere [56]. In addition, there is strong experimental support for microbial community differences existing between the root endosphere, rhizosphere and bulk soil and different microbes can be recruited / switch compartments as situations change [54,57,58].

The soil, rhizosphere and root microbiomes exert significant control over root-invading pathogens such as take-all, during the build-up, disease outbreak and decline phases. Field experiments and synthetic community studies conducted at Rothamsted Research indicate that first year wheat genotypes can impact the rhizosphere communities in the following year. In a culture-based approach which utilised *Pseudomonas* bacteria as an indicator species, *Pseudomonas* species richness was found to be positively correlate with disease pressure. This translated to a reduced control effect against *G. tritici*, which the authors suggest was related to higher levels of take-all disease in the second year of the field experiment, compared to cultivars which supported low *Pseudomonas* species richness [59–61].

The prevalence of fungal species can also be influenced by wheat host genotype. A field study of 40 elite UK winter wheat cultivars demonstrated that wheat genotypes differed in their ability to support natural populations of the beneficial *G. hyphopodioides* in the first wheat crop grown, and the root colonisation ability of the fungus was influenced by the choice of the second wheat cultivar [62].

A feature common to many root-infecting fungi such as *Rhizoctonia solani* [63] and *Fusarium oxysporum* [64], is the occurrence of a disease decline stage caused by suppressive soils. Suppressive soils are characterised by a change in the soil microbial community following high levels of disease, often after several years of continuous cropping [65,66]. Despite a decline of take-all disease symptoms in the cereal host (TAD, see figure 3B), pathogen inoculum levels in the soil can remain high [67]. Even though this phenomenon has been recorded in several sites across the world, TAD is not widespread and seems to be field-dependant [68]. Although there have been extensive and long spanning investigations into suppressive soils, only limited mechanistic information exists. Kwak and Weller (2013) provide a detailed review on take-all disease suppression [68]. The majority of TAD studies have focused on the investigation of antagonistic *Pseudomonas* spp., many of which produce

antimicrobial compounds such as 2,4-diacetylphloroglucinol (2,4-DAPG), which is thought to play a significant role in TAD [69]. The role of other bacterial species in TAD is less well known, but recent studies suggest that endophytic bacteria may be as important as rhizobacterial communities in TAD soils [67] [70].

There is also evidence that wheat cultivars differ in their ability to support the suppression of take-all when grown in a TAD soil [71], suggesting that TAD likely involves a complex interaction between the take-all fungus, wheat host genotype, and endosphere, rhizosphere and soil microbial communities. These studies highlight the important role of the soil microbiome for the control of root-infecting pathogens, both directly through microbial antagonism and indirectly through changes in microbial community structures. Take-all disease represents an important system for the study of the soil microbiome, the careful manipulation of which, could have significant impacts on pathogen populations and/or disease outbreaks.

Molecular interactions between *G. tritici* and wheat

The molecular interactions between pathogenic fungi and roots have been understudied in general compared to the above-ground interactions [72]. This lack of knowledge is in part a result of the difficulty to obtain stable transformants in many root-infecting organisms. Despite this, our knowledge on the molecular interactions in this pathosystem has improved considerably in recent years thanks to the accessibility of new sequencing technologies. The *G. tritici* genome was sequenced in 2015, facilitating the study of genes involved in the pathogen-plant interaction. The genome size and genic content is in the typical range for an Ascomycete species, namely 43.62Mb and is predicted to contain 14,463 protein-coding genes [73]. In the same study the *G. tritici* genome was compared with two other species from the Magnaportheae family, *M. oryzae* and *M. poae*. Surprisingly, despite the larger genome of *G. tritici* only 7% of its genome is composed of repetitive elements, suggesting that other differences in gene copy number and tandem repeats may account for the size difference.

The genome availability opened the door to transcriptomic studies. Shortly after the genome sequence was released, Yang and collaborators performed the first comparative transcriptomic analysis by RNAseq comparing the gene expression of

the fungus growing in axenic culture with the fungus infecting wheat roots in axenic conditions at different time points of infection [67]. This approach allowed the characterization of the transcriptional remodeling across the infection process, pinpointing differentially expressed genes involved in signal transduction pathways, asexual development, plant cell wall degradation, and responses to plant defense compounds. As a result, this study provided new candidate pathogenicity factors, acting at different time points of the infection process [74]. Most recently, a comparative transcriptome profiling of *G. tritici* in wheat roots in the presence and absence of the biocontrol bacteria *Bacillus velezensis*, as well as on axenic culture as a control, has provided additional candidate genes related to pathogenicity. Genes encoding for inhibitors of the Papain-like cysteine protease, which is produced by plants to protect from fungal attack, were upregulated during root infection compared to the axenic culture. Catalase peroxidases that can protect the fungus from reactive oxygen stress generated by wheat plants were also upregulated in the fungus during infection. A gene encoding an enzyme involved in the synthesis of abscisic acid (ABA) was found to be upregulated during *G. tritici* infection, suggesting that the fungus uses ABA to manipulate the host defense response [75]. The study was later extended by a transcriptomic, proteomic and biochemical analysis of the plant response to take-all infection in the presence and absence of *Bacillus velezensis*, revealing that during *G. tritici* infection wheat plants show a strong salicylic acid (SA)-mediated resistance response, which was stronger than the jasmonic acid (JA) accumulation response [76]. These results contradict the role of JA in plant defense observed during infections of leaves, confirming previous observations that defense signaling in roots cannot be extrapolated from research on leaves [77]. The (SA)-mediated resistance response to *G. tritici* infection was confirmed in the most recent published transcriptomic analysis, which also revealed an upregulation of different defense response genes, including MAPK Kinase 1, the transcription factors WRKY4 and WRKY10, and the PR proteins PR3, PR10, PR5 and PR2 [78]. However, none of the gene candidates obtained through different transcriptomic studies have been functionally validated to date.

Several studies have focused on the overexpression of heterologous proteins in wheat to enhance resistance to the fungus. Wheat expression of an antimicrobial peptide from potato (SN1), a MYB transcription factor gene from intermediate wheatgrass (*Thinopyrum intermedium*) (TiMYB2R-1), and a soybean (*Glycine max*)

polygalacturonase-inhibiting protein (GmPGIP3) were shown to confer increased resistance in transgenic wheat [79–81]. HIGS is a promising approach to validate the role of candidate *G. tritici* genes involved in the interaction with wheat. Zhang and collaborators (2019) showed that silencing via HIGS the Barley Powdery Mildew effector gene BEC1019, highly conserved among fungal pathogens, reduced root infection by *G. tritici* [82]. Therefore, this study provided the first characterised virulence effector used by the take-all fungus to promote plant colonisation, opening the way to other functional studies.

Concluding remarks and future perspectives

Take-all disease remains a devastating root disease of cereals, producing significant yield losses worldwide, and for which control mechanisms are limited. This review summarises the recent advances in take-all research. Considerable progress has been made in our understanding of this disease. New sources of genetic resistance have been identified and these are being investigated and transferred into wheat. We now have a better understanding of the soil, rhizosphere and endosphere microbiomes, and their association to different wheat cultivars, which shows the importance of considering all these interactions to achieve efficient disease management practices (Figure 4). Also grass leys harbor related fungal species that are known to restrict in planta Gt infections. The fungus and wheat transcriptome have been characterised during the infection process, providing new gene targets for future antifungal development. Overexpression of heterologous proteins in wheat has produced transgenic wheat with increased resistance. In addition, HIGS has been shown to silence fungal genes, opening the way for future studies to validate the function of candidate virulence genes from a fungus that has previously proven difficult to routine transform using standard approaches. However, there are still many unknowns, especially at the molecular and community population levels. The developments in genome sequencing techniques and comparative genomics, now make it possible to construction a pangenome for this species. The pangenome will allow the determination of the core genome by comparing different strains from different world populations (e.g. wetland and dryland take-all) and from different genetic groups (e.g. T1/T2, A/B, A1/A2, G1/G2 and N/R). The available transcriptome data will allow comparisons with other pathosystems, to compare the plant responses between different organs, and between biotic and abiotic stresses. The recent

advances in genome editing tools, like crispr-cas9, will promote the understanding of the take-all molecular mechanisms of virulence in the near future, which together with virus/host-induced gene silencing and virus-induced over expression of wheat root genes will allow elucidation of the molecular interactions underlying this pathosystem. The future looks promising for this pathosystem, which now provides a premier model system for understanding cereal root diseases caused by fungal pathogens.

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Glossary

Amphiploid: An organism with a genome containing at least one set of diploid chromosomes from each parent species.

Cortex: The outer layer of a stem or root, between the epidermis and the vascular bundles

CRISPR-Cas9: A genome editing technology adapted from a naturally occurring system in bacteria, this technique uses the cas9 protein to cut the DNA at a specific target site.

DaRT Genotyping: Diversity Arrays Technology, allows for high throughput, whole genome genotyping to detect and type variation at several hundred genomic loci in parallel.

Endophytic: An organism that lives within a plant for all or part of its lifecycle without causing damage to the host.

Genomic in Situ Hybridization (GISH): Technique used in molecular cytogenetics to distinguish genomes in a cell

Heterokaryon: A fungal cell or mycelium containing two or more different nuclei of differing genetic constitution.

Host Induced Gene Silencing (HIGS): see VIGS description below

Hydroxamic Acids: Organic compounds with the ability to chelate metal ions and can act as effective enzyme inhibitors.

Hyphopodia: specialised hyphal branch used for attachment and penetration through the root surface.

ITS, LSU, TEF1 and RPB1: The internal transcribed spacer (ITS), large subunit (LSU), translation elongation factor 1 (TEF1) and RNA polymerase II large subunit (RPB1) are all genetic markers used for DNA-based species identification.

Monotelosome: A single nuclear telomere cap located at only one end of a chromosome

Mycelium: The vegetative part of a fungus, comprised of thread-like branching hyphae

Pathosystem: The relationship between a host organism and a disease-causing organism and the conditions in which that relationship develops

Primary, Secondary and Tertiary Gene Pools: A measure of genetic variation in a population. In the primary gene pool organisms are the same species, in the secondary gene pool organisms are different species but closely related and can be crossed to produce fertile offspring. Organisms in the tertiary gene pool are distantly related and cannot cross naturally.

Rhizosphere: The region of soil surrounding the roots in which the roots interact directly or indirectly with the soil microbiome.

Tiller: All stems produced after the parent shoot in grass plants

Triticale: A hybrid cereal crop developed by crossing wheat (*Triticum*) with rye (*Secale*)

Vegetative incompatibility: a genetic mechanism of filamentous fungi that prevents heterokaryosis between genetically incompatible isolates. Hyphal anastomosis between the incompatible isolates results in a programmed cell death reaction to prevent the transfer of cellular contents and /or mycoviruses and viroids.

Virus-Induced Over Expression (VOX): Virus-mediated transient overexpression of a heterologous protein in plants

Virus/Host-Induced Gene Silencing (VIGS or HIGS): The RNA-mediated silencing of a specific plant (VIGS) or plant pathogen (HIGS) gene through the production of small interference RNAs (siRNAs) by the plant host.

Polygalacturonase-inhibiting protein: protein that protects plant cell walls by inhibiting the activity of polygalacturonases secreted by microbial pathogens

Tandem Repeats: directly adjacent repeats in DNA nucleotide sequences

Catalase peroxidases: enzymes that protect cells from oxidative damage by catalysing the decomposition of hydrogen peroxide

RNAseq: transcriptome profiling technology that detects the presence and quantity of RNA in a biological sample using next generation sequencing

Avenacins: saponins found in the roots of *Avena* species, act as a pre-formed fungal growth inhibitor

Table 1: New taxonomic classification of the species within the Gaeumannomyces[a]

SPECIES	PREVIOUS CLASSIFICATIONS	HOST RANGE	CLADE
GAEUMANNOMYCES TRITICI	*Gaeumannomyces graminis var tritici*	Mostly wheat but can also infect rye, triticale and barley as well as other cereals and grasses.	Tritici clade
GAEUMANNOMYCES AVENAE	*Gaeumannomyces graminis var avenae*	Mostly oats but can also infect turfgrasses, wheat, rye and barley.	Tritici clade
GAEUMANNOMYCES ORYZINUS	*Gaeumannomyces graminis var maydis*	Mostly maize, but also *Sorghum* and other cereals.	Oryzinus clade
GAEUMANNOMYCES GRAMINIS	*Gaeumannomyces graminis var graminis*	Rice and turfgrasses, as well as a weak pathogen on cereals, grasses and soybean.	Graminis clade

[a] According to [19]

Figure legends

Figure 1. The cascading direct and indirect consequences of Take-all root disease. Various direct effects occur within the initial take-all disease patch (top blue box) and again in the subsequent wheat or barley crop (bottom blue box). All these direct effects which occur over large tracts of otherwise high-quality arable land are caused by the presence of high levels of Take-all inoculum in the soil combined with conditions conducive to root infections. Various indirect consequences that can occur at whole field to landscape scales are indicated on the white background. Consequences include problems recognised by farmers (shown in red), effects on plants and the soil (shown in black), effects on water quality (shown in blue) and effects on air quality (shown in green). The directional arrows show the interconnected

cascading effects. Although many of these effects are shared with other fungus incited root diseases, the total destruction of the cereal root system mid-season caused by take-all disease together with the fact that wheat is a global arable crop cultivated on a huge scale can result in a bigger impact by this disease and on the scale of tens of millions of hectares annually causes a far bigger impact to global food security and ecosystem health compared with other root disease causing pathogens.

Figure 2. *G. tritici* root infection process. Four main stages: 1) Runner hyphae growing on the root surface. 2) Hyaline hyphae are formed from running hyphae and produce hyphopodia to penetrate the root epidermis and enter to the root cortex. 3) The penetrating hyphae grow inter- and intra-cellularly through the root cortex on their way to the endodermis. The cells of the cortex react to the infection by developing lignitubers (illustrated in red) that enclose the penetrating hyphae, but this fails to stop the fungal infection. The invaded root cells show severe disorganization. 4) The fungus continues growing through the endodermis into the central stele where it colonizes xylem vessels, sieve tubes and paratracheal cells, destroying the plant vascular tissue.

Figure 3. *G. tritici* life cycle. (A) Life cycle within a crop season. The cycle begins when seeds are drilled into soil containing infected crop debris and /or fungal mycelium, the seedlings roots are infected and runner hyphae can be observed on roots. As the infection progresses disease lesions appear on wheat roots and crown tissue. Perithecia, containing asci and ascospores, can be observed on stem bases and stubble. Secondary infections occur by runner hyphae growing through root bridges, which can happen within a plant or between roots from different plants. Diseased plants with stunted growth and whiteheads appear in patches in the field. After the harvest the fungus survives saprophytically in the crop debris. (B) Take-all levels in wheat roots when successive years of wheat crops are grown in the same field. The first wheat crop after a rotation break (i.e. non cereal crop) has low levels of Take-All inoculum Build up (TAB) in the rhizosphere and has low take-all disease levels. In subsequent years, take-all disease in the wheat roots increases and peaks during years 2-4 (depending on local conditions) and then declines. (C) The two-year, synergistic genetic traits concept to reduce take-all root disease in wheat crops. In year 1, the growing of low, intermediate or high Take-All inoculum build up

(TAB) cultivars (respectively, green, blue and red dashed lines) leads to different levels post-harvest of take-all fungal mycelium left in the rhizosphere, even though the roots of the 1st wheat crop remain take-all disease free. In year 2, when a partially resistant wheat cultivar is grown (orange solid line), the disease incidence and severity is lower than when a fully susceptible wheat cultivar is grown (blue solid line). In fields where a high TAB situation has developed in year 1, the economic threshold for severe yield losses is likely to be reached irrespective of second wheat choice.

Figure 4. Recent advances in take-all research are aiding the development of novel control strategies against this highly destructive root disease. The central image shows a typical field patch of wheat plants with whiteheads caused by take-all disease. The left image illustrates a diseased root system caused by and following a severe take-all infection in the absence of any control measures. The close-up inset shows take-all lesions and runner hyphae on a wheat root. The right image shows a healthy root system and the four key recent advances reviewed here that are aiding the development of new multi-disciplinary control strategies to protect wheat root systems from take-all disease.

Take-all disease: New insights into an important wheat root pathogen

Javier Palma-Guerrero, Tania Chancellor, Gail Canning, Jess Hammond, Vanessa McMillan and Kim Hammond-Kosack

Outstanding questions

- How will climate change affect the disease? Will the fungus benefit from the warmer winters and autumns or will the hotter and drier summer and spring weather reduce the disease? Or will climate change affect crop growth and development to permit or impede either disease escape or disease tolerance? Will an increase in temperature favor soil microorganisms antagonistic to *G. tritici?*
- Why is there a lack of recent reports about the global incidence and importance of the disease?
- Can a model-based forecasting approach be developed to predict outbreaks of severe disease and inform rotational/control strategies?
- Are the different *G. tritici* groups identified globally, which routinely co-occur in the same fields, actually cryptic species? Why do different isolates differ in sensitivity to the synthetic chemistry silthiofam?
- Will new fungal genome sequences provide a new reclassification of this group of species?
- Why does rye re-root in response to disease while wheat lacks this desirable trait? Can this trait be transferred from rye into wheat?
- Why do take-all disease patches not form in the same place each year? Is the soil microbiome involved in take-all patch formation?
- Why does the fungus fail to produce significant amounts of root disease in the first-year growing wheat, after a break crop, despite the fungus being detected in the soil? Why is take-all growth restricted in acidic soils?
- What are the molecular factors involved in the fungus-plant interaction?
- Why does take-all only affect grasses while other root necrotrophic pathogens can infect diverse hosts? Why does the take-all fungus fail to infect the aerial parts of the plant whereas other root infecting pathogens do?

- Can the fungus outcross in nature? Do ascospores contribute to the disease? Does outcrossing contribute to the evolution potential of the pathogen? Can different genetic groups outcross? How far can the ascospores travel in the air? What is the role of the asexual spores in nature?
- Is resistance to take-all in the ancestral wheat species associated with a single gene or multiple genes? Can resistance be successfully transferred into modern wheat species?
- Can new knowledge of the microbiome-wheat cultivar interactions be used to achieve efficient disease management strategies?

Figure 1

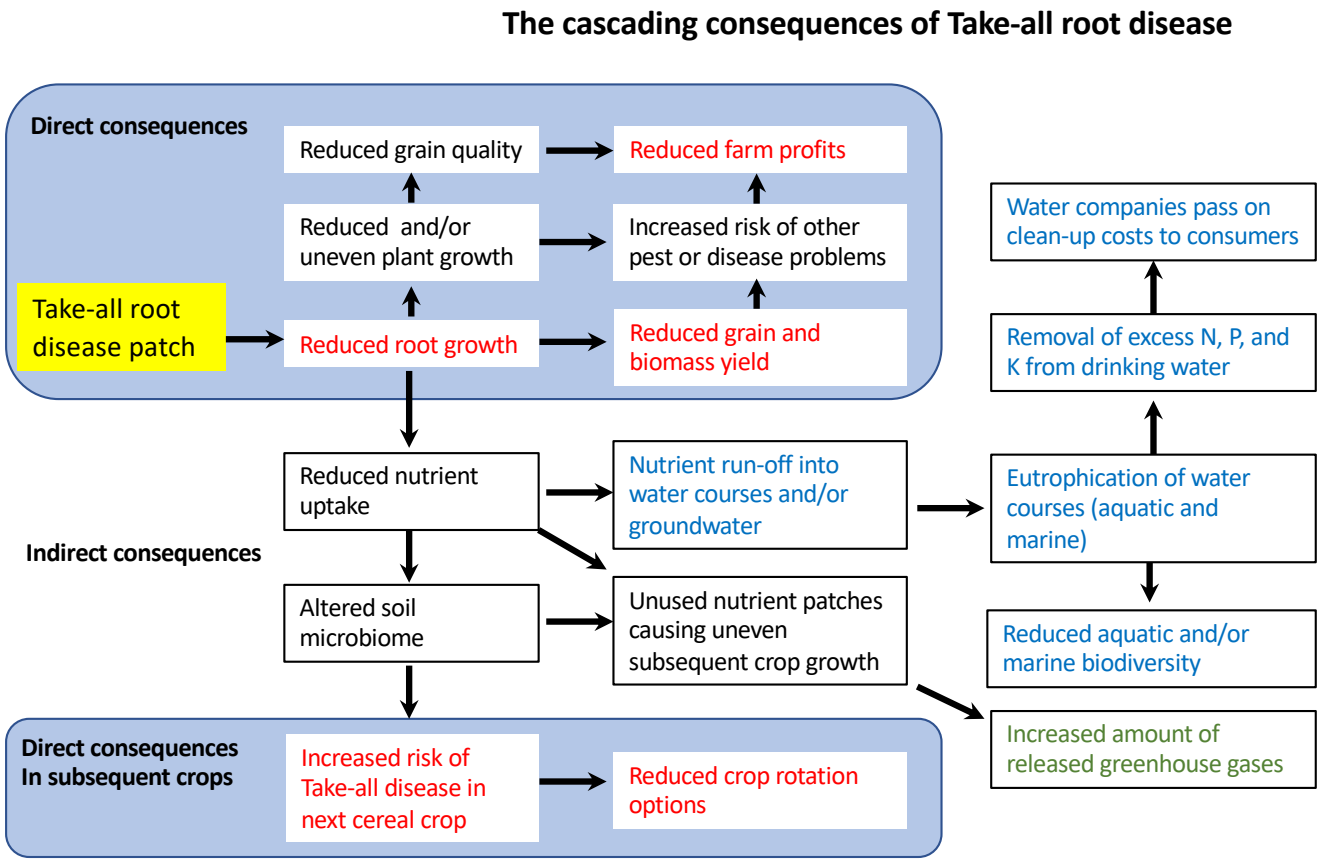

The cascading consequences of Take-all root disease
Direct consequences
Reduced grain quality
Reduced farm profits
Reduced and/or uneven plant growth
Increased risk of other pest or disease problems
Take-all root disease patch
Reduced root growth
Reduced grain and biomass yield
Reduced nutrient uptake
Nutrient run-off into water courses and/or groundwater
Indirect consequences
Altered soil microbiome
Unused nutrient patches causing uneven subsequent crop growth
Direct consequences In subsequent crops
Increased risk of Take-all disease in next cereal crop
Reduced crop rotation options
Water companies pass on clean-up costs to consumers
Removal of excess N, P, and K from drinking water
Eutrophication of water courses (aquatic and marine)
Reduced aquatic and/or marine biodiversity
Increased amount of released greenhouse gases

Figure 2

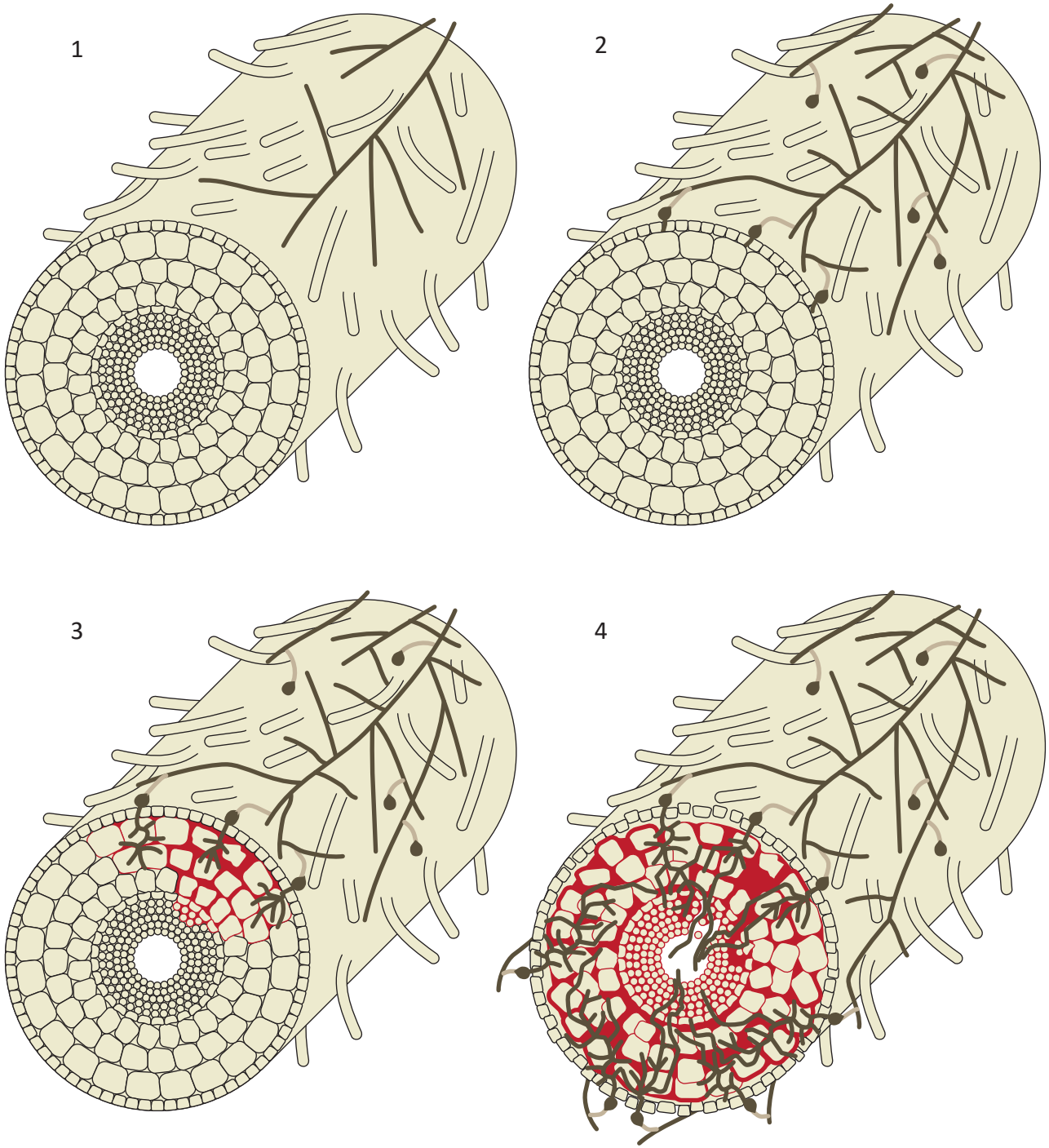
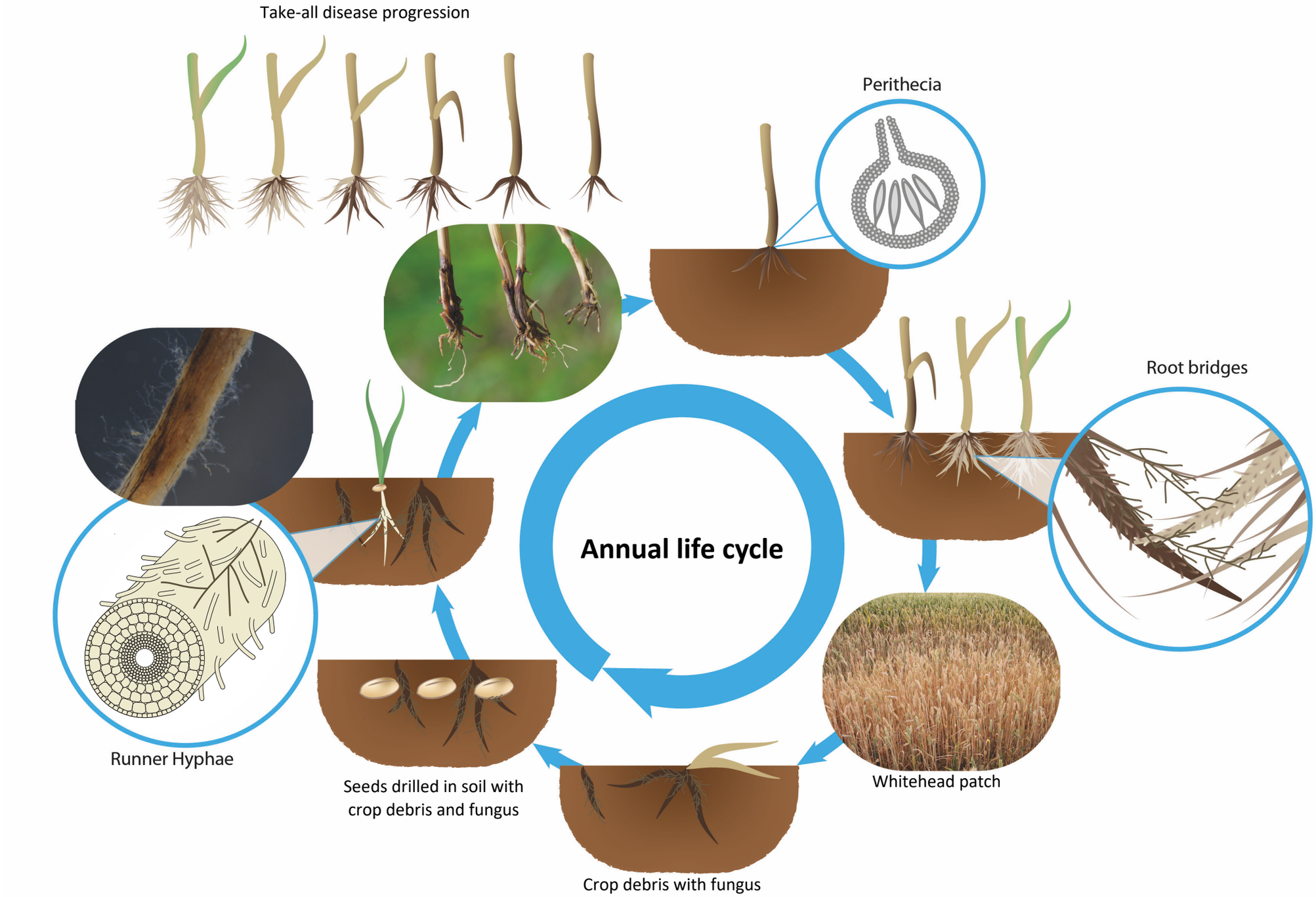
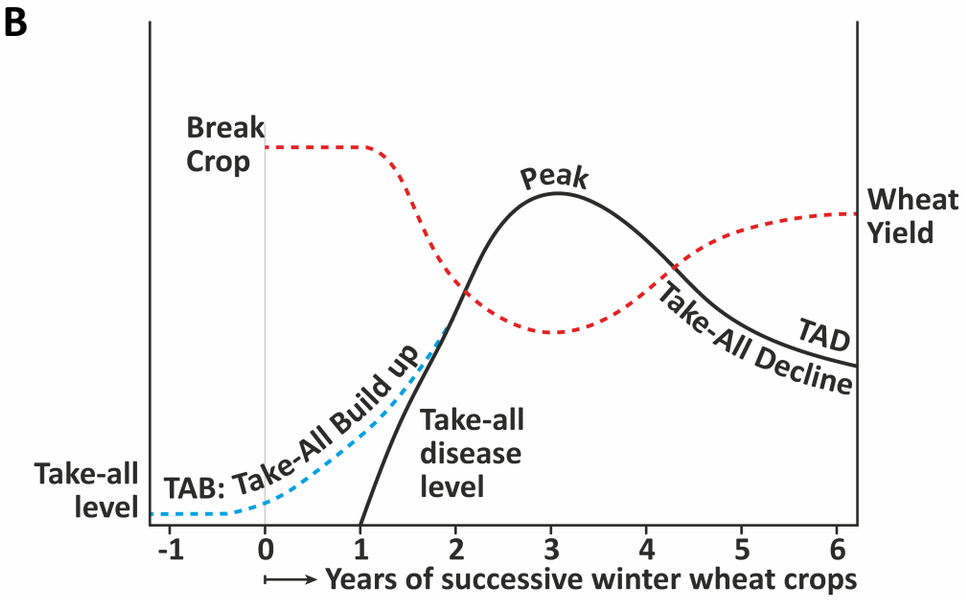


Figure 3

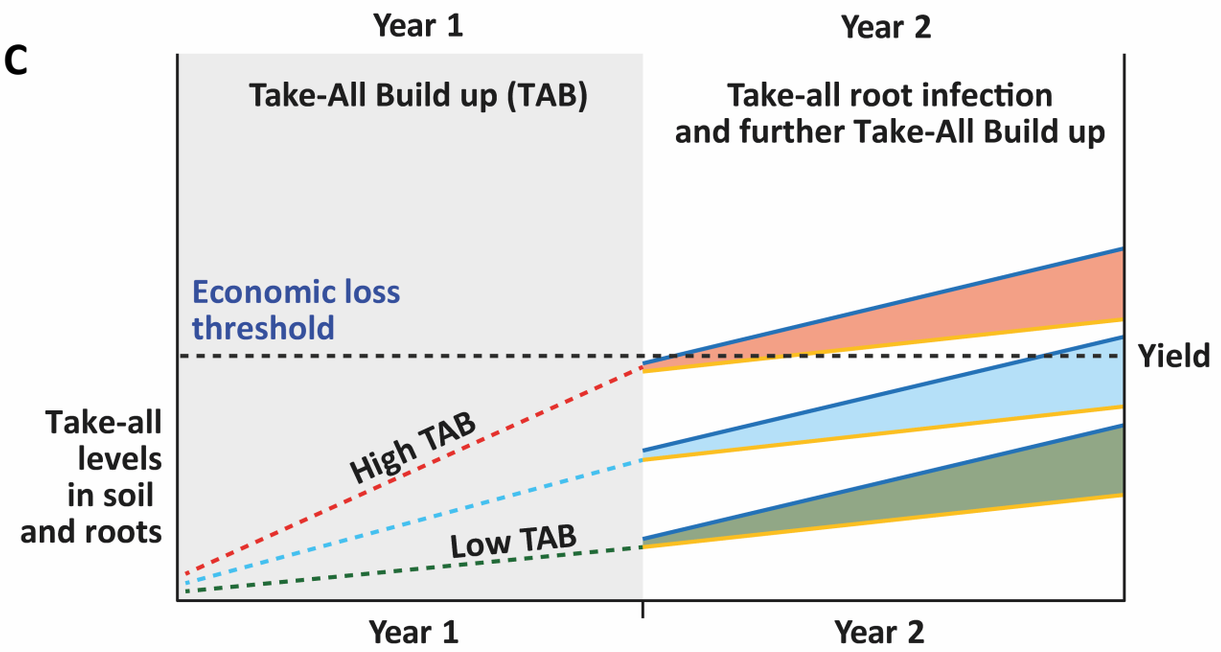
A


Take-all disease progression
Perithecia
Root bridges
Annual life cycle
Runner Hyphae
Seeds drilled in soil with
crop debris and fungus
Whitehead patch
Crop debris with fungus

B


Break
Crop
Peak
Wheat
Yield
Take-All Decline
TAD
Take-All Build up
Take-all
disease
level
Take-all
level
TAB: Take-All Build up
-1
0
1
2
3
4
5
6
Years of successive winter wheat crops

C


Year 1
Year 2
Take-All Build up (TAB)
Take-all root infection
and further Take-All Build up
Economic loss
threshold
Yield
Take-all
levels
in soil
and roots
High TAB
Low TAB
Year 1
Year 2

Key Figure