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Project Acronym: CEREALPATH

Project title: Cereal Pathology – training in innovative and integrated control of cereal diseases

Periodic Technical Report

Part B

Period covered by the report: from [01/09/2017] to [31/12/2019]

Periodic report: 2nd

[1] The term 'project' used in this template equates to an 'action' in certain other Horizon 2020 documentation

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1. Explanation of the work carried out by the beneficiaries and overview of the progress

1.1 Objectives

The major challenge we face today is to ensure food security for the growing world population, and this project Cereal Pathology – training in innovative and integrated control of cereal diseases (CerealPath) derives its motivation from this challenge. The primary objective of CerealPath is to use a multidisciplinary, multisectoral team to train early stage researchers in the broad spectrum of skills and competencies necessary to innovate in the field of sustainable cereal production, thus contributing to the goal of doubling cereal production by 2050.

To achieve this, CerealPath brings together academic, industrial and regulatory expertise from eight European countries, to stimulate innovation in the field of integrated disease control and fill the gap in the field of cereal pathology.

By providing our ESRs with research programmes integrated with network-wide training in creativity and innovation, cereal pathology, socio-economic impacts of crop production, plant diseases and disease control practices, policy and legislation impacts on crop production practices, cutting edge cereal and pathogen technology, plant disease diagnostics and sustainable pesticide usage, it is hoped that they leave CerealPath as true "T-shaped" researchers, with a broad understanding of the issues, concerns and constraints around cereal production and food security while also exhibiting deep discipline-specific knowledge associated with their particular research area.

To achieve this goal, the CerealPath work programme has been developed to ensure that all our ESRs have:

- undertaken cereal disease control research of the highest international quality (WP8, WP9, WP10).
- a broad thematic interdisciplinary knowledge of agriculture, food security and crop production (WP4, WP5, WP6, WP7).
- a portfolio of transferable skills, including excellent communication, networking, innovation and entrepreneurial skills, plus experience of research and working practice in the commercial sector (WP2, WP4, WP8, WP9, WP10, WP11).
- the necessary competencies to inform stakeholders, including policy makers, on strategies for sustainable cereal production (WP2, WP4, WP5, WP6, WP7).

This work programme involves 10 beneficiaries and 11 partner organisations from across Europe, encompassing academia, industry and government agencies, each with specific and complementary competencies in the areas of gene discovery and breeding (WP8), biological and bioactive disease control (WP9) and trade-off and pathogen evolution (WP10). These specific research areas are augmented by a variety of network-wide training events, meetings, and symposia, supported by an extensive management and support function.

In each case, further details are given as part of the associated Deliverables reports submitted via the Participant Portal. Section 1.2 of this report provides an overview of the work described in each work package, and the Deliverables therein.

1.2 Explanation of the work carried per WP

The CerealPath work programme is divided into 11 distinct but complimentary WPs. The WPs are largely divided into 4 categories: management (WP1, WP2); training (WP2, WP4, WP5, WP6, WP7, WP8, WP9, WP10, WP11); outreach and dissemination (WP3, WP11); research (WP8, WP9, WP10).

All of the deliverables and milestones for the management and training components have now been completed, as detailed both in the Participant Portal and in Sections 1.2.1 to 1.2.11 below.

1.2.1 Work Package 1 – Management and Recruitment

The principal objectives of this WP were to establish, implement and oversee the management, governance and mobility of the programme. With these objectives in mind, University College Dublin took the lead but, obviously, all the other beneficiaries were involved. A brief note on each of the deliverables for this Reporting Period is given below.

Individual objectives from this WP were reported as their own Deliverable, as outlined in each subsection below.

1.2.1.1 Consortium Agreement

This part of the WP was discussed in detail in the first Periodic Report (RP1). The Consortium Agreement was submitted as Deliverable D1.1.

1.2.1.2 ESRs Recruitment

All ESRs had been recruited by the summer of 2016 but in early 2017, one ESR withdrew from his position at BOKU. A new recruitment process was undertaken, and a new candidate was appointed to the project. The recruitment process for all ESRs has been discussed in the first Periodic Report.

1.2.1.3 Network meetings

As part of this Deliverable, details of the early meetings of the Consortium have been submitted as part of the Continuous Reporting procedure.

The details of Supervisory Board Meetings and General Assembly Meetings for the Reporting Period 1 (RP1) were discussed in the Periodic Report 1. For Reporting Period 2, the Supervisory Board Meetings and General Assembly Meetings were held in line with the Consortium Agreement. A Supervisory Board Meeting was held on 27 September 2017 as a part of the 2017 Annual Symposium and Mid Term Review Meeting at UCD. Other Supervisory Board Meetings were held on 16 May 2018, 05 December 2018 (as a part of Annual Symposium 2018 at BOKU) and on 2 July 2019. General Assembly Meetings were held on 27 September 2017 and 6 December 2018. A Training Sub Committee meeting was held on 7 March 2018 to discuss the current status of the 15 ESRs within CerealPath with respect to the completion of the core (network-wide) and local training elements of the Action and provided an update on secondment activity.
The report on this Deliverable was submitted as Deliverable D1.2

1.2.1.4 Ethics delivery of documents

This part of the work package requires that all ethics documents be sent to REA at the latest before the related-research starts and all the documents must be valid for the work done within the project Month 6 or before the start of the work. In line with the requirements of the Ethics Review Procedure Screening Report for the CERALPATH programme (reference: Ares(2015)2007995), the consortium members re-affirmed that all relevant ethical standards and guidelines of Horizon 2020 will be rigorously applied, regardless of where the research is carried out.

Equally, in line with the request within the Screening Report, a description of the possible harm to the environment caused by the research was provided. The consortium members noted that the risks to the environment are those due to accidental GMO release (reference: Ares(2015)2226405).

A report detailing the Ethics delivery of documents has been submitted as a Deliverable (D1.3) on 25 May 2016 to the Continuous reporting procedure.

1.2.1.5 Progress Report submitted to REA

As mentioned, an Interim Progress report was submitted in September 2016, outlining activity in the first year of the Action. This report (Deliverable D1.4) was accepted in the Participant Portal on 30 May 2017.

1.2.1.6 Draft Periodic Report

A draft Periodic Report for the purpose of Mid Term Review meeting was submitted as a Deliverable (D1.5) on 11 August 2017 for the reporting period 1 September 2015 to 31 August 2017. This Deliverable was accepted on 25 September 2017.

1.2.1.7 Mid Term Review Meeting

The Mid Term Review Meeting took place in University College Dublin on 28th September 2017 at NovaUCD. The meetings followed the 'standard' model, which involved an introduction by the Co-ordinator [redacted] and the Research Executive Agency's Project Officer [redacted] followed by presentations from the ESRs. For the afternoon session, there were a series of closed sessions between the Project Officer and the ESRs and the Project Officer and co-ordination team. A poster session by ESRs was also held in parallel during the lunch.

The detailed report on the Mid Tern Review meeting which included the presentations of the research work by ESRs was submitted as a Deliverable (D1.6) on the Continuous reporting portal on 31 October 2017 and was approved on 18 December 2017.

1.2.1.8 Website/Social Media

Another significant task was the establishment of a dedicated website and online social media presence. This was important as the website acted as a principal focus for information about applications for positions.

Although this task is listed as a component of WP3, this component is also listed in the relevant Milestones under WP1 as MS2. Further details are provided in Section 1.2.3.

1.2.2 Work Package 2 – Research Career Development

The principal objective of this WP was to ensure that a career plan is developed and implemented to the maximum benefit of all ESRs. While this work package is led by University College Dublin, all the other Beneficiaries and Partners are involved.

Each of the individual components of this WP were designated as separate Deliverables, as outlined below.

1.2.2.1 Researcher Declarations

In line with the Grant Agreement, Researcher Declarations had been completed during the first Reporting Period (RP1) and was discussed in Periodic Report 1. For the newly recruited ESR2 at BOKU the Researcher Declaration was updated in the second Reporting Period. ESR 15 was on maternity leave for two terms from 24 August 2016 to 6 October 2016 and from 15 September 2018 to 24 March 2018. The Researcher Declaration for ESR 15 was updated on the portal taking into consideration of the maternity leave.

In terms of reporting on this component, there was no specific Deliverable, but it is linked with MS3. All the Declarations have now been uploaded.

1.2.2.2 Completion of induction training

Although, this task is listed in WP4 as a Deliverable D4., it is also listed in WP2 as a milestone MS2. A brief description of this milestone was already given in Periodic Report 1.

1.2.2.3 Set up of personal career development plans for all ESRs

This task was completed during the first Reporting Period and was submitted as a part of the Deliverable D2.1.

1.2.2.5 Identification of soft skills deficiencies and provision of training provided

This component of the WP2 was discussed in the first Periodic Report and was submitted as Deliverable D2.2 on 31 December 2016 and subsequently approved on 30 May 2017.
To oversee the progress of the CerealPath Network-wide Core trainings and local Non-Core trainings for ESRs, a Training Sub Committee meeting was held on 7 March 2018. A detailed list of non-core trainings taken by the ESRs are listed in the Annex A.

1.2.3 Work Package 3 – Dissemination and outreach

The principal objectives of this WP are to disseminate the novel outputs produced by CerealPath via presentations at national workshops, world-leading international conferences, publications in journals of high impact factor, reports and outreach activities.

1.2.3.1 Publications

The ESRs have so far published 7 peer-reviewed journal articles, 1 unrefereed preprint, 1 non-peer reviewed technical note and 9 books chapters. Three scientific papers have been submitted to journals and are under revision. More scientific papers are under preparation and expected to be published in the near future. As indicated in the Annex 1 (Part B) of the Grant Agreement, it is expected that at least 2 peer-reviewed scientific journal articles per ESR will be published by February 2021. Besides, the ESRs have also attended several national and international conferences have presented their research work through talks and posters. A list of scientific publications from the CerealPath which includes peer-reviewed scientific papers, technical notes, book chapters, conference abstracts and posters, listed ESR-wise has been submitted on the continuous reporting portal as the Deliverable D3.2 as on 19 December 2019. This list also includes journal articles from other projects in which the ESRs have contributed scientifically. The complete list of publications that have come out of CerealPath is listed in the Publications part of the Continuous Reporting module of the portal.

An editorial was published in *Frontiers in Microbiology* by one of the Scientists-in-Charge at University of Copenhagen, Denmark incorporating the research from CerealPath (open access):
Sarrocco S, Herrera-Estrella A and Collinge DB (2020) Editorial: Plant Disease Management in the Post-genomic Era: From Functional Genomics to Genome Editing. Front. Microbiol. 11:107. doi: 10.3389/fmicb.2020.00107

1.2.3.3 Website and social media

The CerealPath website (http://cerealpath.eu) was established as the principal, public, online face of the CerealPath consortium.

It acts as the focal point for people seeking information about the programme, the network and all of the participants in the programme (Beneficiaries, Partners and ESRs). The website also features News, Events, Research Blogs related to CerealPath programme. On an on-going basis, the CerealPath website is used as an outlet for general news stories about the Action. Details of meetings, training events and publications are listed under the 'Media Centre' section of the site.

The Action is also very active on Twitter (@cerealpath) and has a policy of live-tweeting training events. All the beneficiaries and ESRs are also active through Twitter, serving as a platform to share research experiences, achievements and exchange of ideas. The CerealPath twitter account has so far has 204 followers and has made 326 tweets.

Although mentioned as part of WP1, the principal Deliverable for this component (D3.1) was submitted as part of WP3. The update on this component was also submitted as a part of the Deliverable D3.2.

1.2.3.4 Other outreach (e.g. exhibits, school visits, science though art)

This component of the work package 3 was submitted as a part of the Deliverable D3.2 (Publications and Outreach) on 19 December 2019. A brief description of the outreach activities that were carried out in CerealPath, including ones which were not reported during the previous reporting period (RP1) is given here.

[redacted]

A brief overview of the CerealPath project at the Monogram 2018 – Annual convention for Grass and Grain Research organised at John Innes Centre, Norwich, UK from 24-26, April 2018.

ESR3 (UCD) was selected as one of the MSCA fellows who were present at the Horizon H2020 stand at EUCYS 2018, RDS Dublin, 14-19 September 2018. This was an event open to the public, which allowed the stand to be visited by EUCYS attendees, composed by their contestants, aged 14-20, as well as other visitors and schools.

[redacted]

1.2.4 Work Package 4 – Induction and Innovation

The principal objectives of this WP were to use resources at UCD and the UCD Innovation Academy to induct ESRs into the programme and to provide them with the skills needed to establish entrepreneurial thinking. This WP was facilitated by UCD.

This WP was divided into 2 tasks and both have now been completed. This work package was reported in the previous Periodic Report (RP1) and the Deliverables of this work package D4.1 and D4.2 were submitted on the portal.

1.2.5 Work Package 5 – Winter School 'Agriculture and Society'

The winter school in agriculture and society was run in September 2016 and was a huge success. The details of the winter school were discussed in the first Periodic Report (RP1).

This deliverable is divided into tasks, but reporting is covered under one single Deliverable (D5.1).

1.2.5.1 School

A full report on the School has been provided as part of Deliverable D5.1, including details of the training and the book of abstracts from the talks and was submitted during the first reporting period. Delivery of the School is also associated with MS6 and is now complete.

1.2.5.2 A booklet detailing the School outcomes

The book of papers by the ESRs and other participants in the School, was not completed before the first reporting period due to some logistical issues, including contractual and copyright issues around the papers produced. However, the booklet was published on 5 December 2017 as open access and could be downloaded in the link below.
Zurich-Basel Plant Science Center: PSC Summer Schools 2014 and 2016, Agriculture in transformation – Concepts for agriculture production systems that are socially fair, environmentally safe and economically viable.

1.2.6 Work Package 6 – Theoretical & practical training - 'Cereal Pathology – current practices and advances in disease control'

This Work Package was divided into 3 distinct tasks and covered a number of theoretical and practical exercises in cereal disease control. All three constituent tasks (a) course on cereal pathology and advances in disease control, (b) field workshops at LVH UK and (c) practical field and formal training on pesticide application, testing and sustainable pesticide usage were reported under one Deliverable (D6.1) and discussed in detail in the first Periodic Report (RP1). This WP is also associated with MS7, which has been completed.

1.2.7 Work Package 7 – Workshop on 'Development and sustainable use of Biologicals and Bioactives for disease control'

The objective of this work package was to use both academic and industrial resources within the network to train students in the principles of biological control, its potential and the state-of-the-art methods used to develop and test these agents in the field. The University of Copenhagen organised a specific training event from 06 to 10 March 2017 at Ishøj near Copenhagen. This event was co-hosted by the BestPass MSCA.

All the individual components of this WP were reported collectively as Deliverable 7.1 and was discussed in detail in the first reporting period (RP1). This WP is also associated with MS9, which has been completed.

1.2.8 Work Package 8 – Research Programme – 'Gene Discovery and Breeding'

The principal objectives of this WP are to identify and characterise new candidate genes and genetic loci for disease resistance and determine their potential for disease control.

This work package had the following components:

1. Identification of new wheat genes that show promise for the control of yellow (stripe) rust (ESR1), brown (leaf) rust (ESR5) and root rot diseases (ESR4).
2. Characterization of previously identified genes or genetic loci that contribute to Fusarium head blight resistance (ESR2 and ESR3)

3. Determination of the allelic diversity of the WP complement of genes and development of markers to facilitate crop breeding (ESR2, 3 and 5).
4. Introgression of mutations that enhance resistance of interest from a mutant wheat (TILLING) population into commercial germplasm (ESR1)
5. Development of models to predict disease resistance in a bread and durum wheat breeding populations. (ESR2).
6. Characterization of *Brachypodium distachyon* accessions to identify loci involved in root disease resistance and use wheat TILLING populations to extrapolate findings to the disease responses of wheat. (ESR4).

In terms of specific details for this WP, the following details are provided: the first 2 deliverables associated with this WP, D8.1 - Identification of new candidate wheat genes for yellow rust, leaf rust and root rot resistance and D8.2 - Analysis of allelic variation for FHB and rust disease resistance genes of interest were submitted on the portal during the first reporting period (RP1) and were discussed in the first Periodic Report. The deliverables D8.3, D8.4, D8.5 and D8.6 of this WP were submitted on the portal on the 16 November 2018 and the details of the research work carried out in these deliverables until the end of this reporting period are discussed below.

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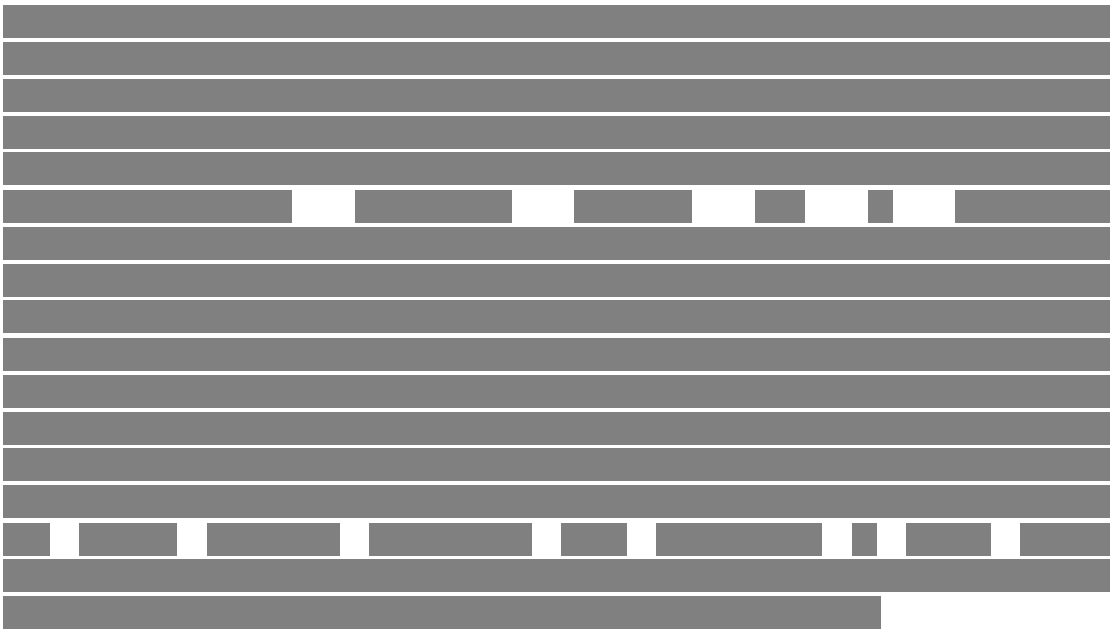
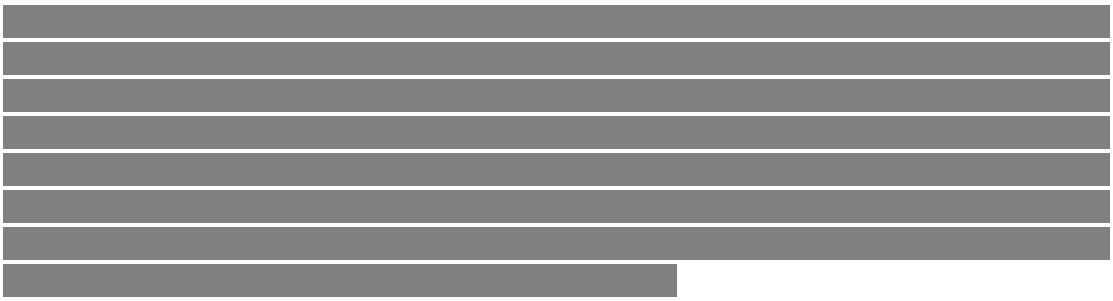
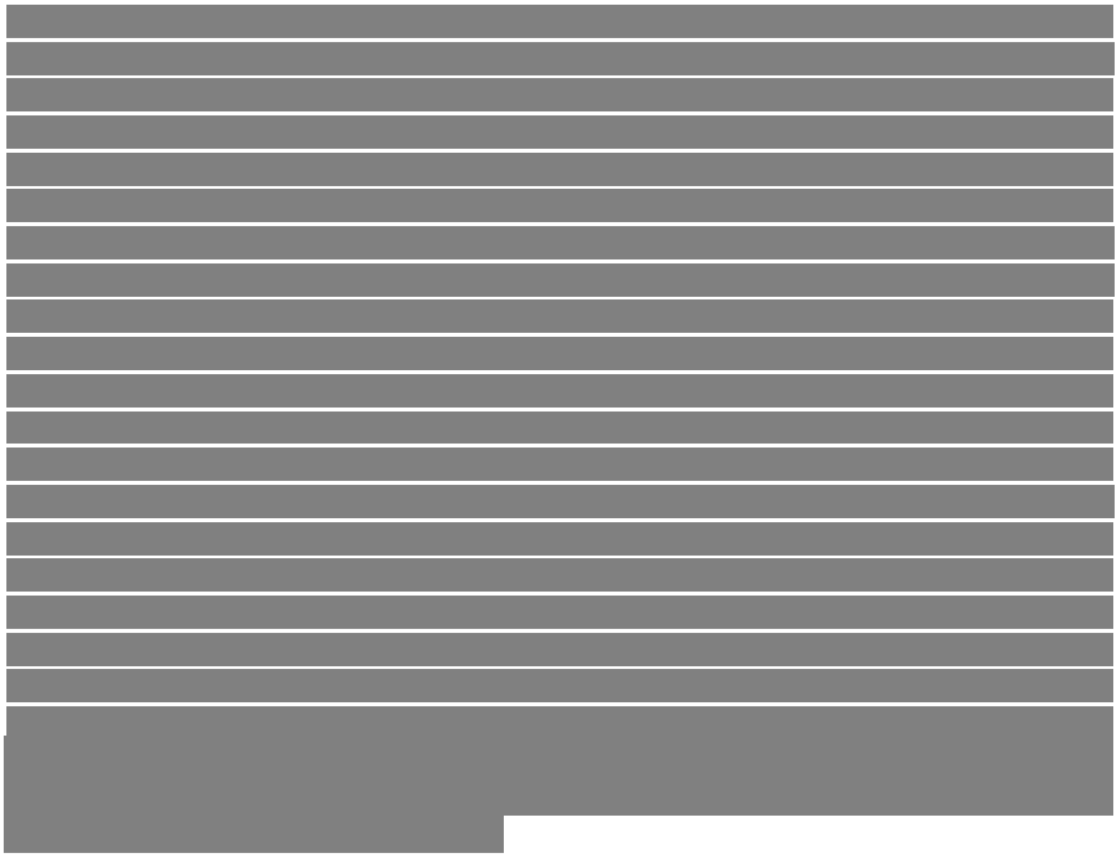






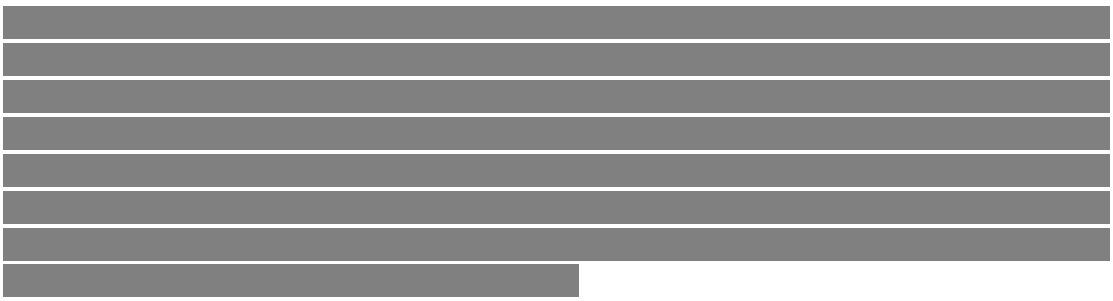
- Introgression of new mutations that enhance rust resistance into commercial wheat germplasm (D8.4)

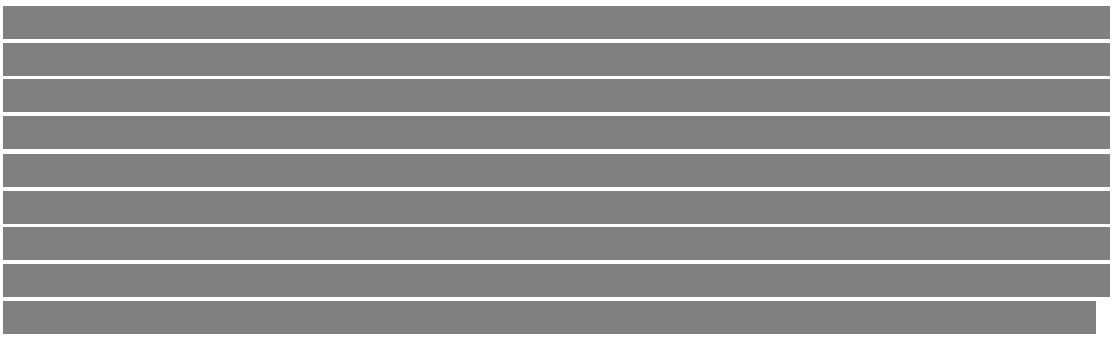
  ESR1 worked on the identification of wheat stem rust effectors by mutational genomics. In this study, ten wheat cultivars containing single dominant stem rust resistance (*Sr*) genes along with their recurrent parents were tested for resistance to a *Puccinia graminis* f. sp. *tritici* (PGT) isolate that was pathotyped as TKTTF. Six effective *Sr* genes where shortlisted as possible targets for cloning their corresponding PGT avirulence effectors. ESR1 then generated an Ethyl methanesulfonate (EMS) mutant population of 12 000 individual pustules. ESR1selected, purified and sequenced random mutants and determined the average mutation density to be 1 single nucleotide variant (SNV) per 90 kb. From this, the number of independently derived gain-of-virulence mutants required to confidently identify a given *Avr* gene was calculated to be five. These mutant urediniospores were inoculated onto three resistance (*Sr*) genes. Independently derived gain-of-virulence mutants on wheat plants carrying *Sr43* (9 mutants) or *Sr45* (14 mutants) were obtained. The selected *avrSr43* and *avrSr45* PGT mutants maintained the same race specificity as the wildtype when tested by inoculating them onto the stem rust standard international differential set. The fitness of *avrSr43* mutants was tested by reading chitin fluorescence from infected leaf tissue of *Sr43* and its recurrent parent Chinese Spring at four timepoints. Loss of *avrSr43* did not seem to affect pathogen fitness compared to the wildtype. This suggests potential lack of durability of PGT resistance conferred by *Sr43* when deployed as a single gene. Sequencing and comparative genomics of *avrSr43* and *avrSr45* mutants and wildtype is underway to identify the corresponding *avr* effector genes. Through this project we hope to clone *avrSr43* and *avrSr45*.

- Development of molecular markers to track genes in breeding programmes and hence expedite the process for the industry (D8.5)

  ESR2 at BOKU in line with the original plans for this Deliverable, contributed to the marker analysis carried out by ESR3 at UCD. These contributions are reflected in outline of results from UCD (please see below).

ESR3 identified allelic variation within FHB/DON responsive genes and their promoters between wheat cultivars CM82036 (FHB resistant) and Remus (FHB susceptible). Sequence analysis revealed the genotype–specific single nucleotide polymorphisms (SNPs) in conserved domains and the promoters of FHB/DON-responsive genes. 15 Single nucleotide polymorphisms (SNP) were identified between FHB resistant and susceptible wheat cultivars. 360 double haploid (DH) lines obtained from BOKU were used for KASPar genotyping for 15 SNP in Biogemma, France. The results of KASPar genotyping exhibited 10 polymorphic SNP in DH lines. Quantitative trait loci (QTL) mapping was performed using the phenotypic data obtained from BOKU for FHB traits. SNPs within *TaNAC-5A* and *TaMPT-5A* were associated with FHB resistance in the field but these genes did not co-localise within known FHB QTL in wheat. Results suggest further validation of candidate gene for FHB resistance in wheat.





- Development and use of a model to predict disease resistance in bread and durum wheat breeding populations (D8.6)

  ESR2 worked on the development of a model to predict disease resistance in wheat breeding populations. Selection for multiple traits is a highly challenging task for breeders due to potential unfavourable associations between characters. Fusarium head blight FHB, being one of the most relevant diseases affecting durum wheat frequently shows in this respect an unfavourable correlation with morpho-agronomical traits like plant height and heading date. In this study, we used a cross-validation scheme to assess the prediction ability of the genomic predictions (GP) for FHB severity relying on genomic best linear unbiased prediction (GBLUP) models in a diverse panel of 178 durum wheat lines evaluated across five environments. Additionally, we compared three types of approaches to include heading date (HD) and plant height (PH) as covariates into the analysis: (i) correcting FHB severity values before training GP models, (ii) tuning the GP model parameters that included multi-trait alternatives, and (iii) adjusting the genomic-based predictions by restriction indexes. Models that weighted genomic estimated breeding values (GEBV) by restriction indexes as well as

models that predicted FHBms values corrected by regression-based methods were efficient alternatives in diminishing the HD trade-off, nonetheless they were also associated with large reductions in prediction ability for FHB severity. After a simulated round of genomic selection, considering HD as fixed effect in the GP model were the most suitable alternative to select a higher proportion of genotypes moderately resistant with lower-than-average heading date and plant height estimations. Hence, an appropriate GP model given unfavourable association between characters should combine high predictabilities and adequate reduction of undesired trade-offs.

This WP is also associated with MS11, regarding Secondments. Details of Secondments have been included in the Continuous Reporting of the portal and are given as an Annex B to this report.

1.2.9 Work Package 9 – Research Programme – 'Biologicals and Bioactives'

The principal objectives of this WP are to identify new endophytes with potential to control cereal diseases, to determine the mode of action of these agents and others currently under investigation, and to determine their disease control potential in the field.

To achieve these objectives, several specific tasks have been identified and are allocated to various individual projects or sub-groups of ESRs. The deliverables D9.2 and D9.3 were submitted on 2 March 2018 and D9.4, D9.5, D9.6 and D9.7 were submitted on 16 November 2018. A brief description the research work carried out in these deliverables until the end of this reporting period are discussed below.

- Identification of new endophytes that show potential for disease control (D9.1)

  A report on this Deliverable (D9.1) has been submitted via the Participant Portal during the first reporting period (RP1) and a brief description of the work of ESR6, ESR7 and ESR8 in identifying endophytes that exhibit potential for disease control was given in first Periodic Report.

- Determination of the mode of action of endophytes that show potential for disease control (D9.2)

  ESR6 identified two endophytes that significantly reduced *Septoria tritici* blotch of wheat in the field. Both strains reduced growth of the pathogen *in vitro* and one strain showed production of antimicrobial secondary metabolites that inhibited pathogen growth under these conditions. (Latz et al 2020 [redacted] A comprehensive analysis was conducted to investigate responses of the host *Triticum aestivum* and the pathogen *Zymoseptoria tritici* to the fungal biocontrol agent *Acremonium alternatum* ML38. The tripartite interaction of host, pathogen and biocontrol agent during disease progression was investigated with quantitative microscopy, β-1,3-glucanase assays, dual-RNA-seq and RT-qPCR. While *A. alternatum* alone did not induce major responses in wheat, it considerably affected the wheat defence responses against *Z tritici*. Thus, here there was an earlier and enhanced host defence response, involving PR-protein expression, hypersensitive responses and anthocyanin biosynthesis, mediated through both JA/ET- and SA-dependent signalling. Evidence was found that *A. alternatum* affected *Z. tritici*

growth through two main mechanisms: antibiosis and induced resistance. Antibiosis is likely responsible for reduced *Z. tritici* spore germination on the leaf surface, but not the final disease reduction, since penetration events were not significantly reduced. Data indicate that the reduced *Z. tritici* biomass is the result of induced resistance in the host, characterised by the earlier and enhanced host defence responses. The sum of defence responses likely restricted *Z. tritici* growth, resulting in reduced necrotic symptoms and number of pycnidia.

ESR7 has isolated endophytic fungi from wheat spikes with potential biocontrol capacity. Twenty different species have been identified using ITS sequencing. These organisms have been assessed for their capacity to produce antifungal compounds by co-culturing with the plant pathogenic fungus *Fusarium graminearum* on standard PDA medium. Although most of the endophytic isolates significantly reduced *F. graminearum* growth compared to control plates, there were no visible inhibition zones. Therefore, it is predicted that direct inhibition is not a mode of action of these organisms against *F. graminearum*. However, at least 3 of these isolates can reduce Fusarium seedling blight caused by *Fusarium culmorum* in spring wheat seedlings after 15 days when they are coated onto the seeds simultaneously with the pathogen. Confrontation tests only show whether the BCA activity in the interaction is based on the production of direct antimicrobial activity, hence, other mechanisms like mycoparasitism, niche competition and induced resistance in the plant need to be further examined. Our subsequent experiments [redacted] where we studied the wheat transcriptome in in two- and three way interactions between the pathogen (*Fusarium graminearum*) a BCA (*Penicillium olsonii*) and the host plant (wheat), have demonstrated that the BCA activity induced by *P. olsonii* is in fact based on induced resistance.

. [redacted]

[redacted]

[redacted]

[redacted]

- Determination of the complementarity of endophytes and bioactive silicon (D9.3)

  ESR6 tested the effect of different silicon products and applications on the disease development of Septoria tritici blotch in wheat seedlings. Silicic acid, silica gel and potassium silicate, which were previously found to reduce disease symptoms on other plants, were applied as seed treatment, watering and foliar application. No disease reducing effect was observed. Furthermore, two silicon products extracted from algae were tested as foliar spray but did not show a protective effect against the disease. Therefore, this part of the project was terminated.

- Elucidation of the effect of host genotype on endophyte efficacy for disease control (D9.4)

  ESR7 has isolated several endophytes from Lantmännen winter wheat cultivar SW 150557 (Nimbus). Two of these isolates were shown to successfully reduce Septoria tritici blotch symptoms in wheat both under controlled conditions in cv. Sevin as well as in field trials in Denmark in cv. Mariboss. Also, one of these isolates is capable of reducing Fusarium head blight symptoms both *in vitro* and in greenhouse tests in spring wheat cultivar Diskett. This endophyte has shown a remarkable robustness in biological control capacity on the different cultivars (Latz et al ibid).

  ESR7 has performed endophyte isolation using wheat spikes from two different cultivars (KW-Nils and SW14308). Despite being grown in two different locations, isolated endophytic communities were alike and community analysis showed that both cultivars possess similar fungal community composition. This shows that fungal communities associated to wheat are more resilient and that host genotype has little influence in the assemblage of endophytic communities compared to other variables such as weather[redacted]

Several lab tests have confirmed that several of these endophytes were able to reduce Fusarium head blight in wheat. It was noted that approximately 70% of them came from the cultivar with greater tolerance to the pathogen. This suggests that these endophytes could be partially associated to the field cultivar tolerance or at least show a preference for this host. The efficiency of these isolates to prevent Fusarium head blight was also tested in field trials and no significant differences in disease severity or in yield were observed. This reveals that host genotype has a small effect on beneficial endophyte colonisation. However, this effect did not influence the efficiency of these endophytes as biocontrol organisms at large scale.

- [redacted]

- Determination of the potential of RNAi for broad spectrum disease control (D9.6)

  ESR 10 examined the bidirectional accumulation of sRNAs in the interaction of the hemibiotrophic rice blast fungus *Magnaporthe oryzae* (*Mo*) with the grass model plant *Brachypodium distachyon* (*Bd*). By comparative deep sequencing of sRNAs and mRNAs from axenic fungal cultures and infected leaves and roots, a wide range of fungal sRNAs that accumulated exclusively in infected tissues were found. Amongst those, 20-21 nt candidate sRNA effectors were predicted *in silico* by selecting those *Mo* reads that had complementary mRNA targets in *Bd*. Many of those mRNAs predicted to be targeted by *Mo* sRNAs were differentially expressed, particularly in the necrotrophic infection phase, including gene transcripts involved in plant defence responses and signalling. Vice versa, by applying the same strategy to identify *Bd* sRNA effectors, it was that found that *Bd* produced sRNAs targeting a variety of fungal transcripts, encoding fungal cell wall components, virulence genes and transcription factors. Consistent with function as effectors of these *Bd* sRNAs, their predicted fungal targets were significantly down-regulated in the infected tissues compared to axenic cultures, and deletion mutants for some of these target genes showed heavily impaired virulence phenotypes. This study is the first experimentally-based evidence for bidirectional ckRNAi in a grass-fungal pathosystem (Zanini et al. 2019).

  Two core protein components, Argonaute (AGO) and Dicer (DCL), are central to the RNAi machinery of eukaryotes. Little is known about the conservation and specific roles of these proteins in cereal crops. ESR10 utilzed *in silico* tools to investigate the

structure and related functions of AGO and DCL proteins from the model grass *Brachypodium distachyon*. Based on the presence of characteristic domains, 16 BdAGO- and 6 BdDCL-predicted proteins were identified. Brachypodium contains a single copy of DCL1 and DCL4 and two copies each of DCL2 and DCL3. Members of the BdAGO family were placed in all three functional clades of AGO proteins previously described in *Arabidopsis*. The catalytic tetrad of the AGO P-element-induced wimpy testis domain (PIWI), which is required for endonuclease activity, is conserved in most BdAGOs, with the exception of BdAGO1, which lacks the last D/H residue. Three-dimensional modelling of BdAGO proteins using tertiary structure prediction software supported the phylogenetic classification. ESR10 also predicted a provisional interactome network for BdAGOs, their localization within the cell, and organ/tissue-specific expression (Šečić et al. 2019).

ESR10 has also successfully explored the potential pathogen range that can be targeted by double stranded (ds)RNA via RNAi-based inhibition in five major crops and five major fungal pathogens relevant for European agriculture. Spray application of a dsRNA, which targets the three fungal genes *FgCYP51A*, *FgCYP51B*, and *FgCYP51C*, inhibited *Fusarium graminearum* (*Fg*) on three different cereal plant species: barley, wheat, and *Brachypodium*. It was confirmed that other Fusarium species, such as *F. culmorum* (*Fc*), also is sensitive to *CYP51*-derived dsRNAs. Treating *Fc* with various dsRNAs targeting the genes *FcCYP51A*, *FcCYP51B* and *FcCYP51C* was destructive to the fungus and resulted in growth retardation in *in vitro* cultures. Moreover, it could be demonstrated that *i.* dsRNAs targeting *MoCYP51* mRNA controls *Magnaporthe oryzae* on wheat, barley and Brachypodium, and *ii.* dsRNA targeting *BcCPY51* mRNA controls *Botrytis cinerea* (*Bc*) on tomato. Moreover, collaborative experiments with Phytoauxilium revealed a potential to use compositions containing the alternative plant protection product "VEGLYS" and dsRNA for the control of *Bc* on strawberry (Okubara et al. 2017). Further experiments are currently being conducted that explore the possibility to enhance the dsRNA activity on greenhouse plants by using formulations on basis of degradable biopolymers. (Koch et al. 2018)

Koch A, Ste n E, Koge KH (2018) RNA-based d sease contro as a comp ementary measure to f ght Fusar um fung through s enc ng of the Azo e target cytochrome P450 anostero C-14 α-demethy ase. Eu J P ant Patho DOI10.1007/s10658-018-1518-4.

Šeč ć E, Zan n S, Koge KH (2019) Further e uc dat on of the ARGONAUTE and DICER prote n fam es n the mode grass spec es *Brachypodium distachyon*. Front P ant Sc do : 10.3389/fp s.2019.01332.

Zan n S, Šeč ć E, Busche T, Ka nowsk J, Koge KH (2019) D scovery of nteract on-re ated sRNAs and the r targets n the *Brachypodium distachyon* and *Magnaporthe oryzae* pathosystem. PLOS Path subm tted; B oRx v do : https://do .org/10.1101/631945.

ESR10 has not conducted the experiments with combined dsRNA with biologicals because during the progress in the work it became clear that a better strategy for future application is to analyze the "structural rules" of dsRNA activity. We found that a very efficient way of improving dsRNA activity is designing molecules that can target more than one gene. Thus, testing different dsRNA designs appeared to be a prerequisite for dsRNA activities under field conditions. The experiments we have done in this respect are very promising (Fig. 1). Using a dsRNA which can target three different genes was very effective on inhibiting the growth of the pathogenic fungus *Fusarium graminearum*, which causes the head blight disease in wheat and barley. We need to extend these experiments to have an excellent basis for a relevant publication that is

now scheduled for end 2020. Once we have assessed the rules for dsRNA design in more detail, we will combine optimized dsRNA with biologicals.

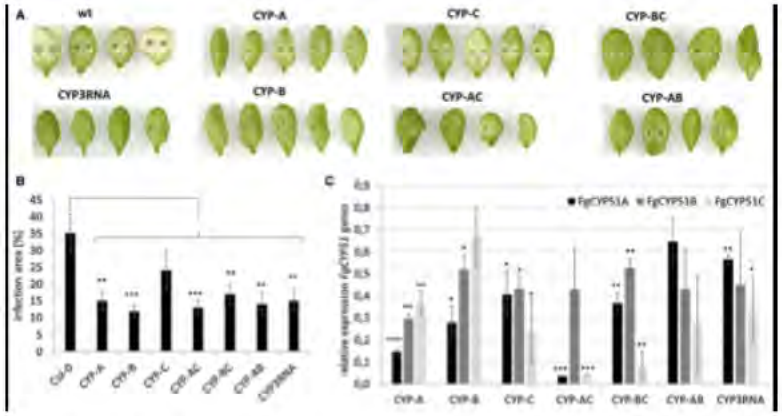


Fig. 1 Host induced gene silencing in Fusarium graminearum upon infection of Arabidopsis expressing CYP51 dsRNAs. (A) Fifteen detached rosette of CYP51 dsRNA expressing Arabidopsis plants (T2 generation) were drop inoculated with 5 × 10^4 conidia/mL. Infection symptoms were evaluated at 5 days post inoculation (dpi). (B) Quantification of the visibly infected area at 5 dpi shown as percentage of the total leaf area. Bars represent standard errors (SE) of two independent experiments, each using 15 leaves of ten different plants for each transgenic line. Asterisks indicate statistically significant (**P < 0.01 ***P < 0.001 Student s t test) differences between CYP51 dsRNA expressing versus wild type (wt) genotypes. (C) Gene specific expression of fungal genes FgCYP51A, FgCYP51B and FgCYP51C was measured by qRT PCR. Gene expression was first normalized against the fungal reference gene EF1 α (FGSG_08811) and subsequently normalized against the Δ ct of the respective control. cDNA was generated after total RNA extraction from infected leaves at 5 dpi. The reduction in fungal gene expression in infected CYP51 dsRNA expressing versus wt leaves was statistically significant. Error bars represent standard deviations (SD) of two independent experiments each using 15 leaves of ten different plants for each transgenic line. Asterisks indicate statistical significance (*P < 0.05 **P < 0.01 ***P < 0.001 Student s t test).

ESR11 tested several naturally occurring small RNAs from Fusarium infected barley for functional validation based on virus-induced gene silencing (VIGS). Barley-derived small (s)RNAs that had sequences homology to fungal target genes in *Fc* were transferred by VIGS to wheat leaves prior to infection with *Fc*. One out of 10 selected candidate target genes governed resistance upon its down-regulation with the appropriate sRNA. To confirm this effect further, experiments are still ongoing.

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As with WP8 and WP10, this WP is also associated with MS11, regarding Secondments.

1.2.10 Work Package 10 – Research Programme – 'Trade-off and Pathogen evolution'

The principal objectives of this WP are to identify genes and genetic loci that have application for broad-spectrum resistance and to determine if enhanced knowledge of pathogen evolution can help develop a smarter farming approach.

Again, to achieve these objectives, a number of specific tasks have been identified and are allocated to various individual projects or sub-groups of ESRs. The deliverables D10.2, D10.3 and D10.4 were submitted on 16 November 2018 and D10.5 was submitted on 9 December 2019. A brief description the research work carried out in these deliverables until the end of this reporting period are discussed below.

- Identification of wheat lineage specific genes (D10.1)

  This Deliverable (D10.1) was submitted in the first reporting period (RP1) on the portal and was discussed in the first Periodic Report. This Deliverable was associated with ESR15.

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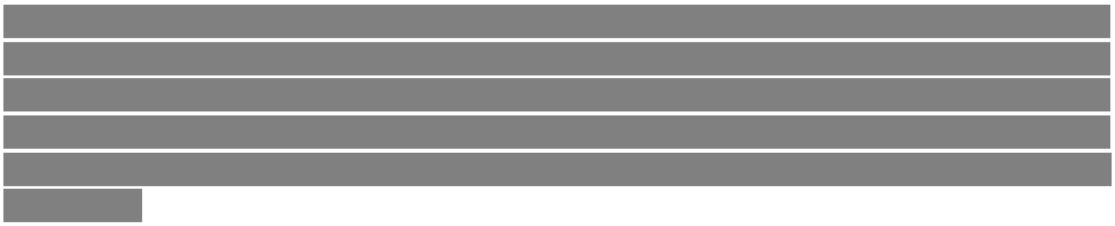

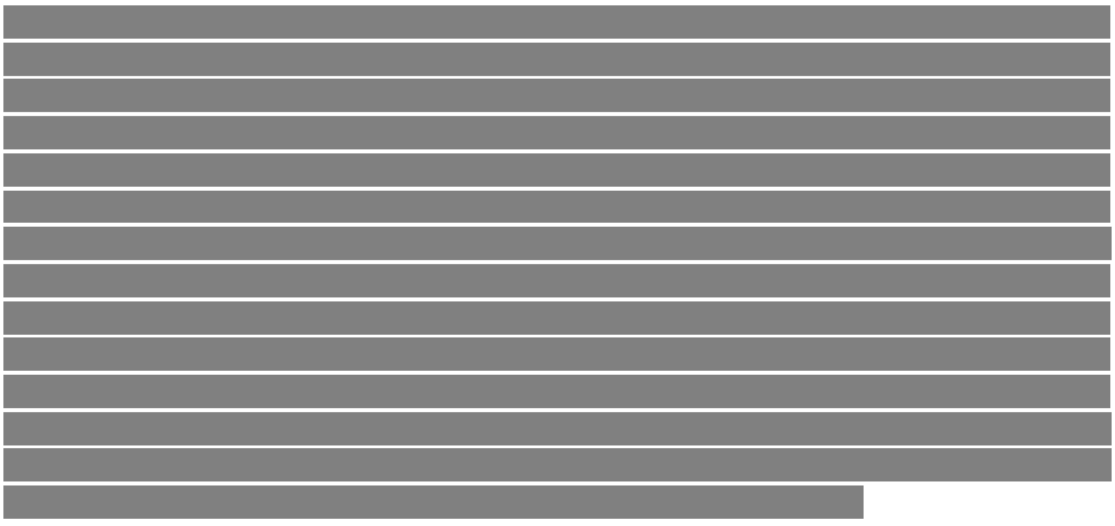

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1.2.11 Work Package 11 – Symposia

The principal objective of this WP is to hold annual symposia that facilitate ESR training in dissemination, outreach and network integration. While BOKU and UCD led these symposia, all the ESRs, the Scientists-in-Charge at the Beneficiaries and representatives of the Partner Organisations attended and contributed in these symposia. Wherever practical, researchers from outside the Consortium were allowed to participate.

1.2.11.1 Annual Symposia

The 2016 Annual Symposium was held in UCD in Dublin on 15 and 16 December 2016. This involved almost a day and half of talks and included the Supervisory Board Meeting for December 2016 and the General Assembly Meeting for 2016. For 2017, the Annual Symposium was scheduled to coincide with the Mid Term Review Meeting for CerealPath and took place on 27 and 28 September 2017. On 27 September, the Annual Symposium was held. The proceedings of these two symposia were discussed in the first Periodic Report (RP1) and the corresponding Deliverable D11.1 was submitted.

The third Annual Symposium in 2018 took place at Universität für Bodenkultur Wien (BOKU), Tulln, Austria from 05 - 07 December 2018. The Symposium started at 12 noon on 5 December 2018 with lunch, followed by a short welcome address by [redacted] A total of 13 ESRs attended this Symposium and presented their work to the Consortium in three blocks that aligned with the three scientific packages of the Action. The schedule of the presentations by the ESRs and their presentations were submitted as a part of the Deliverable 11.1.

A short workshop on Avenues of Future Funding Progression was conducted on 6th December after the ESR presentations. In the post-lunch session on 6th December and on 7th December, each ESR had a series of meetings with their consortium-wide supervisory panels. The purpose of these meetings was to provide bespoke guidance to each ESR on the basis of their specific needs in light of their progress to date.

1.3 Impact

It has always been envisaged that the CerealPath Action would have a significant positive impact not just on research in cereal disease control but also the training and career development of our ESRs and on the prestige and recognition of the Beneficiaries and Partners

as experts in training, education and research. Similarly, there must be a scientific benefit to carrying out the work, having a positive impact on society across Europe and beyond.

The ESRs of the CerealPath project have emerged not only as experts in their own area of research (as might be the norm in "traditional" research programmes) but that the Action has additionally given access to the highest quality training in their respective fields, ensured a spirit of innovation at the outset of the programme, provided an appreciation and understanding of the wider field of integrated disease control, given them hands-on experience in a relevant industry setting, provided industrial awareness, and has facilitated high inter-sectoral and geographic mobility. (See WP2, WP4, WP5, WP6, WP7, WP11 and secondments in WP8, WP9 and WP10)

From a scientific perspective, the Action has materially contributed to our understanding of plant-pathogen interactions. These developments will have direct impact on our ability to protect food supplies at the national, European and global levels.

From the Gene Discovery and Breeding strand (WP8), we have seen the following developments:

- Characterisations of two FHB and DON responsive genes, *TaMPT* and *TaSAM*, which have the potential to enhance both FHB resistance and yield in wheat.
- Identification and characterisation of potential resistance / susceptibility genes that play a significant role in response to Fusarium root rot in the model plant *Brachypodium distachyon*. A multi protein binding factor 1c and an Orphan gene in chromosome 2 which had orthologues in wheat are potential candidates for disease control in wheat.
- Mutant analysis has identified a candidate gene for 7BL QTL for leaf rust resistance and was characterised using virus-induced gene silencing.
- Mutant libraries for a UK stem rust isolate were developed and nine *avrSr43* and 14 *avrSr45* gain of virulence mutants were identified and further characterised. Cloning of avSr43 and avrSr45 and identification of their corresponding *avr* effector genes are underway.
- SNP markers within the FHB responsive genes, TaNAC-5A and TaMPT-5A were identified and these SNP markers were associated with FHB resistance in wheat
- The allelic variation of the leaf rust resistance gene Lr14a in wheat was characterised and two markers were positioned within the Lr14a gene.
- GBLUP approach was used as a genomic prediction model to predict Fusarium head blight resistance in wheat.

Within the Bioactive and Biological Control strand (WP9), a range of biocontrol agents were tested for their use as disease control agents in crops. From these projects, the following developments were seen:

- Identified and characterised several endophytes that showed potential for control of Septoria tritici blotch, Fusarium seedling blight, Fusarium head blight, *Gaeumannomyces graminis* and *Pyrenophora teres in* wheat and barley.
- Bioactive silicon products were tested for their effectiveness against Septoria tritici blotch in wheat seedlings
- Elucidation of the effect of host genotypes on endophyte efficacy in controlling Septoria tritici blotch in wheat.

- Auranta Harvest, a citrus derivative product showed antifungal activity against wheat pathogens and reduced FHB symptoms in wheat plants.
- Identified several fungal sRNAs and plant sRNAs and their possible targets in tissues of *Brachypodium distachyon* infected *Magnaporthe oryzae* and their possible mRNA targets were characterised. This is the first experimental evidence for bidirectional ckRNAi in grass-fungal pathosystem.
- Identified and characterised Argonuate and Dicer components of the RNAi machinery in the model grass *Brachypodium distachyon*
- dsRNA derived from the fungal genes *FgCYP51A*, *FgCYP51B* and *FgCYP51C*, when sprayed on cereal plants effectively reduced the symptoms for several crop diseases.
- Three markers. which significantly correlated with FHB resistance in wheat, were identified in two wheat genes that function either in RNAi processing or in intracellular RNAi transport and secretion. These markers have a potential to be used to enhance HIGS efficiency via breeding.
- Two naturally occurring sRNA molecules in Fusarium infected barley were identified for their potential to control Fusarium disease in wheat.

For the Pathogen Evolution and Trade-Off strand (WP10), we have explored the dual role of genes that confer resistance or susceptibility to disease. From these projects we have seen the following developments:

- Identified that a shorter *Z. tritici* Latent Phase is a primary contributor to wheat yield losses during Septoria tritici blotch. As such, identifying pre-commercial material that support an extended latent period is now seen as an important target for breeding against STB disease in winter wheat.
- Showed that wheat plants containing *Lr34*, a gene for resistance to biotrophic fungi which cause rust and powdery mildew, were highly susceptible to wheat blast, a non-biotrophic disease.
- The trade-offs between powdery mildew and Ramularia leaf spots disease was studied. Experiments confirmed that the use of *mlo* for mildew control in wheat incurs the risk of greater susceptibility to non-biotrophic pathogens.
- Identified several small secreted effector proteins secreted by *Zymoseptoria tritici* (*ZtSSPs*) during its interaction with wheat plants, of which ZtSSP2 might be involved in host defense responses mediated through ubiquitin.
- Identification and characterisation of STB and FHB responsive lineage specific genes were done. Some of these genes were tested for their role in disease resistance.

The Action has been specifically designed to adhere to the *EU Principles for Innovative Doctoral Training* and the training programme, and has demonstrated this commitment:

Innovation and creativity have been a core component of the integrated training for our ESRs from an early stage in the Action (e.g. WP4).

Intersectoral collaboration has been key to the rolling out of the training plan for the ESRs, who have all benefitted from exposure to experts from areas that would not normally interact with academic researchers, such as government officials and industry collaborators (e.g. WP5, WP6 and WP7).

The multidisciplinary nature of the training ensured that the ESRs received the very best of deep, discipline specific training within their research areas while also being exposed to the work of other, complementary scientific approaches (e.g. WP5, WP6, WP7).
There is a significant international profile within CerealPath when one considers both the broad geographic spread of the partners and the cultural and geographic background of the ESRs themselves. Within the ESR cohort, 13 countries from 4 continents are represented, highlighting a diversity of cultural and educational backgrounds. Interactions between and among the ESRs allowed for a broader understanding of real-world needs (as exemplified by group discussions at the WP5 School, for ełłmłłełł

Unlike traditional research projects, CerealPath has extensive academia-industry shared supervision. The fact that some ESRs were based in non-academic research environments (e.g. ESR9) while all of the others have supervisory input from industry collaborators materially contributed to a better understanding of how research can fit into the "real world".

A key component of the CerealPath training programme is exposure to industry, business and complementary skills. This materially contributed to the concept of an "industry ready" candidate who has been provided with an understanding of needs of multiple sectors.

A core skill for any researcher to learn is initiative and versatility. The exposure provided by CerealPath to a variety of different sectors ensured that our ESRs developed this key skill, improving their employability wherever they decide to go.

In terms of CerealPath's ability to contribute structuring doctoral and early stage researcher training at the European level, so far 6 ESRs have successfully completed their doctoral programme and remaining ESRs are at final phase of completion. The approximate dates of PhD thesis submission and defence for the ESRs who have not graduated yet are listed in Annex B. The ESRs have attained the necessary expertise, experience and skills to make them highly valued researchers and employees in the field of plant disease control and related areas of plant science and agriculture, thus making them pioneers who can contribute to global food security.

2. Update of the plan for exploitation and dissemination of result (if applicable)

An update on the plan of exploitation and dissemination of results have been submitted in the first Periodic Report (RP1). In this respect, there is no change proposed for exploitation and disseminations of results.

3. Update of the data management plan (if applicable)

No specific data management plan was developed as part of the CerealPath Action.

However, the Consortium Agreement does require that appropriate data be provided in an appropriate way by the Consortium Members to ensure that the objectives of the action can be achieved. The research data was managed at Beneficiary level and a specific need for a data management plan for the Action didn't arise.

4. Follow-up of recommendations and comments from previous review(s)

There was no follow-up recommendations and comments from the first review. However, the Commission had requested for revisions in the First Periodic report after the initial submission. The Periodic Report was resubmitted within the deadline after making necessary revisions.

5. Deviations from Annex 1 and Annex 2 (if applicable)

Overall, there have been very few deviations from the original plan as laid out in Annex 1 in this reporting period (RP2). There has been some delays and changes in secondment plans from the secondment plan that has been agreed before. The modifications to the secondments are outlined in Annex C. These changes have been communicated to the Project Officer on the portal.

5.1 Tasks

All the tasks of the Action have now been completed.
It is expected that the reminder of the ESRs will be completing their doctoral studies soon and submitting their PhD theses. More peer-reviewed scientific papers are expected to be published soon, as some are already submitted and under review. The beneficiaries will also identify potential outputs from the Action, including genes that enhance disease resistance, molecular markers for breeding, new biocontrol products, and know-how in terms of biocontrol product development, innovative training technologies or approaches for IP exploitation.

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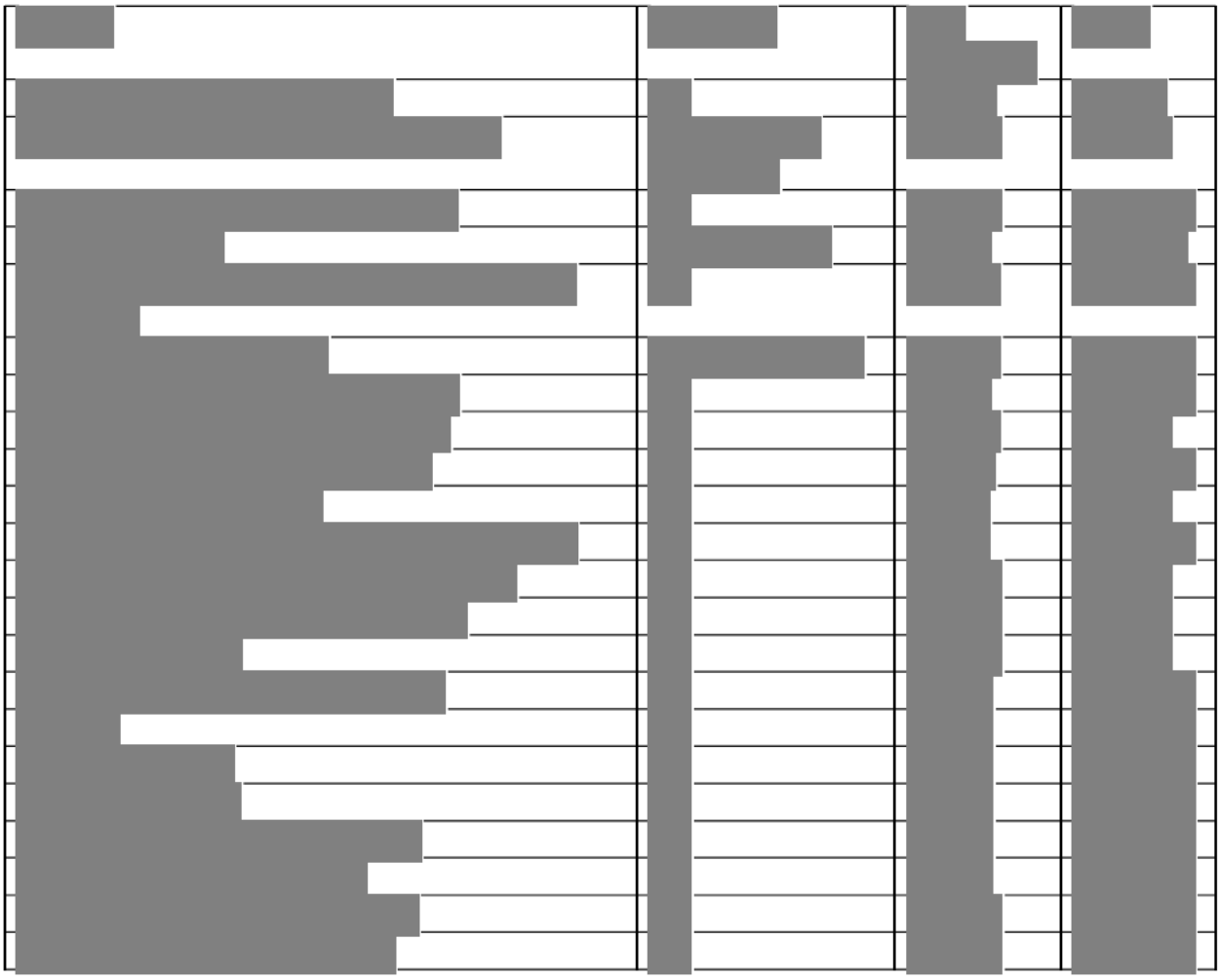

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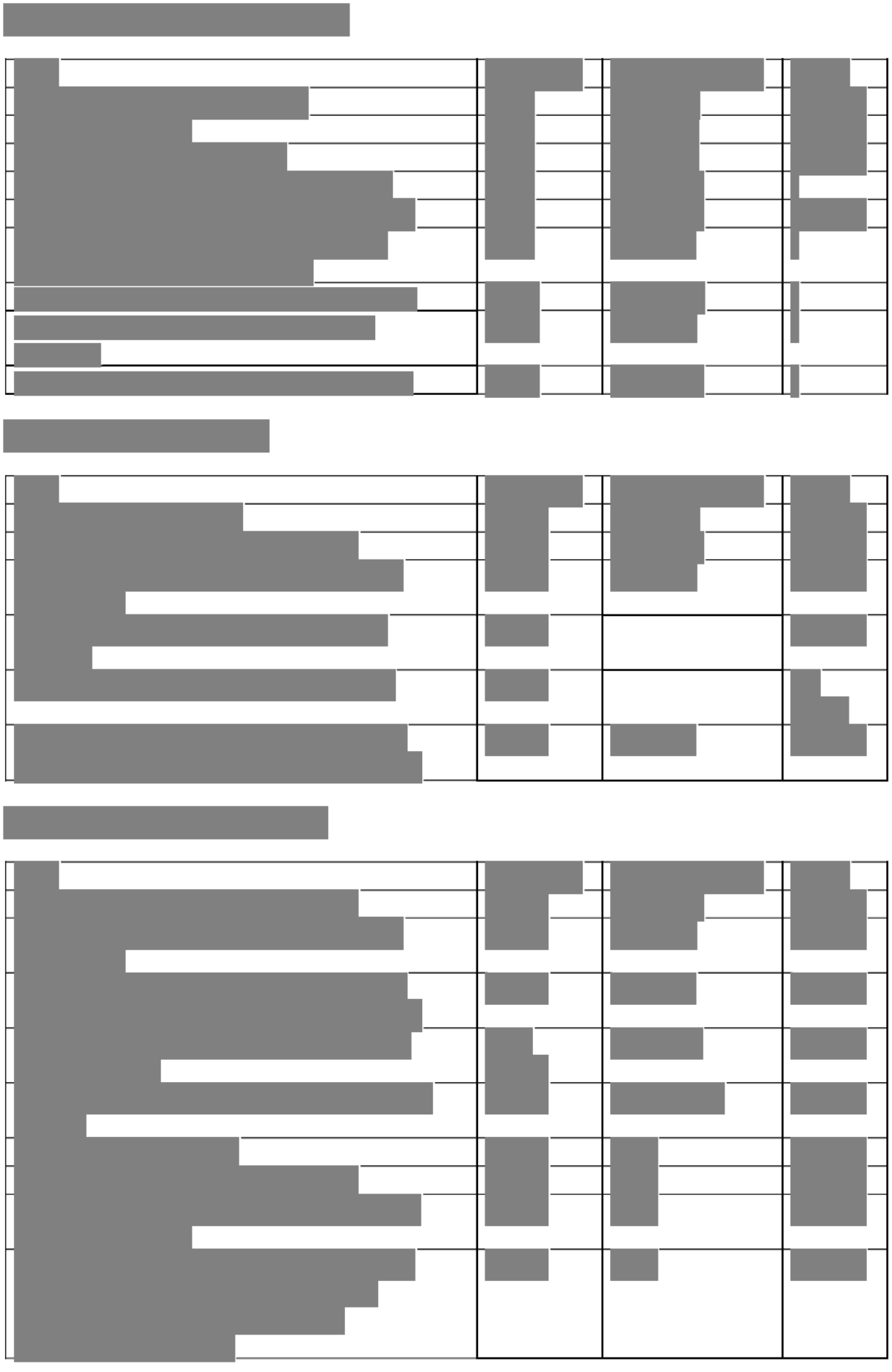

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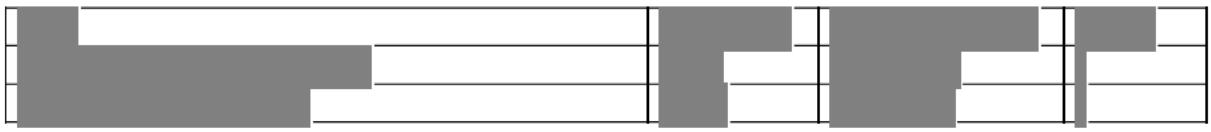

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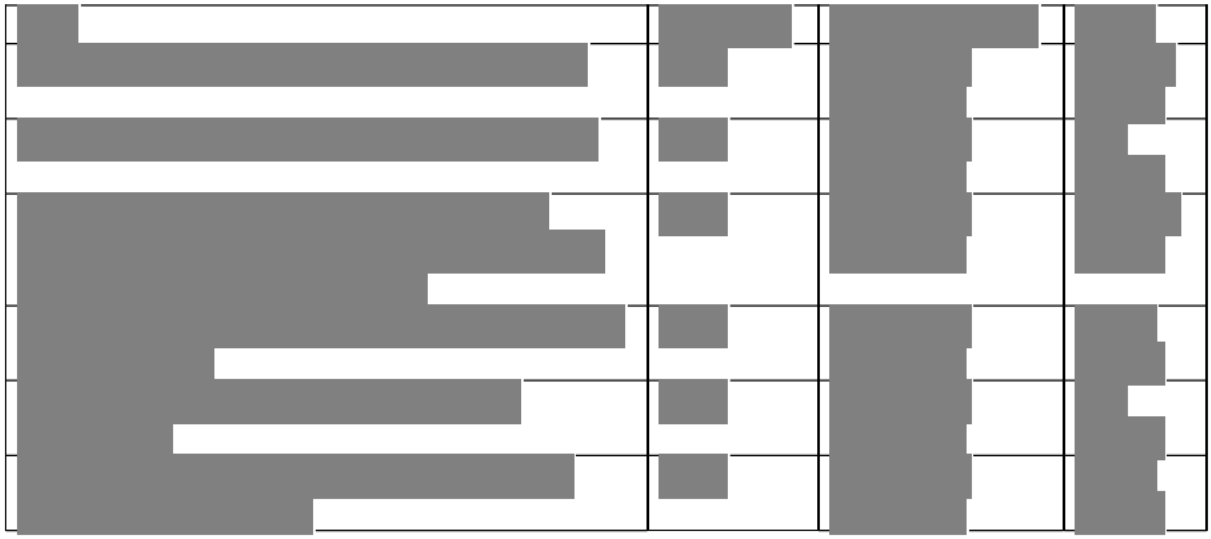

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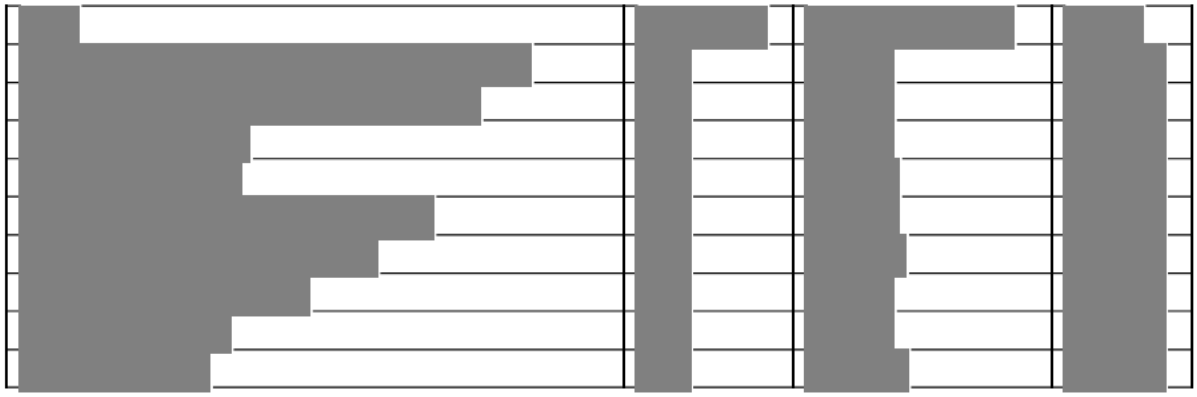

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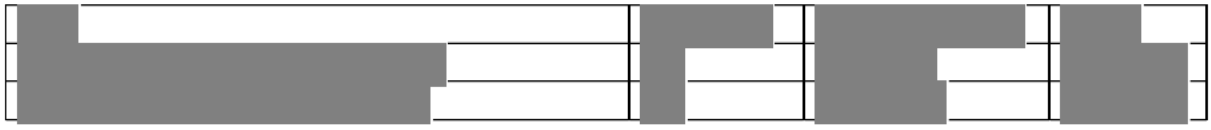

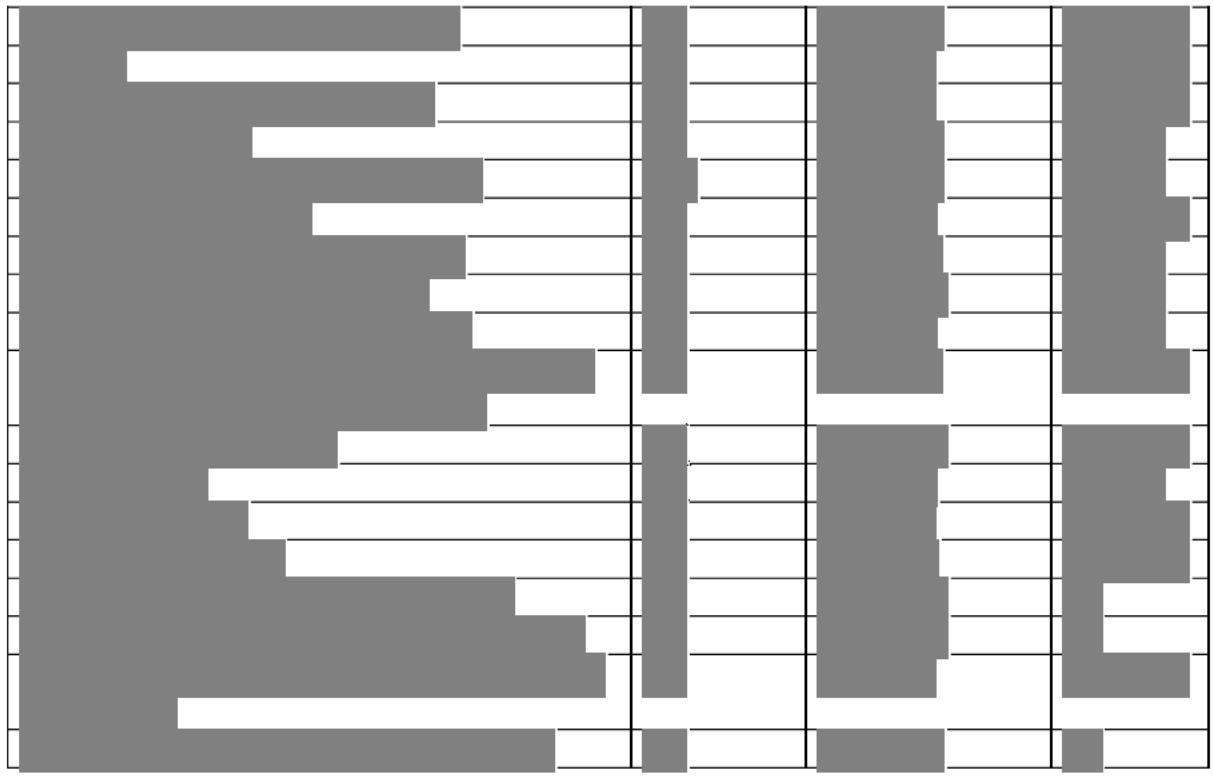

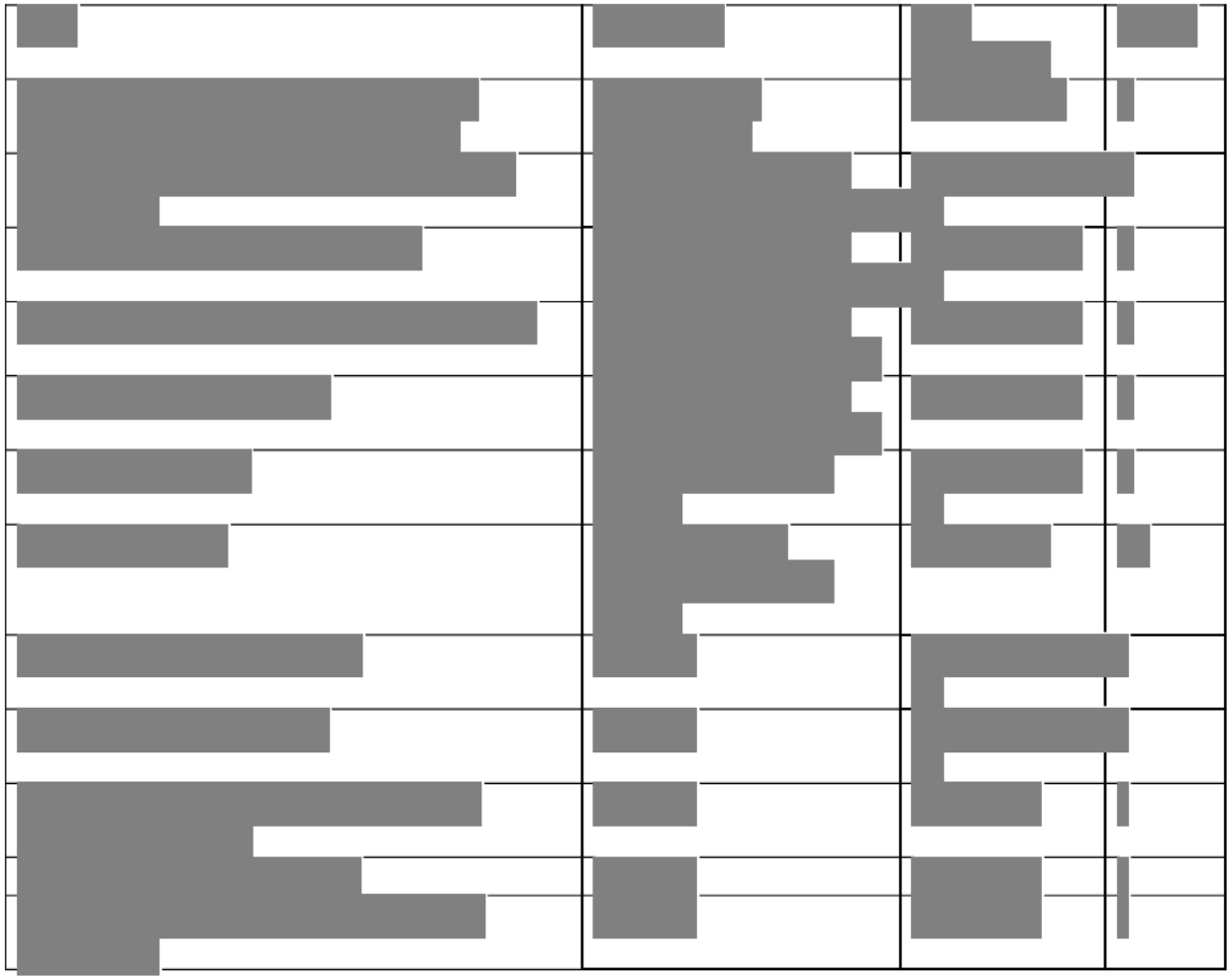

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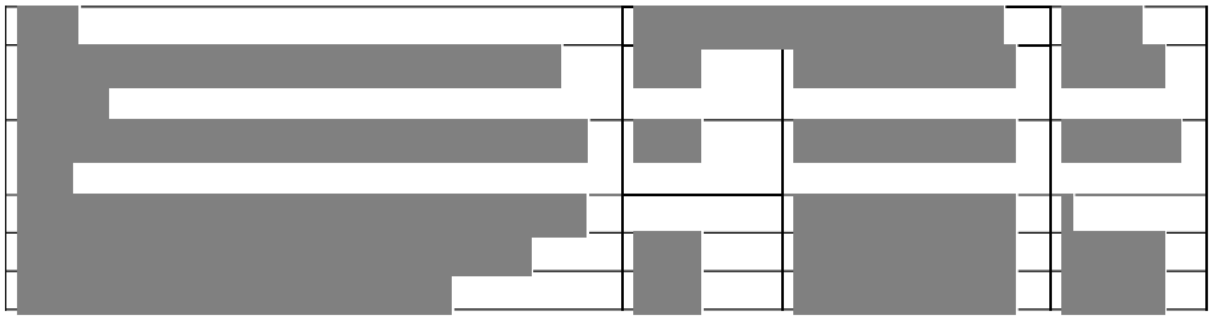

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