

Investigation of artificially applied drought and weather effects on Ramularia Leaf Spot and Fusarium Head Blight of barley

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Summary

Plants often face simultaneous abiotic and biotic stress under natural conditions. Global warming is increasing the strength and severity of abiotic stress, reducing plant growth and productivity. It is further proposed that global warming will increase the abundance of pathogens and the risk of diseases, potentially causing crop yield and quality losses. Therefore, several studies under field and controlled greenhouse conditions were conducted to gain knowledge on Ramularia Leaf Spot disease (caused by *Ramularia collo-cygni*) and Fusarium Head Blight (caused by *Fusarium* spp.) in barley under variable environmental conditions and to investigate the effects of drought stress on basal resistance of barley.

In a first step, two consecutive field studies were conducted on either 15 spring or winter barley genotypes to assess Ramularia Leaf Spot (RLS) severity in a field rainout shelter under artificially applied late-terminal drought or irrigation over several seasons. Different diagnostic methods revealed a variation in quantitative RLS resistance within each barley assortment. Despite strong seasonal effects, it was possible to select genotypes showing reproducible quantitative RLS resistance. In contrast, drought conditions strongly decreased levels of RLS disease parameter, whereby such suppressive drought-effects were less pronounced in winter barley. Here, fungal DNA contents in leaves of several genotypes even exceeded the values of the irrigated plants, indicating variations of RLS disease under persistent drought. Measurement of weather parameters and microclimate within plant canopy revealed low leaf wetness and duration under drought, indicating the high importance of moisture for RLS severity. Taken together, both studies show strong seasonal and environmental effects on RLS disease and identified quantitatively resistant barley genotypes in diverse field environments.

In another five-year field study, quantitative Fusarium Head Blight (FHB) resistance of 17 spring barley genotypes was evaluated after artificial soil surface or spray inoculation with *F. culmorum* and *F. avenaceum*. Visual symptom scorings, quantification of fungal DNA and mycotoxins revealed a genotypic differentiation in FHB infestation, even in hot and dry seasons. Fungal DNA contents positively correlated with species-specific mycotoxin contents in single years and over several seasons. Based on these disease parameters, a genotype ranking was used to normalize year effects and allowed the identification of stable quantitatively FHB-resistant genotypes. Recorded weather data was used for a multiple linear regression model, which identified periods during the reproductive phase of barley when weather conditions significantly influenced the colonisation of barley grains. In a further step, three genotypes showing different quantitative FHB resistance or susceptibility in the field were selected for

head infection with *F. culmorum* spores under irrigation or drought stress in the greenhouse and to study global gene expression under combined stress. Drought stress before infection strongly reduced FHB severity. Quantification of fungal DNA and stress-associated phytohormones or metabolites were utilized as physiological stress markers to correlate with the number and strength of differently expressed genes under single or combined stress. A Weighted Gene Co-Expression Network Analysis revealed 18 stress-responsive modules of co-expressed genes. These modules represent pathogen, drought or genotype-specific responses and correlate with physiological stress markers. An infection-associated module contained various genes involved in defence mechanisms and deoxynivalenol detoxification utilised from the tested genotypes with variable success towards *Fusarium* spp.. Additional drought further increased the number of differentially expressed genes and revealed that responses to single stresses are rather combined by the plant instead of arranging a novel transcriptional answer.

Overall, the present work comprises effects of environmental and weather conditions and specifically drought stress, determining quantitative resistance of barley towards the two important diseases Ramularia Leaf Spot and Fusarium Head Blight. The results intend to increase understanding of the environment-dependent disease resistance for adaptation towards complex stress.

Zusammenfassung

Pflanzen sind häufig gleichzeitig abiotischem und biotischem Stress ausgesetzt. Darüber hinaus erhöht die globale Erwärmung die Stärke und Schwere von Stress-Situationen und verringert somit ein optimales Pflanzenwachstum und Produktivität. Weiter wird angenommen, dass die globale Erwärmung das Vorkommen von Krankheitserregern erhöht, was zu Ertrags- und Qualitätseinbußen an Kulturpflanzen führen kann. Um in diesem Zusammenhang die Auswirkungen einer variablen Umwelt und speziell von Trockenstress auf die basale Krankheitsresistenz von Gerste zu untersuchen, wurden verschiedene Studien unter Feld- und kontrollierten Gewächshausbedingungen durchgeführt, um neue Erkenntnisse über die Krankheitsentwicklung von Ramularia-Blattflecken (verursacht durch *Ramularia collo-cygni*) oder Fusarium Head Blight (verursacht durch *Fusarium* spp.) zu gewinnen.

In einem ersten Schritt wurden zwei Feldstudien mit je 15 Sommer- oder Wintergersten-Genotypen durchgeführt, um Auftreten und Schwere von Ramularia-Blattflecken unter künstlich herbeigeführter Trockenheit oder Bewässerung über mehrere Jahre zu untersuchen. Unterschiedliche Diagnosemethoden zeigten dabei eine Variation in der quantitativen RLS-Resistenz innerhalb der einzelnen Gerstensortimente. Trotz starker saisonaler Effekte konnten Genotypen mit reproduzierbarer, quantitativer Ramularia-Resistenz selektiert werden. Im Gegensatz dazu waren einzelne Krankheitsparameter generell stark unter Trockenheit unterdrückt, wobei diese Effekte in der Wintergerste weniger ausgeprägt waren. Hier überstiegen die Pilz-DNA-Gehalte in den Blättern einiger Genotypen sogar die Werte der bewässerten Pflanzen, was auf eine veränderte Krankheitsentwicklung unter anhaltender Trockenheit hindeutet. Wetterdaten und die Erfassung des Mikroklimas im Bestand ergab eine geringere Blattnässe und -dauer unter Trockenheit, was auf eine Rolle der Feuchtigkeit für die Infektion von *R. collo-cygni* hindeutet. Insgesamt zeigten beide Studien starke saisonale und umweltbedingte Auswirkungen auf die Ramularia-Blattfleckenkrankheit. Darüber hinaus konnten unter verschiedenen Feldumgebungen quantitativ resistente Gerstengenotypen identifiziert werden.

In einer weiteren fünfjährigen Feldstudie wurde die quantitative Resistenz gegenüber Ährenfusariosen (FHB) von 17 Sommergersten-Genotypen nach Boden- oder Sprühhinokulation mit *F. culmorum* und *F. avenaceum* untersucht. Symptombonituren, die Quantifizierung der Pilz-DNA und von Mykotoxinen zeigten eine genotypische Differenzierung des FHB-Befalls, selbst in heißen und trockenen Jahren. Der Gehalt an Pilz-DNA korrelierte positiv mit dem Gehalt an Spezies-spezifischen Mykotoxinen in einzelnen Jahren und über mehrere Jahre

hinweg. Auf Basis dieser Krankheitsparameter wurde ein Genotyp-Ranking erstellt, um Jahreseffekte zu normalisieren und stabile, quantitativ FHB-resistente Genotypen zu identifizieren. Zudem wurden die aufgezeichneten Wetterdaten für ein multiples lineares Regressionsmodell verwendet, mit dem Zeiträume während der Ähren- und Kornentwicklung identifiziert werden konnten, in denen die Wetterbedingungen die Pilzbesiedlung der Ähren signifikant beeinflussten. In einem weiteren Schritt wurden drei Genotypen anhand ihrer variablen quantitative FHB-Resistenz oder Anfälligkeit ausgewählt und im Gewächshaus unter Bewässerung- oder Trockenstress angebaut. Zur Blüte wurde eine Ähreninfektion mit *F. culmorum*-Sporen durchgeführt, um die globale Genexpression unter kombiniertem Stress zu untersuchen. Trockenstress vor der Infektion reduzierte stark die Infektion. Die Mengen von Pilz-DNA und stressassoziierten Phytohormonen oder Metaboliten wurden als physiologische Stressmarker verwendet, um diese mit der Anzahl und Stärke der unterschiedlich differentiell exprimierten Gene unter alleinigem oder kombiniertem Stress zu korrelieren. Eine *Weighted Gene Co-Expression Network Analysis* ergab 18 Module von gemeinsam ko-exprimierten Genen. Diese Module repräsentierten pathogen-, trockenheits- oder genotypspezifische Reaktionen und korrelierten mit physiologischen Stressmarkern. Ein infektionsassoziiertes Modul enthielt verschiedene Gene, die an der Pathogenabwehr und der Detoxifizierung von Deoxynivalenol beteiligt sind und von den Genotypen mit unterschiedlichem Erfolg gegenüber *Fusarium* spp. genutzt wurden. Zusätzliche Trockenheit erhöhte die Anzahl der differentiell exprimierten Gene und zeigte, dass die Reaktionen auf kombinierten Stress eher eine Kombination von Einzelstress-Antworten als eine neuen transkriptionelle Antwort darstellt.

Die vorliegende Arbeit umfasst die Auswirkungen von Umwelt- und Witterungsbedingungen und insbesondere von Trockenstress auf die quantitative Resistenz von Gerste gegenüber der Ramularia-Blattfleckenkrankheit und Ährenfusariosen. Die Ergebnisse sollen dazu beitragen, die umweltbedingte Krankheitsresistenz besser zu verstehen, um Pflanzen an komplexe Stresssituationen anzupassen zu können.

List of publications

Hoheneder, F., Hofer, K., Groth, J., Herz, M., Heß, M., & Hückelhoven, R. (2021). Ramularia leaf spot disease of barley is highly host genotype-dependent and suppressed by continuous drought stress in the field. *Journal of Plant Diseases and Protection*, 128, 749-767. DOI: <https://doi.org/10.1007/s41348-020-00420-z> (**Publication I**)

Hoheneder, F., Groth, J., Herz, M., & Hückelhoven, R. (2023). Artificially applied late terminal drought stress in the field differentially affects Ramularia leaf spot disease in winter barley. *Journal of Plant Diseases and Protection*, 130, 1357-1370. DOI: <https://doi.org/10.1007/s41348-023-00790-0> (**Publication II**)

Hoheneder, F., Biehl, E. M., Hofer, K., Petermeier, J., Groth, J., Herz, M., Rychlik, M., Heß, M., & Hückelhoven, R. (2022). Host Genotype and Weather Effects on Fusarium Head Blight Severity and Mycotoxin Load in Spring Barley. *Toxins*, 14(2), 125. DOI: <https://doi.org/10.3390/toxins14020125> (**Publication III**)

Hoheneder, F., Steidele, C. E., Messerer, M., Mayer, K., Köhler, N., Wurmser, C., Heß, M., Gigl, M., Dawid, C., Stam, R., & Hückelhoven, R. (2023). Barley shows reduced Fusarium Head Blight under drought and modular expression of differentially expressed genes under combined stress. *Journal of Experimental Botany*, 74(21), 6820-6835. DOI: <https://doi.org/10.1093/jxb/erad348> (**Publication IV**)

Abbreviations

ABA	abscisic acid
DEG	differently expressed gene
DON	deoxynivalenol
DRR	Dry Root Rot
FACE	free air CO ₂ enrichment
FCR	Fusarium Crown Rot
FHB	Fusarium Head Blight
FRR	Fusarium Root Rot
IPM	integrated pest management
MAMP	microbe associated molecular pattern
MAPK	mitogen associated phosphorylation kinase
MLO	mildew locus O
MLR	multiple linear regression
MYB	myeloblastosis transcription factor
NAC	NAM, ATAF and CUC transcription factor
PAMP	pathogen associated molecular pattern
PR	pathogenesis related
PRR	pattern recognition receptor
PTI	pattern triggered immunity
qPCR	quantitative polymerase chain reaction
RLS	Ramularia Leaf Spot
ROS	reactive oxygen species
SOTA	self-organizing tree algorithm
WGCNA	weighted gene correlation network analysis
ZEA	zearalenone

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1 Introduction

1.1 Impact of global warming on plant diseases (global scale)

Global warming represents one of the most influential challenges in the near future, causing devastating impacts on world agriculture, food production and security, further changing global natural ecosystems, human health and societal conditions (IPCC, 2021). The extensive emissions of anthropogenic greenhouse gases are raising the global mean temperature and will provoke changes and new dynamics from global to local weather systems. Additionally, environmental conditions occurring in a particular area are strongly dependent on the climate and are sensitive to external influences. Extreme environmental conditions will generally become more likely from a daily to seasonal time range due to global warming. Future climate predictions forecast lower mean precipitation for middle-latitude and subtropical areas with risks of persistent droughts. Simultaneously, single precipitation events will become very likely more frequent and intense in these spatial regions and the tropical areas (IPCC, 2021). Hence, direct weather impacts will produce stronger, more frequent and long-lasting heat waves and droughts, heavy storms or rainfalls, causing flash floods over large areas. Together, varying environmental and weather conditions increase the vulnerability of agricultural systems to multiple abiotic and biotic stress factors, reducing productivity and food safety, increasing land degradation or causing fluctuating food prices (Farooq et al., 2009; Foley et al., 2011; Olesen et al., 2011). In addition, agricultural production and related land uses are significant contributors to global warming (Lynch et al., 2021). Consequently, emissions from the agricultural sector promote its vulnerability to climate change-derived negative impacts on agriculture. With a growing global human population and increasing food demands expected in the following decades, gaps in food supply will likely enlarge. Furthermore, predicted food demand will rise especially in developing countries and put pressure on agricultural systems and food production. Therefore, revealing uncertainties of possible climate change impacts on agriculture and food production, security and distribution support implementing mitigation strategies in agriculture (Farooq et al., 2023).

To meet increasing food demands under unfavourable environmental conditions, novel and comprehensive strategies are necessary to improve climate-resilient global agriculture and climate change mitigations (Malhi et al., 2021). Therefore, multiple strategies are proposed to reduce agricultural vulnerability or to enhance the adaptation of agricultural systems to a changing climate: a stop in the expansion of croplands and land-use change, sustainable

intensification with reformation of farming practices and distribution of production inputs, plant breeding for robust and multiple stress-resistant crops, improved integrated pest management and a reduction of pre- and post-harvest losses represent possible strategies to meet demands on rising food supply and safety, which is increasingly endangered by global warming (Foley et al., 2011; Campbell et al., 2014; Fones et al., 2020; Shahzad et al., 2021).

In the last decades, multiple climate scenarios have been used to model various effects on global to local crop production to better understand future climate-related impacts on agroecosystems and the sensitivity of cropping systems to external changes. Therefore, a focus on future demands and adjustments is discussed with regard to future crop production and food security (Rosenzweig & Parry, 1994; Parry et al., 1999; Fischer et al., 2002; Kang et al., 2009; Lobell et al., 2011; Chaloner et al., 2021). The currently observed trends in climate change support the sensitivity of crop yields to rising temperatures and changes in precipitation patterns in the last decades. Variations in crop yield strongly depend on conditions in the considered region, the used cultivation measures and input levels (Rosenzweig & Parry, 1994; Parry et al., 1999). Consequently, current climate-driven yield losses support the estimation of future scenarios on global yield losses, limitations in regional food supply and fluctuations of respective food prices in the following decades (Lobell & Field, 2007; Lobell et al., 2011; Olesen et al., 2011). In particular, high variability in seasonal weather conditions is hardly predictable but strongly affects the stability of global food supply and prices. For this purpose, risks of crop failure and general vulnerability to climatic factors can be reduced on the farm level, comprising short-term adjustments and long-term adaptations by farmers (Alexandrov et al., 2002; Trnka et al., 2004; Olesen et al., 2011). Specifically, e.g. broad crop rotations, the use of newly climate-adapted cultivars, adjusted sowing date, crop diversification and improved farming management represent measures to spread risks of weather-dependent crop failure, in particular with adjustments to the respective cropping area (Olesen et al., 2011; Henry, 2020).

In addition to climate change-related intensification of abiotic stress factors on crops, pests and pathogens are important threats for agricultural systems worldwide and cause potential annual crop losses of 18 or 16%, respectively, resulting in high economic losses, reduced crop yield and food quality (Oerke, 2006). Furthermore, the high dependency of the world food supply on a few major crops (rice, wheat, maize, soybean, sorghum) on approximately 40% of cultivated land area determines vulnerability to emerging pests and pathogens. Consequently, plant production highly depends on crop protection measures (Lobell & Field, 2007; Savary et al., 2019; Chaloner et al., 2021).

The basic prerequisite for determining disease outbreaks and severity is the mutual interaction of the host plant with the environment and a virulent pathogen (Stevens, 1960). In the context of climate change, dynamics in host-pathogen-environment interactions likely provoke high variations in disease severity and become less predictable. For instance, rising global temperatures and a shift of climatic conditions directly or indirectly, respectively, affect the geographical abundance and spreading of pests and pathogens. Increasing variations in seasonal host plant phenology and further population dynamics of pathogens demonstrate highly variable factors on the host and the pathogen side (Juroszek and von Tiedemann, 2013). Consequently, a globally rising incidence of pests and plant diseases is predicted for the next decades (Chakraborty and Newton, 2011; Olesen et al., 2011; Juroszek and von Tiedemann, 2013; Delgado-Baquerizo et al., 2020). This is caused by increasing mean temperature, relative air humidity and atmospheric CO₂ concentrations and, as a consequence, generally more extreme weather conditions (Chakraborty et al., 2000; Anderson et al., 2004; Luck et al., 2011; Váry et al., 2015; Delgado-Baquerizo et al., 2020; Singh et al., 2023). Additionally, rising temperatures increase areas with optimal conditions, leading to poleward migration of several pests and pathogens within mid-latitudes (Bebber et al., 2013). Noteworthy, shifts in the abundance of specific pests and pathogens in cooler regions coincide with areas with relatively high crop productivity, further aggravating threats to global food security (Bebber et al., 2013). Moreover, temperature-related shifts in population dynamics within a pathogen species complex, broader geographical distributions of vectors or the increase and acceleration of several disease cycles per season could together gain adaptations to new hosts or respective cropping areas (Vaughan et al., 2016a). Hence, the emergence of pre-existing pathogens to become major diseases or invasion of pathogens to new areas will be more likely (Anderson et al., 2004; Bebber et al., 2015). This coincides with possible shifts in plant-pathogen interactions, which could result in different diseases on the same crop caused by a specific pathogen species. For instance, the two fungal diseases Fusarium Head Blight and Fusarium Crown or Root Rot (FCR, FRR) in cereal crops are caused by the same *Fusarium* species (Liu & Ogbonnaya, 2015), though FRR is mostly abundant and economically relevant in semi-dry areas. Despite dry conditions possibly enhancing the disease severity (Liu & Liu, 2016), there is evidence for an association of Fusarium root rot resistance with tolerance to abiotic stress on a genetic level (Su et al., 2021). Furthermore, plant phenology strongly affects plant-pathogen interactions (Develey & Rivière, 2007). Hence, infection and disease outbreaks are often dependent on specific growth stages (e.g. seed germination, flowering), plant age (ripening) or the development of the target organ (e.g. fruit) for infection representing variable phases of host susceptibility or resistance

(Whalen, 2005; Krokene et al., 2011; Simon et al., 2015). With warmer conditions, plant phenology stages occur earlier, resulting in premature outbreaks of diseases, where infection is strongly associated with specific growth stages as described e.g. for FHB in cereal crops (West et al., 2012). As a consequence, earlier disease outbreaks and new diseases of currently present pathogens will further need alignments in disease management, targeted resistance breeding and novel forecast and protection strategies which integrate abiotic stress factors and impacts from predicted future environmental conditions.

Another aspect of rising temperatures or elevated concentrations of atmospheric CO₂, respectively, are possible direct effects on fungal growth and accumulation of inoculum for the following crop within the soil or crop debris (Ainsworth & Long, 2005; Melloy et al., 2010), hence leading to contaminations of upper soil layers and increasing disease pressure (Delgado-Baquerizo et al., 2020). Several studies focussing on future climate conditions (e.g. raised temperature, elevated CO₂ concentrations) on pathogen abundance, disease severity and host resistance revealed that future conditions will likely gain pathogen fitness, aggressiveness, or modulate basal disease resistance (Jwa & Walling, 2001; Chakraborty & Datta, 2003; Lake & Wade, 2009; Melloy et al., 2010; Váry et al., 2015), possibly in favour of soil-borne diseases (Delgado-Baquerizo et al., 2020). Consequently, increasing pathogen abundance and infections may lead to relevant contaminations of crops pre- and postharvest with mycotoxins provoking risks for animal feed and human consumption worldwide (Patterson & Lima, 2010; Magan et al., 2011; Zingales et al., 2022). Proper management of pathogens pre-harvest will become more decisive in avoiding mycotoxigenic fungi on the field. Higher efforts are also needed to reduce mycotoxin production during postharvest storage by preventing crops from high temperatures and moisture or insufficient gas exchange, which are conditions conducive to the growth of moulds in crop batches stored in silos (Magan et al., 2011). In particular, the latter factors lead to higher costs and food prices, especially relevant for developing countries where food safety is of disproportionately high concern (Shephard, 2008).

Global warming will also negatively affect pests and pathogens, comprising conditions that may not be in the optimal range for compatible host-parasite interaction or proper pathogen spread (Luck et al., 2011; Chakraborty et al., 2000; Matic' et al., 2018) unless pathogens would not adapt to changing conditions. Nevertheless, disease monitoring and implementation of forecasting systems from a local to international scale might uncover pathways in geographical distribution and early abundance of pathogens, hosts and vectors in non-native regions. In addition, the geographic distribution of host plants and seeds by global trade, further introducing crops and vectors to non-native cropping areas are important anthropogenic drivers

of emerging pathogens (Shaw & Osbourne, 2011). However, this problem goes back to when global trade began and probably will undergo uncertainties in the context of globalization and global warming in the near future. Consequently, prevention and timely management of emerging plant diseases will likely mitigate possible epidemics and crop losses but increase expense and costs for control measurements (Chakraborty et al., 2000; Olesen et al., 2011; Shaw & Osbourne, 2011). In the framework of integrated pest management (IPM), the application of pesticides is widely used and effective when other measures failed to keep disease pressure under the economic threshold. However, several studies revealed that fungicide treatment efficacy is negatively affected by higher temperatures, either directly or indirectly (Juroszek et al., 2022). Namely, direct effects are e.g. higher pathogen fitness, growth and sporulation. Indirect effects comprise higher volatility and reduced uptake of fungicides, further slower conversion of inactive compounds to active forms (Ypema & Gubler, 1997) or potential temperature-mediated accelerated evolution of fungicide resistance, which increases risk in a broad reduction of control efficiency (He et al., 2018). This raises the need for novel strategies in the frame of integrated and climate-adapted pest management (Stenberg, 2017).

1.2 Manipulation of environmental factors in plant-pathogen interactions (field scale)

The global warming-derived impacts on agriculture will generally change environmental conditions and its variability over large areas, further enhancing abiotic stress situations with multiple influences on pests and pathogens. Understanding the direct impact of the environment on host plant resistance and pathogenic microbes is crucial for the mitigation of plant stress and to improve crop protection in the field. Furthermore, knowledge of the interaction of two or more stresses is highly important to adopt pest management directly in the field or to improve the practical breeding and selection of multiple resistant plant genotypes (Juroszek & von Tiedemann, 2013). The epidemiology of many different pathogens is well studied under relatively controlled conditions in climate chambers or greenhouses, reducing errors by external influences. Inoculation nurseries in the field are widely used to artificially increase disease pressure by applying pathogenic inoculum, either directly on the plant or indirectly on the soil surface. Hence, inoculation nurseries reveal plant-pathogen interactions and genotype resistance in the field, but with bias from the environment. However, a direct transition of plant-pathogen interaction studies to field environments is often highly complex and superimposed by multiple interconnected influencing factors, i.e. through highly variable weather conditions during and between seasons, inhomogeneous soil and field sites, abundance of populations of

competing pathogens or beneficial microorganisms, respectively, further whole plant and soil microbiomes. Nevertheless, besides the high complexity of conditions, open-field experiments are essential tools to unravel research questions with regard to pathogen control in applied agriculture. Despite the high complexity of field environments, the robustness of field data increases with the number of replications over locations and field seasons. Furthermore, in addition to inoculation nurseries in the field, many different technical devices are available to control single environmental factors or to directly set predicted environmental conditions (e.g. elevated atmospheric CO₂ concentrations). For instance, the free-air CO₂ enrichment (FACE) technique (Hendrey et al., 1993; Ainsworth & Long, 2020) enables studies on the impact of elevated atmospheric gas concentrations on crop plants, pathogen growth and accumulation of fungal inoculum under open-field conditions, further on specific host-pathogen interactions in the field (Kobayashi et al., 2006; Oldenburg et al., 2009; Eastburn et al., 2010; Melloy et al., 2010; Khudhair et al., 2013). However, according to the considered pathosystem and lifestyle, increased CO₂ concentrations in the field are probably promotive (Kobayashi et al., 2006; Melloy et al., 2010) or suppressive (Eastburn et al., 2010). By adding rainout shelters (drought; Gray et al., 2016) or infrared heaters (heat; Ruiz-Vera et al., 2013), additional abiotic stress factors can be applied to simulate more complex environments (Ainsworth & Long, 2020). Despite rainout shelters being widely used to study plant performance under drought conditions in the field (Wenzel et al., 2015; Hoover et al., 2018), the impact of abiotic stress conditions on disease resistance is rarely studied in open-field experiments. Nevertheless, the controlled exclusion of natural rainfalls represents an opportunity to investigate pathogen abundance and disease severity under dry field conditions to assess possible impacts of climate change on plant health and genotype resistance on a field scale (Sinha et al., 2019; Hoheneder et al., 2021, 2023a). Furthermore, artificially modified soil or canopy temperatures in the field represent tools to assess the effects of global warming, in particular on soil-borne diseases in detail (Pivonia et al., 2002; Delgado-Baquerizo et al., 2020).

Water availability is one of the most critical factors affecting plant infections by pathogens. In particular, rainfalls, relative air humidity and soil moisture determine water availability in the field. The level of air humidity and duration of leaf wetness are decisive epidemiological factors for pathogen growth, spore germination, sporulation and further disease progression (Huber & Gallspie, 1992; Velásquez et al., 2018). Due to the importance of available moisture in plant canopy, disease warning systems mainly incorporate the amount and duration of leaf wetness as a critical factor to implement risk warnings to farmers (Rowlandson et al., 2015). However, irrigation usually aims to improve crop growth and yield during dry periods in the field with

effects on microclimate relevant for disease development (Olanya et al., 2007). Studies on pathogen's epidemiology and their response to environmental conditions often rely on the manipulation of wetness in inoculation nurseries in the field. Therefore, sprinkler irrigation is a widely used method to promote pathogen growth and proliferation for increased disease pressure (e.g. Campbell & Lipps, 1998; Lemmens et al., 2004; Radecka-Janusik & Czembor, 2014; Cope et al., 2021). In contrast, irrigation management needs to balance the promotion of plant growth by increasing moisture in the field without a possible gain of pathogen growth (Juroszek & von Tiedemann, 2011).

In the context of global warming and especially during phases with lower rainfalls, accumulation of dew in the night likely provides sufficient humidity and leaf wetness for pathogen infections. Because warmer air can hold more water vapour, higher temperatures increase the possibility and amount of dew accumulation, given the condition that the surface temperature of the plant canopy decreases below dew point temperature during the night. Consequently, dew accumulation is an important source of humidity besides rainfall events and will likely become more decisive for risk assessment of pathogens which infect aerial plant tissues (Rowlandson et al., 2015; Velásquez et al., 2018). However, modelling leaf wetness within plant canopy is highly complex (Pangga et al., 2011). On the other hand, increasing the likelihood of extreme rainfalls elongate phases of sudden high leaf wetness or flooding stress with significant impacts on disease severity.

Taken together, modifying single or combined abiotic factors to study the response of either the plant, the pathogen or the specific interactions under the given conditions mainly comprise effects in the context of the considered agricultural system and microclimate within the plant canopy. The latter needs to be considered as a function of variable environmental or weather conditions and ecological and evolutionary responses of pathogens resulting in plant disease risk. Further, the precision of plant disease risk models and prediction of disease severity will require comprehensive monitoring of possible abiotically derived shifts in field conditions in the following decades (Laine, 2023). Consequently, data on pathogen epidemiology with very close relations of its infection biology with environmental factors is highly valuable to gain the general understanding of environment-dependent pathogen resistance of crop plants under rapidly changing conditions in a warming world.

The following paragraphs describe exemplary pathosystems of two important fungal diseases, which possess a strong association between epidemiology and environmental and weather conditions.

1.2.1 Interplay of RLS disease with abiotic factors and host stress physiology

The ascomycete fungus *Ramularia collo-cygni* causes Ramularia Leaf Spot disease of barley and provokes economically relevant yield and grain quality losses in many barley-growing countries worldwide. RLS disease has become an emerging disease since the 1980s, and its epidemiology and infection biology are exemplary to be strongly linked with environmental factors. To date, fully resistant genotypes are not available, thus only moderate quantitative resistance is usually observed (Havis et al., 2015). Despite the epidemiology of *R. collo-cygni* and specific conditions crucial for disease outbreaks, are still not fully understood, there is increasing evidence that different abiotic factors affect the formation of symptoms and sporulation and strongly interfere with host physiology, which likely has an impact on stress mitigation and defence against the fungus (Walters et al., 2008; McGrann & Brown, 2017). Originating from infected seeds or infection of young plants, the fungus undergoes an endophytic phase within the barley host (Havis et al., 2014; Matusinksy et al., 2011). During the relatively long endophytic phase, the fungus is detectable within leaf tissue but hardly produces any visible symptoms. During the transition of barley from vegetative to generative growth and with the onset of tiller elongation, spike emergence and flowering, necrotic leaf symptoms rapidly appear. Hence, significant disease outbreaks are mostly recognized during the post-flowering phase. The dark brown-reddish spots with yellow halo are often margined from leaf veins, which gives the lesions a rectangular shape. The leaf spots mostly become visible in a speckled pattern on the sunlight-exposed sides of the leaves, causing rapid leaf senescence. Simultaneously, the fungus releases the photodynamic anthraquinone toxins, called rubellins, into the apoplast, which function as pathogenicity factors (Heiser et al., 2004). The light-induced activation of rubellins is involved in the formation of necrosis. Therefore, the toxigenic rubellins were shown to induce oxidative stress within the host tissue by the induction of reactive oxygen species (ROS) in a light-exposure dependent manner. Rubellins further damage cell membranes by fatty acid peroxidation. Infected leaves exhibit a decrease in photosynthesis, accelerated loss of green leaf area and premature ripening, followed by leaf senescence (Miethbauer et al., 2003; Heiser et al., 2004; Walters et al., 2008). Furthermore, a decline in the antioxidative protection system of the host is likely to be associated with enhanced disease severity due to the physiological stress of the host (Schützendübel et al., 2008; McGrann & Brown, 2017). As a consequence, accelerated cell death eases the ability of the necrotrophic pathogen to nourish from dead tissue. In addition to cell-toxic rubellins, the fungus exploits cell wall degrading enzymes to damage leaf tissue and to release nutrients (Sjokvist et al., 2019). The light-dependent activation of the specific pathogenicity factor rubellin

demonstrates the strong interaction of the disease with abiotic factors (McGrann & Brown, 2017). In addition, the relatively late RLS disease outbreaks and the assumed development/ripening- and plant age-associated conjunction of pathogenesis with a described decline of the antioxidative protection system during late growth stages is still not thoroughly investigated (Schützendübel et al., 2008; Havis et al., 2015; McGrann & Brown, 2017). Hence, apart from plant development, identification of the role of environmental factors as either trigger or enhancer of the RLS disease outbreak is crucial to assess the epidemiological consequences, which are decisive for disease severity risk modelling (Havis et al., 2018) and breeding of RLS resistant genotypes, additionally showing high tolerance towards abiotic stress (Brown & Rant, 2011). McGrann & Brown (2017) found increased RLS symptom development in response to abiotic stress, which was associated with increased formation of ROS in leaves of barley seedlings. These results suggest that abiotic stress factors might increase the formation of ROS in the host and thus lead to the promotion of RLS symptoms. The strength of this effect varied between differently susceptible barley lines, indicating a possible relationship between the host-specific capacity to mitigate physiological stress and the expression of RLS symptoms.

After successful infection, spreading within the host and induction of leaf spots, sporulation of *R. collo-cygni* is usually observed on senescing leaf surfaces. Compared with other fungal pathogens, *R. collo-cygni* generally produces relatively high amounts of small conidiospores, which are then extensively released in the air as wind-borne inoculum (Havis et al., 2023). The increased amount of generated spores suggests the high relevance of wind-borne inoculum for the epidemiology and long-distance spreading of the fungus. Further, spore release levels from spring barley or crop debris are probably associated with disease severity in the following winter barley crop and vice versa (Frei et al., 2007; Schützendübel et al., 2008; Zamani-Noor, 2011; Havis et al., 2023). Meteorological factors proposed to have a significant effect on spore production, release and disease severity are relative air humidity, leaf wetness, temperature and precipitation pattern. However, none of these factors was identified to consistently promote spore release and RLS symptoms over different field locations and seasons in both barley crops (Salamati & Reitan, 2006; Schützendübel et al., 2008; Zamani-Noor, 2011; Havis et al., 2018), although leaf wetness seems to be the most crucial factor to initiate RLS disease epidemics (Havis et al., 2023). These uncertain associations between the pathogen, weather and microclimate conditions and disease severity demonstrate the highly sensitive associations and interactions of the RLS disease with environmental factors.

According to the current state of knowledge, the following schematic representation summarizes the proposed interaction between the fungus *R. collo-cygni*, different abiotic factors

and stress physiology of barley causing an imbalance in the antioxidative state followed by oxidative stress in host tissue (Fig. 1). In parallel, physiological processes involved during plant ripening and ontogenesis in the post-flowering phase of barley are characterized by a switch in the distribution of nutrients between the source (leaves) and sink tissues (grain), aging of leaves and ongoing senescence. The latter processes during plant maturity probably interfere with damage of cell membrane components by the release and activation of fungal rubellins, resulting in the formation of symptoms. In a later stage, spore-carrying conidiophores emerge through stomata representing inoculum for secondary infections (Formayer et al., 2002; Miethbauer et al., 2003; Heiser et al., 2004; Zimmermann & Zentgraf, 2005; Miethbauer et al., 2006; Schützendübel et al., 2008; Newton et al., 2010; Brown et al., 2014, Havis et al, 2015; McGrann et al., 2015; McGrann & Brown, 2017; Sjökvist et al., 2019; Hoheneder et al., 2021; Lemke et al., 2021; Havis et al., 2023; Hoheneder et al., 2023a).

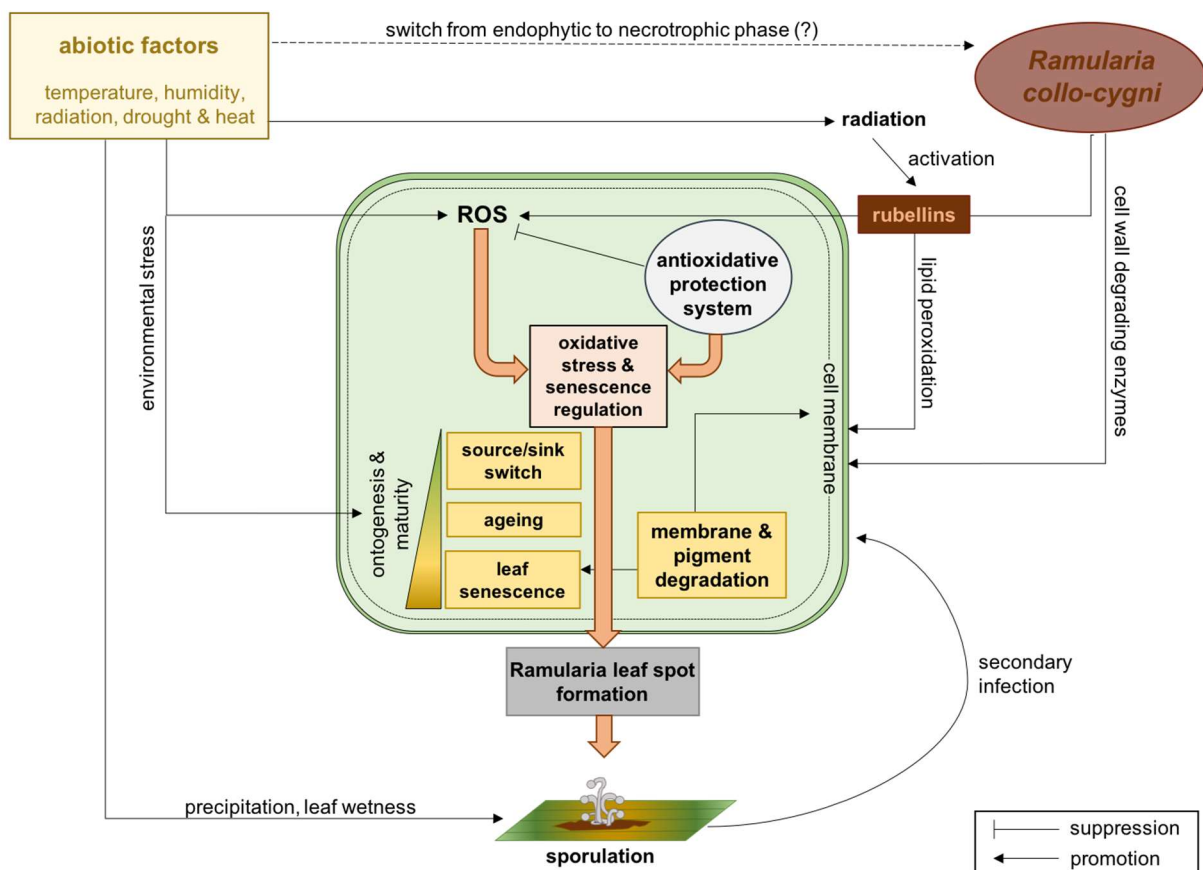


Figure 1: Schematic representation of the hypothesized interaction of abiotic factors with *Ramularia collo-cygni* during infection and progression of Ramularia Leaf Spot disease in the barley host with effects on plant physiology. The proposed model summarizes current knowledge on the barley-*Ramularia collo-cygni* pathosystem under the influence of environmental factors affecting plant physiology and pathogen defence.

1.2.2 Climate and weather effects shape *Fusarium* spp. severity in cereal crops

Pathogenic fungi of the genus *Fusarium* are causal agents of the diseases FHB or ‘scab’, seedling blight and FCR or FRR in many cereal crops, like wheat, barley, oat and maize (Parry et al., 1995). *Fusarium* diseases of cereal crops are associated with a complex of different *Fusarium* species (*Fusarium*-complex’) showing variable host preferences, response to environmental conditions, virulence and geographic distributions (Parry et al., 1995; Bai & Shaner, 2004). Among others, the most important species infecting cereal crops and primarily associated with FHB and FCR are *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *F. sporotrichoides*, *F. triticum*, *F. asiaticum* and *F. pseudograminearum* (Osborne & Stein, 2007; Scherm et al., 2013; Pasquali et al., 2016). However, *F. graminearum* is the most considered species associated with FHB in small-grain cereals and maize (Parry et al., 1995; Goswami & Kistler, 2004).

FHB and FCR threaten global cereal production in a multifactorial manner. Hence, effective control in the field is relatively challenging. *Fusarium* infections cause significant yield losses and contamination of grains with a broad range of hazardous mycotoxins and have adverse effects on human and animal health when used in the food supply chain or for animal feeding (Parry et al., 1995; Miller, 2008; McMullen et al., 2012). Among several different *Fusarium* mycotoxins and their derivatives, zearalenone (ZEA) and the type B trichothecene deoxynivalenol (DON) are predominantly associated with *Fusarium* spp. infections (Bottalico & Perrone, 2002; Foroud et al., 2019).

The epidemiology of *F. graminearum* causing FHB and FCR in wheat is relatively well investigated, though only partially representative for related *Fusarium* species and their interactions with other small-grain cereals. Originating from colonized crop debris in the soil or at the soil surface, primary infections are usually observed on seedlings, causing seedling blight and roots causing FCR. Therefore, the fungus directly penetrates host tissue via soil-borne mycelia or germinating spores in the soil. During systemic colonization of the plant, roots and stem tissue is killed off and shows necrotic discolorations (root rot). In addition, spores emerge on infected or dead plant tissue and are distributed over long distances by wind or rain splashes within the plant canopy. After spike emergence and mostly during flowering, spores infect the inflorescence and damage single spikelets or the entire spike, considered as FHB. Infected heads show brown discolorations, necrotic spots, grain abortions or become blight above the site of infection (wheat FHB). Later, salmon to reddish-coloured spore carrying

conidiophores emerge on husks, being the inoculum for secondary infections (Parry et al., 1995; Osborne and Stein, 2007; Alisaac & Mahlein, 2023).

The epidemiology of *Fusarium* spp. is strongly influenced by several environmental parameters, which determine pathogen incidence and disease severity. Moreover, weather and microclimatic conditions during anthesis of the cereal host, where the open-flowering spikelets are most sensitive to a *Fusarium* spp. infection, highly determine FHB severity in wheat (Kubo et al., 2010) and barley (Yoshida et al., 2005). Predominantly, several *Fusarium* species simultaneously coexist in fields and thus occupy different agroecological niches as a function of the environment and the individual demands on growth conditions of each individual *Fusarium* species (Xu et al., 2008). In particular, temperature (Brennan et al., 2005) and duration of moist conditions (Xu et al., 2003) are associated as factors with significant impact on fungal growth, sporulation, spore germination and production of mycotoxins as virulence factors (Beyer et al., 2004; Beyer et al., 2005; Cowger et al., 2009; Linkmeyer et al., 2016). For instance, *F. graminearum* is associated with warm and humid conditions, whereas *F. culmorum* and *F. avenaceum* prefer cool and humid conditions (Xu, 2003; Xu et al., 2008). Consequently, the high variability in weather conditions differently promotes species of the *Fusarium* complex in each individual season, revealing competition between single species but also high plasticity within the species community (Xu et al., 2008). Furthermore, environmental factors drive the dynamics of airborne sporulation patterns, even with daily periodicity (Fernando et al., 2000). Depending on specific environmental conditions, there is a high likelihood that one *Fusarium* species fills in a potential ecological niche of another species, which is limited due to unsuitable growth conditions. Hence, despite strong shifts in *Fusarium* species communities within a field or small area, FHB disease risks remain probably high and show plasticity in response to the environment and seasonal weather conditions (Osborne & Stein, 2007). Moreover, depending on multiple factors, shifts in *Fusarium* species compositions, infecting cereal crops were observed in a wide range over large geographic regions (Ward et al., 2008; Hofer et al., 2019; Valverde-Bogantes et al., 2019) or are even predicted in the near future (Madgwick et al., 2011; Parikka et al., 2012). Such shifts in *Fusarium* species composition are associated with a changing climate and, thus, variations in meteorological factors. Additionally, adaptations of crop management to changing environmental conditions or an increase in the cultivation of susceptible hosts adapted to warmer conditions (maize) favor risk of FHB epidemics (Parikka et al., 2012; West et al., 2012). Expected warmer temperatures in the early season lead to earlier anthesis, which will probably coincide with more humid conditions, finally increasing the risk of FHB disease in wheat (Madgwick et al., 2011). Changing conditions might favor

Fusarium species to become more dominant in a particular area, out-competing other *Fusarium* species, which are probably less toxigenic or virulent. Consequently, there is an increasing risk of disease severity and mycotoxin contaminations of cereal products (Ward et al., 2008; Magan et al., 2011). For instance, there is evidence for a shift in the abundance of *F. graminearum* over *F. culmorum* as the most dominant species associated with FHB in Northern and Western Europe (Waalwijk et al., 2003; Jennings et al., 2004; Parikka et al., 2012; van der Lee et al., 2015). While the growth of *F. culmorum* is optimal under cool conditions, *F. graminearum* is adapted to a broader temperature range and still grows under relatively warm conditions (Xu et al., 2005; Nielsen et al., 2011). In addition, an increasing dominance of *F. poae* and a decreasing abundance of *F. graminearum* was found in Italy and the Czech Republic, which was speculated to coincide with warmer temperatures and dryer conditions promoting infections with *F. poae*. Consequently, other hazardous mycotoxins likely become more prevalent in those areas (Xu et al., 2008; Sumíková et al., 2017).

According to different field studies, changing climatic conditions and strong weather anomalies around anthesis reveal dynamics in FHB abundance and severity, demonstrating how *Fusarium* species populations in the field respond to variations in seasonal weather conditions (Xu et al., 2008; Cowger et al., 2009; Beyer et al., 2014; Zhang et al., 2014). For instance, *F. pseudograminearum* is prevalent mainly in semi-arid and arid cropping regions, causing root diseases (FCR, FRR). FCR recently became more severe in those regions due to cultivation practices preserving soil moisture (minimum tillage, stubble retention). Adaptive cultivation measures in response to changing environmental conditions, together with shifts in climate and weather conditions, reveal high dynamics in the adaptation of pathogens to environmental factors in relation to the management of agricultural systems (Poole et al., 2013; Kazan & Gardiner, 2018). However, it is still hardly understood whether *Fusarium* species probably tend to more likely cause root diseases when conditions for spike infections are not optimal by lacking rainfalls and humidity or remain latent in soils. Under warmer conditions, the abundance of soil-borne diseases, including *Fusarium* spp., will become more likely (Manici et al., 2014; Delgado-Baquerizo et al., 2020; Ejaz et al., 2023). As hypothesized by Beyer et al. (2014), more free moisture at the soil surface than within the upper plant canopy, even under dry conditions, could favour conditions for root and stem base rather than spike infections. According to the literature, there is increasing evidence that dry and warm conditions could probably provide conditions more suitable for FCR and FRR diseases in cropping areas, where FHB is currently the most abundant *Fusarium* disease (Chakraborty & Newton, 2011; Manici et al., 2014). However, the epidemiology of both diseases is linked together. However, it is still

hardly understood whether *Fusarium* species probably evade unsuitable conditions for a complete life cycle by causing root diseases in favour of FHB.

As described before, the rising global temperature and atmospheric CO₂ concentrations will have direct effects on both, host plant resistance (Khudhair et al., 2013; Vaughan et al., 2014; Sabburg et al., 2015) and pathogen fitness or abundance, respectively (Kobayashi et al., 2006; Sabburg et al., 2015; Manici et al., 2014; Delgado-Baquerizo et al., 2020; Ejaz et al., 2023). Results from free-air CO₂ enrichment experiments revealed increased aggressiveness of *F. pseudograminearum* under artificially increased CO₂ and altered temperatures in the field (Melloy et al., 2010; Khudhair et al., 2013). For instance, under increased atmospheric CO₂ concentrations, maize shows increased susceptibility to *F. verticillioides* (Vaughan et al., 2014), but only simultaneously applied drought conditions further increased accumulations of its associated mycotoxin fumonisin (Vaughan et al., 2016b). The results demonstrate the complex effect of changing environmental conditions on *Fusarium* spp.. By adding more variable factors, their effects on disease severity become more unpredictable, such as pathogen aggressiveness, mycotoxin production and host resistance. Hence, the understanding of weather effects in a warming world on *Fusarium* diseases is still limited, but conditions tend to likely increase the risk and severity of FHB (Paterson & Lima, 2010; Chakraborty & Newton, 2011; Madgwick et al., 2011; Zhang et al., 2014).

1.3 Plant physiology in complex stress situations (plant scale)

Plants are permanently influenced by combinations of abiotic and biotic stressors, triggering various responses from a molecular to a physiological level. These responses to stresses are often complex and orchestrate different signalling pathways or the entire plant metabolism. Consequently, stress responses often employ costly resources for the plant and there is often a trade-off observed in balancing between different stresses (Brown & Rant, 2013; Pandey & Senthil-Kumar, 2019; Leisner et al., 2022). The exposure to individual stresses often have synergistic or antagonistic relationships between each single stress response, resulting in either enhanced or mitigated stress. Hence, exposure to simultaneous stresses can also lead to tailored regulatory responses with unique transcriptomic profiles, which are frequently different from those under single stresses (Gupta et al., 2016). However, such mutual effects between different stress responses are often superimposed by the variable strength of single stressors and the possible dominance of one stressor over another or the sequence in the occurrence of certain stressors. This increases complexity of antagonistic or synergistic effects between different

stresses on a physiological level (Zandalinas & Mittler, 2022). Nevertheless, neutral interactions between responses to different stresses are also occurring in planta, when both stresses address completely independent transduction pathways and response mechanisms (Suzuki et al., 2014; Pandey et al., 2015; Ramegowda & Senthil-Kumar, 2015; Zhang & Sonnewald, 2017). The co-occurrence of simultaneous stress factors often results in significant crop losses (Farooq et al., 2009; Zandalinas & Mittler, 2022) and investigating such complex stress responses is crucial for the breeding of stress-tolerant and healthy crops (Kissoudis et al., 2014; Rivero et al., 2022). The complex nature of multiple-stress interactions still needs to be sufficiently understood due to the simultaneous integration of a multidimensional spectrum of molecular and physiological responses affecting each other in a sophisticated defence network (Nejat & Mantri, 2017; Zandalinas et al., 2021a, 2021b). Moreover, complexity increases with the number of simultaneously impacting stresses, which together often decline plant health and vitality (Suzuki et al., 2014; Zandalinas & Mittler, 2022). Thus, plants often undergo situations which require appropriate coordination of conflicting stress signals when exposed to simultaneous biotic and abiotic stressors. This includes responses on a transcriptional level by expression and regulation of a large number of stress-responsive genes. Under combined stress, there is increasing evidence suggesting shared functions of various genes commonly involved in biotic or abiotic stress responses, acting as multiple stress-responsive genes (Shaik & Ramakrishna, 2013; Nejat & Mantri, 2017; Panigrahi et al., 2021). Beyond that, several studies demonstrate a tailored transcriptional response to combined biotic or abiotic stress, which is different to the responses towards single stress factors (Mantri et al., 2010; Mittler & Blumwald, 2010; Rivero et al., 2014; Gupta et al., 2016). Such unique responses comprise extensive transcriptional reprogramming, and besides a significant overlap in several multi-stress-responsive genes, plants are further able to construe an individual program to cope with complex stress combinations (Mantri et al., 2010; Atkinson et al., 2013; Rasmussen et al., 2013).

In addition to general responses on a transcriptomic level, plants need to transmit different signals in a local to systemic manner and between a particular cellular tissue to plant organs, further within the entire plant (Pandey et al., 2015). Following sensing of external stimuli via various pattern recognition receptor (PRR) proteins located at the cellular periphery, calcium ion fluctuations in the cytosol, production of ROS (Mittler et al., 2022) as well as the phosphorylation of mitogen-activated protein kinase (MAPK) cascade are regarded as initial interphase between environmental signals and the intracellular signal transmission towards the nucleus triggering defence gene expression. Interestingly, these signalling pathways are major

components of pattern-triggered immunity (PTI) in response to the perception of highly conserved microbe- or pathogen associated molecular patterns (MAMPs or PAMPs). However, signalling pathways involved in PTI are similarly activated in response to abiotic stress stimuli. Hence, it is proposed that PTI has initially evolved as relatively unspecific ‘stress-triggered immunity’ because PTI responses comprise several relatively similar and evolutionary conserved molecular signatures and were then probably shaped towards the more specific perception of individual pathogen stresses (Nejat & Mantri, 2017). Taken together, there is a broad overlap in the recognition and processing of abiotic and biotic stress factors and, hence, a strong cross-talk between stress physiology originating from both factors. The mutual effects between different stress-related signals need a fine-tuned and very sophisticated downstream signaling network to determine the level of tolerance to abiotic or resistance to biotic stress. Further, the efficiency in stress mitigation under simultaneously impacting stresses depends on the ability to effectively translate external signals into specific physiological responses (Verma et al., 2016).

Following the sensing of a particular external stress, downstream signaling in plants represents a critical but complex component in processing various stress responses. Therefore, among others, plants use several different chemicals (phytohormones) as signals for controlling responses to external stimuli and for regulation of physiological and developmental processes (Atkinson & Urwin, 2012; Rejeb et al., 2014; Ku et al., 2018). In particular, plant hormones often represent convergent components in the transduction of abiotic and biotic stress signals. For instance, abscisic acid (ABA) is a major hormone for drought and salt stress responses (Zhu, 2002). In contrast, the salicylic acid (SA) signalling pathway is mainly involved in pathogen stress and mediates responses critical for disease resistance. Despite their divergent roles regarding different stress stimuli, both hormones address, e.g. stomatal closure under different stresses, which either regulates water loss via transpiration or prevents the plant from entering pathogens (Montillet & Hirt, 2013; Verma et al., 2016; Ku et al., 2018). Additionally, both hormone signalling pathways show specific cross-talks and mutual effects on either tolerance to water deficit or disease resistance. While salicylic acid is involved in PTI responses, additional drought stress and accumulation of ABA interfere with the downstream signalling regarding pathogen defence (Verma et al., 2016; Ku et al., 2018). The major stress hormones ABA, salicylic acid and jasmonic acid (JA) are further connected with the gaseous plant hormone ethylene (ET) (Verma et al., 2016; Kissoudis et al., 2017), which is regarded as a critical regulator in many different developmental and physiological processes in plants and is mainly upregulated in response to abiotic and biotic stress (Morgan & Drew, 1997; Pierik et

al., 2006). Hence, ethylene is another very crucial mediator for plant-microbe interactions, stress regulation and mitigation of various environmental stresses (Shekhawat et al., 2023).

Such inter-connections between hormonal signalling pathways, mainly regulating responses to different stresses, represent key points for ‘decision making’/prioritization of a plant facing simultaneous stressors while minimizing extended fitness costs. However, this requires accurate sensing and fine-tuned orchestration of specific and targeted molecular components, e.g. regulating gene expression (via transcription factors) leading to transcriptional reprogramming or appropriate accumulation of signalling molecules (Rizhsky et al., 2004; Atkinson & Urwin, 2012; Rasmussen et al., 2013). Additionally, the characterization of regulatory gene sets, which show a shared expression under different stresses, and identification of their role in stress response mechanisms likely enhance understanding of multiple stress resistance (Shaik & Ramakrishna, 2013). This suggests the selection of genotypes possessing high genomic plasticity and prioritization to specifically economize metabolism and physiology, thus superior in staying vital and healthy under complex stress (Rizhsky et al., 2004).

1.3.1 Effects of stress combinations on plant immunity

As described above, pattern-triggered immunity (PTI) comprises a complex signalling cascade from the initial sensing of conserved molecular microbial patterns towards signal processing and transduction, resulting in an immune response (Bigeard et al., 2015). Moreover, environmental stress affects PAMP/MAMP-induced signalling and immune responses, thus modulating pathogenesis and disease resistance (Cheng et al., 2013; Shaik & Ramakrishna, 2013; Janda et al., 2019). Consequently, abiotic stress can tie plant resources and energy, which are necessary for effective defence against invading pathogens. Conversely, abiotic stress responses, which overlap with those involved in pathogen resistance, may also support defence mechanisms when the plant is in a primarily stressed state. The observation of increased resistance towards a specific stress due to exposure to another stress is described as cross-tolerance, which led plants to effectively adapt to a wide range of environmental stimuli (Rejeb et al., 2014). For instance, abiotic stress-mediated expression of transcription factors conversely enhances the expression of pathogenesis-related (PR) genes, thus promoting pathogen resistance (Tsutsui et al., 2009). Among many more examples, drought stress induces ABA-mediated stomatal closure to prevent plants from excessive water loss, further inhibiting pathogen entry on a physiological layer (Gupta et al., 2016). Hence, such cross-tolerance

mechanisms probably decrease disease severity under abiotic stress, even though such effects further depend on the combination, sequence and strength of the impacting stresses (Atkinson & Urwin, 2012; Kissoudis et al., 2016; Zandalinas & Mittler, 2022). This represents factors for creating a multitude of possible stress profiles in plants which experience complex stress situations. On a transcriptomic level, a primary stress leaves significant signatures from the processing of the first stress and affects the responses and gene expression profiles of a second stress (Coolen et al., 2016) with comprehensive impacts on plant immunity. For instance, a short heat stress treatment before bacterial infections strongly modulates PTI-mediated responses and plant metabolism, increasing susceptibility (Janda et al., 2019). Similarly, Prasad & Sonnewald (2013) observed that heat and drought reduced gene expression involved in defense responses to virus infections and led to specific alterations in transcriptomic and metabolomic networks associated with responses to those stresses. The study revealed that gene expression under complex stress situations is hardly predictable from gene expression patterns in response to single stresses. Consequently, combinations, timing and strength of responses towards a subsequent stress are often affected by a prior stress, which represents physiological adjustments by the plant to prepare and adapt towards future stress impacts (Coolen et al., 2016).

1.3.2 Drought stress modifies disease resistance and response to pathogens

Plant growth and productivity strongly depend on water availability in soil, provoking comprehensive impacts on various plant functionalities. The following paragraph exemplary describes the possible impacts of the increasingly important abiotic factor ‘drought stress’ (Farooq et al., 2009), which has comprehensive effects on plant growth, physiology and productivity. In the context of plant immunity, drought stress notably alters the response to pathogen infections and defence gene expression, affecting disease resistance and progression (Gupta et al., 2016; Nejat & Mantri, 2017; Panigrahi et al., 2021). Drought stress usually has a high impact on various basal physiological functionalities and thus creates conditions strongly divergent from the plant’s regular equilibrium (Gaspar et al., 2002). Under the presence of a pathogen, the drought-stressed plant is often limited in using resources important for defence mechanisms, which are usually available under irrigated conditions. Despite the globally high relevance of drought on agriculture, the impact of drought stress on plant resistance on a molecular level is still poorly understood (Gupta et al., 2016).

For instance, among several others, globally important diseases favored by drought conditions are Dry Root Rot of chickpeas and FCR and FRR in cereal crops like wheat and barley. These soil-borne fungal diseases cause significant damage of the vascular root system and mainly occur in semi-arid to arid cropping areas. The infection and severity of both diseases is associated with the weakening of plant growth and defense due to water deficit (Liu & Liu et al., 2016; Sinha et al., 2019; Rai et al., 2022). FCR causes yield losses of 10 to 35% in cereals, though occasionally greater losses are possible (Kazan & Gardiner, 2018). Combined drought and DRR in chickpea increases yield losses from 40 to 50% under drought alone to up to 100% (Sinha et al., 2019; Irrulappan et al., 2022). Under drought stress and infection, colonization and the ongoing progression of DRR in chickpeas are commonly enhanced due to a dampening of the host defense program in the root tissue. Compared to irrigated chickpea plants, down-regulated genes involved in root endodermal cell layer enforcement, production of ROS, and regulation of jasmonic acid and ethylene pathways reveal a broad and drought-dependent weakening of plant defense mechanisms. Plant growth and morphology of the root system are further affected by both, drought and infection. The combinatory stress decreases the number of lateral roots, an important morphological trait related to tolerance to DRR, due to altered gene expression responsible for lateral root formation (Irulappan et al., 2022). Similarly, drought stress in rice reduces the expression of PR genes, increasing susceptibility to the fungal pathogen *Magnaporthe oryza*, causing leaf blast. Moreover, transcriptomic responses of the fungus showed enhanced secretion of cell wall degrading enzymes, increasing host damage (Bidzinski et al., 2016). For wheat and barley, both crops show a strong relationship between sets of differently expressed genes responsive to drought and FCR. This demonstrates overlapping response mechanisms towards both stresses on a transcriptomic level and confirms the close linkage between tolerance to drought and pathogen resistance. A possible exploitation of genes conferring tolerance to drought could similarly enhance resistance to FCR (Ma et al., 2015; Su et al., 2021). Therefore, understanding the highly modulated expression of defense genes and transcriptional reprogramming in response to drought is highly important for breeding healthy crops for water-limited environments (Gupta et al., 2016; Panigrahi et al., 2021). Several studies provide examples of the primary function of single regulatory genes simultaneously mediating drought tolerance and pathogen resistance. For example, the overexpression of a single NAC transcription factor in barley, involved in drought tolerance, confers enhanced resistance to RLS disease in barley (McGrann et al., 2015). Similarly, a MYB transcription factor mediates defense to *Bipolaris sorokiniana*, causing common root rot, and enhanced drought stress tolerance when overexpressed in transgenic wheat. In detail, the

transcription factor regulates a subset of genes involved in the ABA- and salicylic acid-signaling pathways and thus acts as a molecular linker positively balancing hormonal responses regarding stress mediation (Zhang et al., 2012). Although a single mutation of the Mildew Locus O (MLO) confers genetic resistance to powdery mildew infection in barley, it was shown that re-watering of drought-stressed barley expresses a temporarily decreased powdery mildew resistance (Newton & Young, 1996). The modulation of genetic resistance via abiotic impacts possess a potential risk of disease outbreaks and break of pathogen resistance due to great variability in environmental conditions, in particular when resistance is mediated via a single gene (Baker et al., 1998). On the other hand, exploiting genes effective in the simultaneous mediation of pathogen and drought stress is essential to successfully enhance marker-assisted selection for breeding healthy plants, which possess high robustness under unfavorable environmental conditions. In particular, in combination with pyramiding several of those genes effectively increase the durability of stress-related traits, which ideally is combined with high yield and yield stability, thus accelerating response to selection (Dixit et al., 2020; Dormatey et al., 2020; Haque et al., 2021).

1.4 Objectives and methodology

This dissertation aims to investigate interactions of abiotic factors with the resistance of barley towards the fungal pathogens *Ramularia collo-cygni* and *Fusarium* spp. with a focus on the impact of drought stress on disease severity and resistance. Therefore, this work focused on disease assessment of different barley genotypes under open and semi-controlled field conditions towards studying transcriptomic and physiological responses of barley suffering from combined drought stress and *Fusarium* infection under controlled greenhouse conditions. The following objectives and the used methodology are addressed in detail:

1. To assess the effects of drought conditions on RLS severity and respective associations of drought tolerance with basal quantitative resistance of several barley genotypes, plants were grown in a field rainout shelter and under open field conditions. Different diagnostic tools were used to evaluate foliar symptoms, spore production and fungal colonization of leaves. Disease phenotypic data was used to calculate disease severity ranking balancing year-effects on disease parameters. Plant characteristics and agronomic traits were further assessed to evaluate genotype performance under drought, irrigated or rain-fed conditions in the field. Recording of weather and microclimate conditions within the plant canopy was conducted to

reveal parameters relevant for RLS epidemiology in the context of persistent late-terminal droughts and micro-climate within the canopy. Two separate field studies were conducted for spring or winter barley, respectively, to reveal drought effects on RLS disease development and genotype-specific quantitative resistance in different field environments. The findings are presented in publications I and II.

2. The second objective was to increase the understanding of the genotype-specific dependency of quantitative FHB resistance of barley on specific weather and microclimate conditions. The expected results aimed to improve the methodology of assessing and selecting FHB-resistant genotypes in the field for practical breeding purposes. In a five-year field study, both inoculation with bruised grain material on the soil and spray inoculations of heads were conducted with either *F. culmorum* or *F. avenaceum* on 17 different barley genotypes to increase disease pressure and to provoke genotypic variations in disease severity. Quantification of fungal DNA and multiple *Fusarium* toxins in harvested grain was conducted to allow assessment of reproducible quantitative FHB resistance/susceptibility of different barley genotypes. Based on disease parameters, disease severity ranking, as described in publications I and II, was used to balance the effects of variable weather conditions over several seasons. Further, agronomic traits were measured to find associations of plant morphology with FHB resistance. Following the recording of environmental conditions in each field season, a multiple linear regression model was generated to identify specific periods during the reproductive stages of barley, during which environmental conditions show the strongest associations with fungal colonization of mature grains. The findings from this study are discussed in publication III.
3. In the context of investigating the impact of drought on disease resistance, a global gene expression study aimed to reveal transcriptomic responses of barley to *Fusarium culmorum* infection under drought and associations with pathophenotypes discovered in publication III. Therefore, three differently susceptible genotypes were exposed to drought stress before infection to measure transcriptomic regulations with global 3'RNA-sequencing technology. Weighted gene correlation network analysis was used to investigate regulatory networks of differently expressed genes under either infection, drought stress or under stress combination. Measurement of fungal DNA, physiological stress markers and associated plant

hormones in barley heads were used to evaluate and confirm physiological responses under complex environmental stress situations in the context of co-regulated differentially regulated genes (DEGs). The findings of this study are presented in publication IV.

Investigating the effects of environmental conditions on the epidemiology of pathogens and the impact of abiotic stress factors on disease severity can unravel the understanding of environment-dependent disease resistance of crop plants. Hence, the gained knowledge supports the improvement of integrated pest management and breeding strategies under variable and increasingly unfavourable growth conditions. This dissertation work provides differently scaled approaches from field to controlled environments to study the interaction of environmental factors, weather conditions and specifically the impact of drought stress on disease resistance against two important fungal pathogens of barley.

2 Results and embedded publications

2.1 *Ramularia* leaf spot disease of barley is highly host genotype-dependent and suppressed by continuous drought stress in the field

Summary

Ramularia collo-cygni has recently become one of the most important fungal pathogens in barley, causing RLS disease. A recent and broad decline in fungicide efficacy and the lack of fully resistant barley cultivars makes effective disease control challenging. The fungus typically causes a rapid disease outbreak late in the season following a relatively long endophytic stage without causing symptoms. Hence, the initial phase of the infection event and the appearance of the first symptoms remain incoherent, pointing out the complex epidemiology of *R. collo-cygni*. Further, strong interactions of environmental conditions and plant maturity with epidemiology are suspected but are still little understood.

To study basal RLS resistance and the effects of long-lasting drought periods late in the season on RLS abundance and severity, a field rainout shelter and parallel field trials were used. Quantitative RLS resistance of 15 spring barley genotypes was evaluated under drought, controlled irrigation and open field conditions. In addition, measurement of weather conditions and parameters regarding microclimate within plant canopy revealed that conditions provoked through long-lasting dry conditions were strongly suppressive for RLS disease, and *vice versa*; moisture appears crucial for disease progression. In this context, data suggest that the extent and duration of leaf wetness is crucial. A relatively high disease severity under open field conditions in dry and warm seasons showed that the formation of dew and single rain events are sufficient for high disease outbreaks even in overall dry seasons. Furthermore, the assessment of different disease parameters and calculation of disease severity ranking could identify differentiation in basal quantitative RLS resistance between genotypes, which allowed the identification of relatively stable RLS-resistant barley genotypes. The use of complementary diagnostic tools was further proved and is discussed to be valuable for reliable evaluation and selection of genotypes under breeding perspectives.

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Authors: Felix Hoheneder, Katharina Hofer, Jennifer Groth, Markus Herz, Michael Heß & Ralph Hückelhoven

Contributions

I conducted field trials and visual assessments on RLS disease parameters in the field and quantified fungal DNA and leaf area carrying conidiophores in the laboratory, except for season 2016 and with support from Katharina Hofer and Michael Heß in season 2017. I measured microclimate conditions within plant canopy and leaf wetness with the help of Michael Heß and analysed the data. I assessed the grain quality parameter of harvested kernels of open field trials and provided the data for yield stability analysis. I wrote the initial manuscript and generated all tables and figures. With critical feedback from the other authors and editing by Ralph Hückelhoven, I finalized and submitted the manuscript for publication together with Ralph Hückelhoven.

2.2 Artificially applied late terminal drought stress in the field differentially affects *Ramularia* leaf spot disease in winter barley

Summary

One of the most dominating fungal diseases in barley is caused by the pathogen *R. collo-cygni*, resulting in RLS disease. Little knowledge about the genetic resistance of barley to RLS and the influence of variable weather conditions on disease severity still impedes breeding progress and the release of quantitative RLS-resistant barley cultivars. In this context, genotype performance is often superimposed by multivariable effects of weather conditions, hampering accurate disease phenotyping. Global warming will probably provoke adverse environmental conditions for optimal crop growth, which could enhance RLS epidemics. Hence, phenotyping must address both, abiotic and biotic stress factors to select for genotypes resistant to multiple stress situations.

Analogous to the study on RLS disease in spring barley under drought conditions (Publication I), the effects of persistent late-terminal drought stress on RLS incidence and severity in winter barley were studied in the field. Again, a field rainout shelter was used to apply controlled drought or irrigated conditions, respectively. Further, various diagnostic tools were used to assess differences in quantitative RLS resistance between 15 winter barley cultivars over three consecutive seasons. Evaluation of RLS severity revealed reproducible differences in quantitative field resistance of several genotypes despite high variations between consecutive seasons and variable suppression of RLS under drought conditions. Differentiation in RLS severity was less distinct and strongly varied in winter barley compared to results in spring barley. In addition, a general drought-mediated suppression was not evident for leaf symptoms, fungal sporulation and DNA contents in leaves, as observed for spring barley. Fungal DNA contents even exceeded levels under irrigated conditions in several genotypes, suggesting that drought conditions differently affected RLS disease severity in spring and winter barley. Measurement of weather conditions revealed strong seasonal effects on overall RLS severity over the three consecutive field seasons, which additively altered disease progression in each individual season. In addition, drought conditions resulted in minor differences in the growth stages of individual cultivars. No clear correlation was found between the severity of different RLS disease parameters and plant ripening in both field environments. Similarly, correlations of RLS disease parameters with yield data did not show a significant trade-off between genotype performance and basal RLS resistance. Furthermore, the calculation of two-way ANOVA revealed significant effects of the environment on RLS

symptoms and leaf area carrying conidiophores in winter barley. Differences in RLS-environment interaction between spring and winter barley are discussed.

Taken together, this study found partial suppressive effects of long-lasting drought conditions on RLS disease in winter barley over three consecutive seasons. Despite variations between genotypes, moderate differentiation of RLS severity suggests a more complex and season-dependent interaction of the environment with the epidemiology of RLS in winter barley. In addition, this study could identify cultivars with reliable and stable quantitative RLS resistance recommendable as parents for further breeding programs.

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Authors: Felix Hoheneder, Jennifer Groth, Markus Herz & Ralph Hüchelhoven

Contributions

I designed the study with major support in genotype selection from Jennifer Groth and Markus Herz. I assessed RLS symptoms in the field and scored leaf area with clusters of conidiophores on leaf samples in the laboratory. Further, I extracted total genomic DNA and performed a qPCR assay to quantify fungal DNA contents in leaf samples. I analysed the data and generated all figures and tables. I drafted the initial manuscript with additions of the other authors and critical evaluation and editing by Ralph Hüchelhoven. After critical comments from the reviewers, I completely revised all figures, tables and the entire manuscript together with Ralph Hüchelhoven before final resubmission and publication.

2.3 Host genotype and weather effects on Fusarium Head Blight severity and mycotoxin load in spring barley

Summary

FHB of barley is a widespread disease and provokes losses in grain yield, product quality and leads to hazardous mycotoxin contaminations. Disease abundance and severity show strong interactions with environmental factors and weather conditions. Genetically mediated FHB resistance is relatively complex and incomplete in barley. The possible effects of unfavourable weather conditions, which influence FHB resistance in the field, are hardly understood. Hence, breeding for quantitative FHB resistance of barley is challenging. This asks for an extensive understanding of specific interactions of the disease with environmental factors and tools for accurate path-phenotyping in the field. For this purpose, quantitative FHB resistance of 17 barley genotypes was studied in the field over five years with variable weather conditions. Two different inoculation methods using the species *F. culmorum* and *F. avenaceum* were compared to evaluate stable field resistance in interaction with variable weather conditions.

Inoculations in the field increased disease pressure and provoked differentiation in fungal DNA contents and mycotoxin load in grains of different genotypes. This allowed to identify stable quantitative FHB resistance/susceptibility despite weather conditions varied between individual seasons. On this basis, the effects of variable weather conditions across individual seasons were balanced with a disease ranking approach. Therefore, the overall FHB resistance of a particular genotype was ranked in relation to the resistance of the whole genotype assortment using detected fungal DNA or multiple mycotoxin contents in mature grain. Pearson's correlation analyses revealed positive associations between fungal DNA contents and species-specific *Fusarium* toxins in individual years and over three consecutive seasons after soil surface inoculation. Similarly, plant height reduced FHB severity in plots with soil inoculation but not after spray inoculation.

To find relations between weather conditions during the reproductive phase of barley and fungal grain colonization, a multiple linear regression model was built based on recorded weather parameters (temperature, precipitation, relative air humidity) and fungal DNA contents measured in mature barley grain over five consecutive field seasons. The MLR model indicated that weather conditions were decisive for FHB severity during periods before heading and late after anthesis. Such weather-dependent associations were not clearly found for *F. avenaceum* DNA contents and with genotype-dependent variations, indicating variable environmental effects on individual *Fusarium* species. The assessed data over several seasons allowed to

identify spring barley genotypes with either stable resistance or susceptibility to FHB, respectively, despite variable environmental conditions in the field. Therefore, methods for artificial inoculation were proven to be reliable for genotype selection in practical disease phenotyping programs under variable weather conditions. This study further provides a first approach which might support the prediction modelling of FHB severity in barley under variable weather conditions.

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Authors: Felix Hoheneder, Eva M. Biehl, Katharina Hofer, Johannes Petermeier, Jennifer Groth, Markus Herz, Michael Rychlik, Michael Heß and Ralph Hückelhoven

Contributions

I performed all field inoculation trails, except for field season 2016 and with the help of Michael Heß in season 2017. I prepared field inoculum, conducted visual assessments of head symptoms and measured plant height in the field. I extracted genomic DNA and performed a qPCR assay for quantification of fungal DNA and provided grain material for toxin measurements. I measured microclimate conditions within the plant canopy and retrieved raw data from a nearby weather recording station to process data on environmental and weather conditions. I generated a multiple linear regression model of fungal DNA contents and weather conditions over five years with critical input from Johannes Petermeier. I calculated Pearson's correlations and disease severity rankings according to data on fungal DNA and mycotoxin contents. I generated all figures (except S3) and tables (except tables S7 and S8). I wrote the first manuscript draft (except for the methods section regarding the quantification of multiple *Fusarium* toxins) and implemented input and comments from all other authors. After editing by Ralph Hückelhoven, Eva Biehl, Michael Heß and feedback from all authors and reviewers, I finalized the manuscript for publication.

2.4 Barley shows reduced Fusarium Head Blight under drought and modular expression of differentially expressed genes under combined stress

Summary

Under natural conditions, plants are usually confronted with complex abiotic and biotic stress combinations. The simultaneous occurrence of different stresses activates regulatory processes to express and balance stress responses. The interaction between two different abiotic and biotic stress factors often results in synergistic, antagonistic or neutral effects on plant growth, health and productivity. However, *in planta*, physiological and transcriptional regulations under combined stress situations are little understood. Moreover, global warming provokes severe and frequent droughts, which will increasingly endanger crop productivity and might affect plant diseases and genotype resistance. In this context, the present study investigated stress regulation and quantitative resistance of spring barley to *Fusarium* head infection under drought stress. Therefore, three differently FHB susceptible spring barley genotypes were drought-stressed before *F. culmorum* spray infection of heads under controlled greenhouse conditions.

Quantification of fungal DNA contents showed reduced FHB severity under drought compared to respective watered control plants. Measurement of several stress-related phytohormones and metabolites showed specific physiological responses to infection or drought stress. Furthermore, RNA extraction, global 3'mRNA-sequencing and mapping of transcripts on the barley reference genome revealed stress-specific and genotype-dependent differential gene expression represented by 12,818 DEGs. A self-organizing tree algorithm (SOTA) uncovered differential gene expression patterns over all treatments and genotypes. Quantification of DEGs and strength of transcriptomic regulation reflected concentrations of fungal DNA and stress-associated physiological markers, notably abscisic acid. Under infection, a number of pathogenesis-related DEGs clustered together and expression strength indicates that these co-expressed DEGs likely reflect susceptibility rather than resistance. The combination of both stress factors resulted in the highest number of DEGs (10,188). Patterns of co-expressed gene clusters suggest a modular stress response pattern consisting of combinations of genes similarly expressed under either infection or drought stress alone. Additionally, infection-related DEGs predominantly showed a lower responsiveness under combined stress. This suggests that the firstly applied drought stress led to strong alterations in gene expression under combined stress and resulted in reduced fungal colonization in all three varieties.

In a further step, a weighted gene correlation network analysis sorted similarly co-expressed genes over all treatments, samples and time points into 18 different gene modules, reflecting

drought or *Fusarium*-specific stress responses of the three different barley genotypes. An infection-associated module contained co-expressed genes with functions for defence, programmed cell death or detoxification of mycotoxins for all three genotypes. Although the diverse genotypes exploited similar genes under infection, gene expression resulted in variable success in defence. Further, drought or genotype-related modules showed correlations with phytohormones and the osmolyte proline. The largest module showed enrichment of mainly downregulated genes under drought with functions in photosynthesis according to gene ontology, outlining the comprehensive impact of drought on basal physiological functions.

Taken together, the combination of *Fusarium* infection under drought stress incorporated the highest number of DEGs and consisted of modular responses to single stresses rather than a specifically recomposed gene expression pattern. Further, networks of co-expressed DEGs clustered in modules showed strong associations with physiological stress markers, which revealed to be useful for the interpretation of complex stress combination studies and for the possible development of stress-related genetic markers for breeding approaches.

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Contributions

I developed the experimental design for *Fusarium* infections of barley under drought stress together with Michael Heß. I performed the greenhouse experiment, spray infections, drought application and sampling of barley heads. I extracted DNA for quantification of fungal DNA contents and extracted total mRNA for 3'RNA-sequencing. I analysed raw data on fungal DNA contents and physiological stress markers and suggested weighted gene co-expression network analysis for analysing gene expression data. I wrote the manuscript with Christina Steidele and

Ralph Hückelhoven and gave input and ideas on data visualization. After critical input from other authors, I submitted the finalized manuscript together with Christina Steidele.

3 Discussion

The environment creates conditions in which plants simultaneously face multiple abiotic and biotic stresses. Combined exposure to drought or heat and invading pathogens enhances the complexity of plant responses and respective stress mitigation. There is growing evidence that global warming is increasing the strength and severity of abiotic stress situations and the abundance of pathogens provoking disease risks and crop losses in world agriculture (Velásquez et al., 2018; Delgado-Baquerizo et al., 2020; Farooq et al., 2023). For instance, shifts in climatic conditions strongly affect the host-pathogen-environment interactions, resulting in less predictable severity of plant diseases. In this context, changes in climatic conditions alter plant growth and health, pathogen abundance and create field conditions probably unsuitable or promotive for a disease outbreak.

The epidemiology of the two fungal pathogens *R. collo-cygni* and *Fusarium* spp. in barley is exemplary for plant diseases strongly associated with environmental factors. Studies on the epidemiology and stress physiology possess two model plant-patho-systems to unravel weather and environment-dependent modulations of disease resistance, which is of increasingly high importance in terms of adapting crops to complex environmental conditions and to ensure global food security and safety under a changing climate (Singh et al., 2023).

The present work studied the impact of drought conditions on RLS in the field and assessed the effects of drought stress on FHB of barley on a physiological and transcriptomic level. Moreover, different diagnostic tools were adapted and validated to assess the quantitative RLS and FHB resistance of barley under diverse field environments or weather conditions and over several consecutive seasons. The findings support the assessment of global gene expression and physiological responses towards multiple stresses and further diagnostic methods to identify durable pathogen resistance under abiotic stress. The results contribute to our understanding the diverse impacts of environmental factors on disease resistance of barley for future adaptation towards complex stress situations and possibly allow translation to other crop plants.

3.1 Severity of RLS is strongly shaped by the environment and abiotic stress

Studies on the effects of environmental factors on RLS are rare. Hence, there is still a large gap in knowledge concerning the epidemiology of this increasingly emerging pathogen in barley, hampering effective control and selection of quantitative RLS-resistant barley genotypes. Furthermore, environmental conditions play a major role in the epidemiology of the disease.

However, the impact of certain field conditions on RLS severity and the expression of quantitative RLS resistance of barley, specifically under abiotic stress is still unknown. Therefore, the two conducted field studies on RLS disease in barley under drought (Publication I and II) aimed to assess the influence of drought stress on RLS severity and basal genotype resistance under field conditions.

Both field studies revealed a general suppression of RLS disease severity among 15 spring and 15 winter barley genotypes under late-terminal drought stress in the field (Publication I and II). Therefore, different assessed disease parameters indicated differences in RLS severity between genotypes under irrigation and to a lower extent also under drought conditions. Although the level of disease parameters varied over consecutive seasons, genotypes showing a robust basal RLS resistance could be identified in both barley assortments. In particular, drought conditions strongly changed the microclimate within the plant canopy, which can be associated with the strong suppression of RLS disease under persistent drought conditions. Undoubtedly, the recording of leaf wetness and leaf wetness duration revealed a dramatic decrease under drought conditions (Publication I), which likely provides adverse conditions for the fungus to grow and infect leaves. This is in line with findings from Formayer et al. (2002), where manual prevention of dew formation on barley plants during the night reduced leaf wetness and strongly decreased RLS leaf symptoms compared to plants permanently growing outdoors. For this purpose, plants were grown in pots under a polytunnel in the field and were placed indoors during the night. The polytunnel also prevented the plants from natural rainfalls, thus exclusively effects of leaf wetness on RLS were studied. Similarly, although the field seasons in 2018 and 2019 were hot and dry, the spring barley assortment grown under open field conditions was noticeably diseased with RLS (Publication I) but also with FHB (Publication III). According to observations in the field during symptom scoring for both diseases, the plant canopy was frequently wet in the morning, creating sufficient moisture to maintain fungal infestations despite the absence of rain over several weeks. This indicates that fungal diseases will probably not be fully absent in persistent dry seasons, because dew formation and single rain events could be still sufficient for disease outbreaks.

The recording of leaf wetness confirmed to be very sensitive to environmental (drought vs. irrigated) conditions in a very short time (Publication I), which is likely representative of assessing optimal conditions for the release of spore inoculum (Havis et al., 2023) and secondary infections via stomata, thus affecting RLS incidence and leaf infestation. In addition, Brown et al. (2014) found a positive relation between RLS severity and leaf wetness duration over several field sites. Investigation of minimum leaf wetness duration in relation to RLS

disease level could probably depict agro-meteorological thresholds with significant effects on disease risks over diverse field environments. In contrast, the mean temperature was increased within the canopy growing under drought (Publication I) and is likely affecting RLS disease over a longer term (seasonal scale), This might rather influence final disease levels in individual seasons. Warmer temperatures probably be additive towards drought conditions, which creates microclimate conditions strongly suppressing RLS (Publication I and II). Hence, this draws attention to the suitability of leaf wetness and leaf wetness duration for model-based disease warning and risk assessment, which is still lacking confirmation of robust parameters to construct RLS disease forecast (Havis et al., 2018; Havis et al., 2023). Results from publications I and II revealed the high sensitivity of RLS on environmental conditions, which created persistent droughts in the rainout shelter. Although drought comprehensively suppressed RLS in spring and winter barley, this was only consistent for all three assessed disease parameters in spring barley. Interestingly, fungal DNA contents in drought-stressed winter barley genotypes even exceeded the values of the respective irrigated plants. In addition, the increased amounts of fungal DNA in several genotypes under drought did not increase RLS symptoms. This suggests that the winter barley genotypes differently expressed symptoms under drought stress and environmental factors might differently affect the level of disease parameter. This raises the question of why fungal colonization was more variable in winter barley despite drought stress suppressed other disease parameters. It remains speculative, which environmental factors most significantly affected the epidemiology of *R. collo-cygni* with regard to the different field conditions and growth seasons of spring and winter barley. However, such comparisons could gain future understanding of specific interactions and disease resistance (genotype x environment interaction) of the same pathogen with two different types of the same host in different seasonal and environmental contexts. A study by Dreseitl (2011) could directly compare and further reveal a 40% lower susceptibility of winter barley in relation to spring barley towards powdery mildew infections when converting data on assessed powdery mildew disease scores of several spring and winter barley genotypes over a period of 30 years. On a physiological layer, plant characteristics and variable adaptation of spring or winter types, respectively, towards different growth habits result in altered responses to environmental conditions via differently regulated gene expression, protein or metabolite accumulation in leaf and grain tissue up to entire hormonal stress regulation (Stanca et al., 1996; Karimzadeh et al., 2005; Looseley et al., 2017). Such an interplay with molecular, biochemical and physiological characteristics might provoke variable disease resistance of spring and winter barley, which probably is further superimposed by growth period-dependent environmental conditions or

abiotic stress, respectively. The suppressive effect of the long-lasting drought conditions on RLS severity had been more consistent in spring barley (Publication I). Under conditions in Germany, spring barley usually flowers between the end of May and the beginning of June, whereas winter barley already flowers in mid of May, resulting in a time offset from this growth stage of about three weeks. Hence, the most RLS-sensitive post-flowering phase of spring barley is during periods with different weather conditions when compared to the same growth phase of winter barley. This may explain the more generally observed suppression of RLS disease of spring barley under drought (Publication I and II). For winter barley, mean leaf area showing clusters of conidiophores, fungal DNA contents and RLS symptoms were less strongly suppressed over all tested winter barley cultivars or even exceeded detected values of irrigated plants. However, the assortment of winter barley cultivars likely has a lower basal field resistance towards RLS than the tested spring barley assortment, resulting in higher mean DNA contents in leaves of winter barley (Publication I and II). In addition, RLS severity differed less in winter than in spring barley under both field conditions. Linear regressions between different disease parameters revealed a positive relationship between each single RLS disease parameter under drought conditions. Interestingly, the results again differ from the findings in spring barley, where such positive relations were only found for irrigated plants. Possibly, drought conditions in winter barley were less suppressive for the fungus than in spring barley, where microclimate conditions were characterized by increased mean temperature and lower leaf wetness in the plant canopy (Publication I). This leads to the assumption that drought conditions similarly altered microclimate in winter barley, but with probably less impact on epidemiology through overall different environmental conditions for winter barley crops. Besides, it can be assumed that conditions for winter barley generally create greater leaf moisture and leaf wetness duration than under spring conditions. This could explain why drought conditions strongly superposed relations between disease parameters in spring, but not in winter barley. Additionally, leaf moisture was recently determined as most crucial parameter to gain spore release of *R. collo-cygni* (Havis et al., 2023), which supports the suppressive effect on leaf area with clusters of conidiophores under drought (Publication I and II). Moreover, other studies indicate a positive relationship between leaf wetness and RLS leaf symptoms (Formayer et al., 2002; Brown et al., 2014). Finally, the putative variations in RLS epidemiology and disease severity between spring and winter barley must be proven in more detail. This could be investigated through parallel growth of both barley types, which rules out differences in seasonal conditions on genotype resistance of both barley types, although requiring artificial vernalisation of winter barley for cultivation in spring or growth during winter season under

mild (frost-free) conditions. Such experiments could give more insights into the genotype x environment interaction of the two barley types affecting RLS severity. However, to unravel the effects of drought stress on barley – *R. collo-cygni* interaction needs more accurate experiments under controlled conditions comprising functional analyses of the transcriptome and hormonal status. Here, there remains still a missing link between the physiology of barley and the impact of environmental stress on the expression of defence mechanisms on a physiological level. In this context, McGrann et al. (2015) could show that overexpression of a single stress-responsive NAC transcription factor (HvSNAC1) involved in drought tolerance and suppressing leaf senescence increases resistance to *R. collo-cygni*. This example shows how regulatory genes are pivotal linkers between mitigation of abiotic and biotic stresses and genotypes likely differ in utilization of genes with multiple beneficial effects on plant health. Such regulatory genes pose potential key points to understand the crosstalk between different stress-responsive pathways important for multi-stress mitigation. Similarly to the experimental setup conducted in publication IV, a transcriptomic study of RLS-infected barley under irrigation and drought grown under controlled conditions could gain new insights into the genotype-specific responses of barley and resolve physiological reasons for the observed suppression of RLS under drought (Publication I and II). Measurement of stress- and defence-associated phytohormones would support understanding of the specific stress regulations in response to the pathogen or both stresses, which is even hardly investigated for the *R. collo-cygni*-barley interaction. However, it should be noted at this point that the response of barley towards *R. collo-cygni* infection is not well investigated on a transcriptional or regulatory level and only relies on artificial infection of young plants (Sjokvist et al., 2019; Lemke et al., 2021). Hence, the previous transcriptomic studies do not cover the growth stages of barley during the transition towards generative growth (ear emergence and later), where the endophytic fungus usually initiates a pathogenic lifestyle for RLS disease outbreak (Walters et al., 2008). Indeed, the physiological interactions between host plants and endophytes living in a balanced antagonism without causing a disease are highly complex (Schulz & Boyle, 2005). Moreover, environmental stress is presumed to weaken the well-balanced defence status in such host-endophyte interactions, thus inducing a pathogenic lifestyle (Schulz et al., 1999), which is presumed for the barley-*R. collo-cygni* interaction (McGrann & Brown, 2017). Hence, the proposed subsequent experiments could primarily decipher physiological alterations in adult barley leading to the induction of pathogen lifestyle and could answer the question whether and how additional drought stress might have suppressed the pathogenesis of *R. collo-cygni* in spring and winter barley. For this purpose, the transcriptome analysis and methods as utilized

in publication IV are recommendable to uncover clusters of co-expressed DEGs in a physiological context to identify genetic markers or transcription factors (McGrann et al., 2015) which probably control quantitative RLS resistance of barley.

3.2 Evaluation of methods to assess quantitative FHB resistance under variable field conditions

Similar to RLS, FHB is regarded as a disease with strong associations with environmental and weather conditions affecting disease incidence and severity (Osborne and Stein, 2007). From the field studies conducted in publication III, a strong differentiation was found for quantitative FHB resistance of different spring barley genotypes after increasing pathogen pressure through bruised grain and spray inoculation over several years with variable weather conditions. In contrast, natural infection without inoculation did not result in appropriate differences in FHB severity between the tested genotypes, which was similarly reported in other field studies on FHB in barley (Linkmeyer et al., 2016; Khanal et al., 2021). Among the five years of field trials, even in hot and dry seasons, a strong *Fusarium* infection was detected after inoculation but also under natural infection. This led to the identification of quantitatively FHB-resistant spring barley genotypes adapted to Central European and German growth conditions. In particular, the quantitative resistance of these genotypes was further stable over several seasons despite variable weather conditions. Ranking of different disease parameters could balance variability in the amount of fungal DNA and multiple *Fusarium* spp.-associated mycotoxins in mature grain between different seasons. Hence, those genotypes possessing ranks, which were associated with resistance could be used for further crossing and selection in practical barley breeding programs. Probably, germplasm derived from those lines could show high quantitative FHB resistance. However, full FHB resistance is hardly existing in wild and cultivated barley (Huang et al., 2013) and not well exploited in practical plant breeding due to limitations in combining several quantitative trait loci, each contributing a relatively small effect on FHB resistance, in one genotype. This demonstrates the complex inheritance of resistance and the need for intensive FHB resistance phenotyping (Takeda, 1990; Steffenson, 2003; Arruda et al., 2016). Consequently, there is still a lack of highly quantitative resistant cultivars for sufficient management of FHB and DON accumulation in barley (Wegulo et al., 2015), instead often relying on fungicide treatments (Cowger et al., 2019). As recently shown in a meta-analysis of Sallam et al. (2023), several identified QTL contributing to resistance to FHB or DON accumulation in barley hardly explain genotypic variations in resistance to *Fusarium*

pathogens. These QTLs further did not consistently contribute to FHB resistance over multiple locations or seasons. This hampers selection approaches using genetic markers and marker-trait associations, as utilized e.g. in marker-assisted selection and genomic selection, however, with different accuracy for FHB resistance (Arruda et al., 2016). This likely indicates a strong genotype x environment interaction affecting FHB resistance of a genotype. In addition, most of the mapped QTL regions associated with FHB resistance show tight linkage to morphological traits, indirectly affecting infection of barley with *Fusarium* species. However, it is still not fully clear whether these traits have pleiotropic effects on resistance or how plant morphology traits influence the level of infection (Sallam et al., 2023). There are findings indicating FHB resistance-associated QTLs coincide with QTLs for morphological traits in barley, including inflorescence traits and plant height (Zhu et al., 1999). Plant height is one of the most studied morphological traits influencing resistance towards *Fusarium* spp. (Jenkinson & Parry, 1994; Yan et al., 2011). Despite the genotype assortment consisted genotypes showing relative homogenous height over multiple seasons and even under variable weather conditions, due to previous selection for different agronomic traits, plant height negatively correlated with fungal DNA contents of both utilized *Fusarium* species after soil surface inoculation (Publication III). This effect was not present for spray-inoculated or naturally infected plants. Notably, this FHB-associated trait only became assessable through artificial soil inoculation, which needs to be considered for field selections in breeding nurseries. Overall, the genetic FHB resistance is complex and often interconnected with morphological traits. Indeed, there are likely more morphological traits like row and flowering type (Yoshida et al., 2005), the angle of the head, the density of grains, the distance between leaves and head or thickness of husks determining an 'ideo-type' in barley morphology limiting or avoiding *Fusarium* infections, namely independent from environmental conditions. This would need more fine-tuned field evaluations over multiple field locations and weather conditions based on the findings in publication III.

Unlike wheat, barley is frequently infected by a heterogeneous complex of multiple *Fusarium* species. Those species produce diverse and hazardous mycotoxins and show different optima in environmental conditions for growth and proliferation (Nielsen et al., 2011; Linkmeyer et al., 2016). This complicates the assessment and control of FHB in barley, and genotypes probably do not incorporate resistance similarly effective towards diverse *Fusarium* species and their associated mycotoxins. In particular, seasonal weather effects further create high variations in the abundance of different species in barley (Hofer et al., 2019). In the present study (Publication III), the field screening for FHB resistance was assessed using only two

Fusarium species as inoculum and resulted in variations in incidence between genotypes in response to increased disease pressure, the two *Fusarium* species and their associated mycotoxins. However, FHB is associated with a complex of more species with individual optima for growth conditions and distribution or variable mycotoxin spectra. In addition, different *Fusarium* species cause unspecific symptoms or infections remain inconspicuous in barley (Linkmeyer et al., 2013; Geißinger et al., 2017). In publication III, inoculation with *F. culmorum* and *F. avenaceum* produced variations in the severity of head symptoms and grain symptom characteristics, disease severity (fungal DNA contents) and levels of different mycotoxins over consecutive seasons. These effects were likely due to variable responses to weather conditions and microclimate and are consistent with other studies (Nielsen et al., 2011; Linkmeyer et al., 2016). However, the above mentioned complexity of FHB in barley suggests paying more attention to genotype responses towards multiple *Fusarium* species in barley, which should comprise comprehensive inoculation trials comparing disease incidence and weather conditions. This would resolve specific environmental conditions promoting individual *Fusarium* species in barley. For that reason, inoculation nurseries and highly accurate diagnostic tools are indispensable (Nicolaisen et al., 2009) and were validated despite variable weather effects in publication III. On the one hand, the inoculation in the field revealed quantitative resistance despite the strong influence of the environment. Spray inoculation in the greenhouse could control environmental conditions and exclude external factors that interfere with the present pathogen, the host plant or both, thus affecting accuracy in FHB resistance scoring (Geddes et al., 2008). On the other hand, the obtained results on FHB resistance were used to identify differently susceptible genotypes for the greenhouse experiment in publication IV. Here, the irrigated, *Fusarium*-infected plants again showed similar resistance levels as assessed in the field (Publication III and IV).

Weather conditions strongly affect the distribution of *Fusarium* species and influence conditions for successful infection of the host (Jenkinson & Parry, 1994; Xu et al., 2008). For effective management of barley FHB disease and mycotoxin contaminations in the field, determining specific conditions for the epidemiology of *Fusarium* species is of high importance. However, high variability in weather conditions complicates predicting infection events, especially for different *Fusarium* species (Hooker et al., 2002). Despite several FHB forecasting models being implemented for wheat FHB and DON often using epidemiology of *F. graminearum* (Musa et al., 2007; Prandini et al., 2009), implementations for barley remain more complex (Bondalapati et al., 2012). In contrast to wheat, the lower economic relevance of barley products might have kept risk assessment of barley FHB out of focus. Out of this reason,

a multiple linear regression model was used to determine specific weather conditions before and post flowering, which had the most significant effect on fungal colonization of mature barley grains of different genotypes (Publication III). The defined model combined mean temperature, relative air humidity, precipitation during the two weeks before and four weeks after heading date with fungal DNA contents among the 17 tested genotypes. The obtained data revealed strong weather effects for the two weeks before anthesis and the fourth week after on infestation of grain with *F. culmorum* but found more genotype-specific and time-dependent association of weather conditions with *F. avenaceum* DNA contents. This reflects the difficulties for FHB risk assessments in barley due to the species-specific optima in weather conditions. However, the genotype-dependent manner reflecting *F. avenaceum* DNA contents in MLR model analysis suggests species-specific interactions with variable susceptible barley genotypes under the given weather conditions. The findings indicate that various weather parameters might need to be differently weighted according to estimated optimal conditions for individual *Fusarium* species in barley (Osborne & Stein, 2007; Schöneberg et al., 2018). However, the obtained model is only based on a five-year assessment and might not sufficiently reflect the full complexity of field conditions relevant for FHB development in barley. For instance, mycotoxins were only measured for three years, hence MLR analysis was not computable lacking enough data. Therefore, the MLR analyses in publication III support the understanding of epidemiology within the barley-*Fusarium* species complex and might be a first step towards implementing a barley-specific FHB risk forecast in Germany. Overall, this might need more meteorological variables (e.g. number of days with precipitation, sum of temperature, global radiation, days with humidity above a certain threshold, leaf wetness) over multiple consecutive seasons and sites in relation to monitoring various disease parameters over the entire seasons. In addition, such data might also be useful to attain risk assessment for RLS.

Global warming rapidly changes the abundance and severity of conditions creating complex stress situations for plants (Chaloner et al., 2021; Laine, 2023), which raises the need to adapt crops to multiple and variable stresses (Lasky et al., 2023). Therefore, one important aspect is combining multiple stress resistances in one genotype, either qualitatively or quantitatively (Wiesner-Hanks & Nelson, 2016; Munns and Millar, 2023). As mentioned above, resistance towards RLS and FHB is of quantitative nature and ideally stable across diverse environmental conditions (Publication I and III). By ranking assessed disease parameters of different field-grown spring barley genotypes for RLS (Publication I) and FHB (Publication III), differentiation in basal resistance between the most susceptible and the most resistant genotype became depictable. The disease rankings balanced season-dependent variations and combined

different parameters to a final rank. Indeed, a few genotypes were ranked with low ranks for RLS disease parameters and for infection of *F. culmorum* and *F. avenaceum*, which was associated with high quantitative resistance (Eunova, Grace, IPZ 24727, Umbrella). Hence, these genotypes represent suitable germplasm combining resistance towards two important barley diseases and three different fungi under variable open field or even adverse environmental conditions created in the rainout shelter. In addition, global SNP data (Publication III; supplementary data) could genetically cluster those genotypes together, which indicates a partially common genetic origin and relationship. This suggests that these genotypes might carry genes controlling quantitative resistance to both diseases, which appeared robust towards variable weather effects. In a further approach, the identified genotypes could probably be used as crossing parents providing breeding lines for QTL mapping to identify chromosomal regions associated with resistance or for genomic selection to improve quantitative FHB and RLS resistance of barley.

3.3 Drought stress modulates FHB disease resistance of barley

A recently increasing number of stress combination studies allows deeper insights into complex plant stress responses, which gain understanding of plant disease resistance in an environment-dependent context (Pandey et al., 2015). From the findings in the field experiments on FHB (Publication III), barley genotypes were selected for their variable quantitative resistance to investigate the specific impact of drought stress on the defence of barley at a transcriptomic and physiological level (Publication IV). For this purpose, barley plants were grown under controlled greenhouse conditions and exposed to drought stress one week before anthesis and then spray inoculated with *F. culmorum* spore solution. The obtained data revealed a strong decrease in FHB severity of drought stressed compared to irrigated plants, at least for susceptible genotypes. Transcriptomic data showed that combined stress led to the highest quantity of DEGs, which reflected the composition of transcriptomic regulations towards the applied single stresses, rather than a newly orchestrated genetic response. Besides, Weighted Gene Co-expression Network Analysis (Zhang & Horvat, 2005) successfully identified different co-expression gene clusters (modules) containing typical infection- and drought-specific co-expressed DEGs, which correlated with different drought and pathogen-related physiological markers.

In particular, Weighted Gene Co-expression Network Analysis identified a gene module reflecting multiple defence-response associated DEGs with large overlaps between the three

genotypes. The module contained genes responsible for general pathogen defence, disease resistance proteins (including a 'Fusarium resistance orphan protein' (Perochon et al., 2019)), regulation of programmed cell death or immune responses, and mycotoxin detoxification. Interestingly, the gene encoding for the enzyme UGT glycosyltransferase (HvUGT13248) was identified as most strongly co-regulated with other infection-associated genes in the blue module. This module contains various co-expressed defence-associated genes and exclusively showed a significant positive correlation with the amounts of fungal DNA in barley heads under infection or drought stress and infection. According to Weighted Gene Co-expression Network Analysis and subsequent network analysis, the 25 gene edges with the highest co-expression values in the blue module were selected to evaluate inter-connection and connection strength (edge weight) between most co-regulated infection/defence-associated genes. This produced a gene co-expression regulatory network showing most heavy edges connected to the UGT13248 glycosyltransferase. This suggests that mitigation of the DON virulence factor is highly important in barley in response to *Fusarium* infections. Barley suffering from DON-producing *Fusarium* species utilizes several glycosyltransferases for glycosylation of DON to the less phytotoxic DON-3-glucoside (Schweiger et al., 2010). Previous studies confirmed the high relevance of the identified barley glycosyltransferase (HvUGT13248) in type II resistance in cereals thus reducing pathogen spread in host tissue and severity of FHB and FCR. This holds true over several plant species in transgenic overexpression approaches in *Arabidopsis thaliana* (Shin et al., 2012), wheat (Li et al., 2015, 2017; Mandalà et al., 2019) and barley (Bethke et al., 2023), which consistently resulted in enhanced resistance towards *Fusarium graminearum* and *F. culmorum* by protective conversion of deoxynivalenol or nivalenol towards less phytotoxic DON-3-glucoside or nivalenol-3-O- β -D-glucoside, respectively (Li et al., 2017). In addition, two oat UGT glycosyltransferases, orthologous to barley HvUGT13248, were identified by Khairullina et al. (2022), which are functional in DON detoxification. Hence, mitigation of these decisive virulence factors effectively reduces FHB and FCR (Mandalà et al., 2019; He et al., 2020). The expression data in publication IV show upregulation of HvUGT13248 in response to *Fusarium* infection of all three tested genotypes under well-watered conditions. Noteworthy, and among most of the 25 selected genes, the HvUGT13248 gene remains significantly upregulated under drought stress and infection in the cultivars Barke and Palmella Blue but was less strongly upregulated in Morex. Consequently, the upregulation of the UGT glycosyltransferase alone did not correspond to the infection levels neither under irrigation nor drought stress. Morex was the least FHB susceptible under drought, suggesting that other defence-associated genes contributed to less successful pathogenesis. Hence, DON

detoxification was probably less important for Morex, being least infected under combined stress, indicating that the response to specific virulence factors is strongly shaped by abiotic stress factors. However, the contents of DON or DON-3-glucoside were not measured. Thus, it remains speculative whether the expression pattern of one or multiple glycosyltransferases could reflect the attenuation of the virulence factor DON to explain the level of FHB resistance either under irrigation or combined stress. In contrast, a positive correlation between fungal DNA contents (fungal colonization) and DON-3-glucoside and a positive trend between fungal colonization and DON contents were found among 17 barley genotypes in three consecutive seasons in the field (Publication III). Despite variable weather conditions in each season, the results indicate that the severity of fungal colonization is associated with higher loads of DON and conversion of DON towards DON-3-glucoside. Moreover, the contents of DON positively correlated with DON-3-glucoside (Publication III; data not shown). Hence, susceptible genotypes likely compensate higher loads in fungal colonisation and associated DON in terms of tolerance rather than to defend against the fungus. Conversely, only genotypes governing effective suppression of initial DON synthesis likely show general FHB resistance (Mesterházy et al., 1999). In this context, the utilization of genes for mycotoxin detoxification represents a substantial tool for crop improvement regarding food safety. Moreover, UGT-glycosyltransferases constitute a group of potential marker genes for the assessment and selection of genetic resistance to FHB mycotoxins in cereal crops (Faltusová et al., 2019). Hence, detection of DON and DON-3-glucoside should be incorporated in future stress-combination experiments, as conducted in publication IV, to resolve the role of DON detoxification in defence responses of barley. The relatively uniform expression of several glycosyltransferases under infection and even under combined stress (Publication IV; supplementary data D1.1) possess high potential for pyramiding these class of genes in a single genotype to address enhanced and environment-independent quantitative FHB resistance in barley and other cereals. Therefore, FHB resistance breeding needs a more extended understanding of the above mentioned resistance components even under complex combination stress, which is likely genotype-specific and depends on the expression of multiple genes. Indeed, this becomes increasingly relevant for phenotyping and plant breeding under changing climatic conditions with frequently increasing unfavourable growth conditions. Besides, there is evidence that disease resistance to *Fusarium* spp. and tolerance to drought are compatible on a transcriptomic level (Su et al., 2021).

The Self Organizing Tree Analysis (Publication IV) clustered DEGs according to different conditions, which overall identified gene expression clusters. Several clusters regulated in

response to drought or infection alone showed similar regulation patterns under combined stress, which indicates that barley generally utilizes a composition of specific responses to the single stresses rather than a unique composition of regulated genes. However, some clusters in Morex under combined stress showed similar responses under infection or drought alone, indicating Morex specific responses, which probably reduced the success of infection. On the other hand, infection under well-watered conditions resulted in the highest numbers of upregulated DEGs corresponding to the level of infection of each genotype, suggesting that strength of gene expression can be associated with fungal success in infection. Nevertheless, the clusters of infection-responsive DEGs generally showed lower expression under combined stress, suggesting other factors might have limited pathogenesis under drought.

Measurement of the drought stress-associated phytohormone ABA and associated metabolites, like ABA glucoside, phaseic acid, as well as the osmolyte proline, revealed that the strong drought stress altered plant physiology and hormonal regulation compared to watered plants. This highlights that physiological alterations had an impact on infection under drought. Drought-associated up-regulation of physiological products such as phytoalexins (Vaughan et al., 2015) could possibly suppress pathogen infection, posing a potential biochemical defence. In particular, an increase in proline contents in plant tissue is a common physiological marker for abiotic stress, including drought stress. Proline is considered an osmolyte mediating osmo-protection of cell tissue and is known to scavenge ROS under stress (Hayat et al., 2012). Proline metabolism and upregulation are also involved in plant-pathogen interactions and associated stress responses (Qamar et al., 2015). In *Arabidopsis thaliana*, it was previously found that proline metabolism mediated by proline dehydrogenase and pyrroline-5-carboxylate dehydrogenase affects pathogen defence responses (Cecchini et al., 2011; Qamar & Senthil-Kumar, 2019). The transcriptomic data (Publication IV; supplementary data D1.1) reveal strong upregulation of a single pyrroline-5-carboxylate dehydrogenase (HORVU.MOREX.r2.5HG0429800) in response to *Fusarium* infection under both, watered and drought conditions, but not under drought stress. Similarly, *F. culmorum* infection and DON treatment induced proline accumulation in wheat seedlings (Bandurska et al., 1994). Altered proline contents were also found by Manghwar et al. (2021) in wheat growing in *Fusarium equiseti* inoculated soil. Increased proline contents were found for a resistant genotype, while a susceptible wheat line showed decreased contents. Hence, proline metabolism may represent a valid stress marker, which is further relevant in response to *Fusarium* infections.

Stress combinations often cause conflicting signals for the plant. As a result, plants need to prioritize and economize their specific responses to mitigate stress exposition and further damage (Verma et al., 2016). Drought stress before inoculation resulted in strong regulation of e.g. ABA and ABA-derivates or salicylic acid, which depict typical hormone signalling towards drought or pathogen stress. At this point, drought responses and regulation of ABA signalling might also affect pathogen responses. However, drought increased ABA contents, but this may not have enhanced FHB susceptibility as previously described (Qi et al., 2016; Wang et al., 2018). ABA-responses under drought rather affected auxin regulation in a manner that likely decreased the proposed effects on susceptibility (Luo et al., 2016; Wang et al., 2018; Brauer et al., 2019). The infection-related (WGCNA) module positively correlated with auxin contents, whereas the abiotic stress-related salmon module showed strong positive associations with drought-associated hormone signalling (ABA, ABA derivates, phaseic acid, dihydrophaseic acid). The many contrary correlations of co-expression modules with different hormones may indicate that the plants utilized different gene regulatory pathways, which control or interfere with hormonal regulations. This gives insights into how the plants probably prioritized particular stress responses and how tight *Fusarium* spp. infections interacted with plant physiology (Ding et al., 2011; Makandar et al., 2010, 2012). Measurements of jasmonic acid, gibberellic acid and ethylene were not conducted in publication IV, but could in the future complement the physiological responses of barley towards the applied stress combinations due to the proposed relevance of jasmonic acid and gibberellic acid in resistance (Sun et al., 2016; Buhrow et al., 2021) and ethylene in susceptibility towards *Fusarium* spp. (Chen et al., 2009; Xiao et al., 2013). However, several DEGs related to ethylene and jasmonic acid synthesis or respective responses were significantly regulated in response to infection strength and primarily clustered in the infection-related module (Publication IV; supplementary data D.1.8, D1.9). In contrast, ethylene is associated with ripening processes, which were clearly accelerated under drought stress and probably early grain maturity retarded the ongoing pathogenesis and thus limited fungal grain colonisation.

3.4 Plant resistance and disease severity depend on temporal influence of stresses

The observed responses towards multiple stresses provided detailed insights into the complex barley-*Fusarium* spp.-interaction under abiotic stress. Together, the investigated impact of drought stress before inoculation on FHB severity (Publication IV) and the highly variable weather conditions differently affecting quantitative FHB resistance of barley genotypes

(Publication III) raises attention to review the relevance of such a drought scenario in the context of the epidemiology of FHB. Drought before infection led to strong suppression of FHB in the greenhouse, despite infection conditions were considered optimal due to covering barley heads to maintain high relative air humidity for two days post inoculation with polythene bags. In addition, the stop of irrigation and very reduced watering provoked a sudden and strong decrease in available water in the pot substrate, hence the plants quickly suffered severe drought stress. In nature, soils do not desiccate so quickly and strong and sudden abiotic stress will only appear in combination with a relatively quick rise in temperature in the field, which adds heat stress. The rainout shelter experiments (Publication I and II) created persistent drought conditions between spike emergence and grain maturity. As a result, drought stress increased gradually and the plants had more time to adapt and mitigate dehydration stress. The adverse conditions in the plant canopy suppressed RLS in spring and winter barley. Under such persistent drought conditions, short rainfalls could provide moisture and splash dispersal of *Fusarium* spores, creating a scenario similar to that in the stress combination experiment in the greenhouse (Publication IV). Such conditions were partially observed in hot and dry seasons in 2018 and 2019 but did not suppress *Fusarium* spp. infections (Publication III). The experimental setup in the greenhouse provoked strong and relatively distinct responses to single and combined stresses, which made conclusive answers to this point unknown transcriptional and physiological readouts possible. Undoubtedly, this raises the question whether less severe drought stress before inoculation would produce similar results or simply weaker effects on gene expression, hormone signalling or physiological alterations and thus pathogen resistance. According to the literature, disease severity is related to the strength of abiotic stress, but not generally in a simple linear connection. Consequently, this leads to a variable final outcome in each scenario (Kissoudis et al., 2016). For FHB of barley under drought, it remains elusive and would further require multiple experiments with differently severe drought stress. Moreover, mild drought stress would need more time to establish and cause significant responses of the plant. However, preliminary experiments resulted in enhanced FHB severity when drought stress began to increase with simultaneous infection. As a result of this alternative scenario, the plants need to mitigate drought and pathogen stress simultaneously. Especially stress signals that require conflicting transcriptional answers could possibly limit the plant to effectively mitigate the combined stress, which increases FHB susceptibility. For this reason, genotypes with high genomic and physiological plasticity might better prioritize utilization of resources under complex stress and thus perform stable yield and crop quality.

4 Concluding remarks

This dissertation aimed to assess the quantitative resistance of different barley genotypes towards RLS disease and FHB under variable environmental and weather conditions in open and semi-controlled field trials using a rainout shelter. In addition, the effects of drought stress on the basal resistance of barley were studied under controlled conditions by analysing the global gene expression in response to combined drought stress and *Fusarium culmorum* infection.

The results achieved insights into the weather and environment-dependent quantitative pathogen resistance of barley towards RLS and FHB disease in the field. Persistent drought conditions resulted in the suppression of RLS. A Multiple Linear Regression model revealed that weather conditions during the reproductive growth stages of barley showed associations with grain infection of *Fusarium* spp. after soil inoculation. Several barley genotypes showed high quantitative RLS and FHB resistance over several seasons and variable weather conditions.

The global gene expression analysis on the combination of *Fusarium* infection under drought stress led to the highest number of differentially regulated genes compared to the single stresses. The stress combination resulted in a modular gene expression pattern composed of DEGs expressed in response to both single stresses. The clustering of genes in co-expression networks showed strong associations with physiological markers, which supported the interpretation of the gene expression responses under single and combined stress.

The findings of this work contribute to the understanding of genotype responses and weather effects which modulate disease incidence and resistance. In conclusion, increasingly adverse weather conditions, intensified by global warming, will provoke more complex stress conditions for crop plants. Hence, various stress scenarios and their impact on the epidemiology of pathogens, as well as the resistance of crops, need to be better understood in the near future. The effect of the climate and environmental conditions on plant-pathogen interactions requires more attention. Therefore, patho-phenotyping from individual plants to whole fields enables better adaptation and represents effective solution steps to meet the complex challenges in global agriculture.

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Appendix

The original publications, supplementary figures and tables are accessible via the following web links below:

Publication I:

<https://link.springer.com/article/10.1007/s41348-020-00420-z>

(last accessed: 04 December 2023)

Supplementary data - publication I:

https://static-content.springer.com/esm/art%3A10.1007%2Fs41348-020-00420-z/MediaObjects/41348_2020_420_MOESM1_ESM.pdf

(last accessed: 04 December 2023)

Publication II:

<https://link.springer.com/article/10.1007/s41348-023-00790-0>

(last accessed: 04 December 2023)

Supplementary data - publication II:

https://static-content.springer.com/esm/art%3A10.1007%2Fs41348-023-00790-0/MediaObjects/41348_2023_790_MOESM2_ESM.xlsx

(last accessed: 04 December 2023)

Publication III:

<https://www.mdpi.com/2072-6651/14/2/125>

(last accessed: 04 December 2023)

Supplementary data - publication III:

<https://doi.org/10.6084/m9.figshare.c.5798849>

(last accessed on 04 December 2023)

Publication IV:

<https://academic.oup.com/jxb/article/74/21/6820/7260321>

(last accessed 04 December 2023)

Supplementary data - publication IV:

<http://academic.oup.com/jxb/article-lookup/doi/10.1093/jxb/erad348#supplementary-data>

(last accessed: 04 December 2023)