Characterisation of 40 barley genotypes regarding powdery mildew susceptibility and responses of selected genotypes to biological control agents

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Summary

Global agriculture is confronted with increasing weather extremes due to climate change and is facing broadening fungicide resistance. As part of EU's Green Deal the use of synthetic chemical pesticides needs to be reduced and their availability is already decreasing. Therefore, sustainable biological solutions are highly demanded to stabilise harvests. In barley production, powdery mildew (*Blumeria graminis* f.sp. *hordei*) is one of the major threats for overall yield and therefore a focus in cultivar selection and breeding processes. To study the host-pathogen system and to develop promising cultivation strategies, methods for artificial inoculation are just as important as knowledge about the susceptibility of individual genotypes and their responsiveness to biological control agents.

Therefore, this study introduces an inoculation protocol including an easy-to-build, low-cost inoculation tower whose characteristics are statistically described. The method was applied to examine 40 spring barley genotypes for their susceptibility towards *Blumeria graminis* f.sp. *hordei* race A6. A susceptibility continuum from resistant to very susceptible genotypes was found, with 18 genotypes being classified as very resistant. The results partly complemented the descriptions of previous studies, but also solved the so far unanswered question of resistance for nine genotypes.

With a systematic investigation of several genotypes and biological control agents this study revealed diverse effects of these products against powdery mildew. They ranged from genotype-independent mildew reduction to genotype-dependent infection enhancement. Expression studies showed not only a direct expression-enhancing effect on pathogen-associated genes but also priming effects, reflecting the variability of reactions found. This expands the so far little knowledge of the genetic basis and the mode of action of biological control agents. The possibilities and limits of the products were also diverse under simultaneous drought stress and infection with powdery mildew. Besides, a further spraying at the onset of the first symptoms of infection provided indications on enhanced efficiency against powdery mildew after respraying. In addition to infection, the biological control agents influenced the above-ground dry biomass, plant height, developmental stage, as well as the chlorophyll content and the nitrogen balance index. These traits showed great variability depending on the product, the occurrence of drought stress and the genotype.

This work demonstrates that the isolated consideration of the mildew-reducing effect of biological control agents does not reflect their overall potential or their limitations. Therefore, future experiments on biological effectiveness should include combinations of simultaneous stressors, investigations of physiological and growth parameters as well as comparisons of different genotypes.

Zusammenfassung

Die globale Landwirtschaft ist aufgrund des Klimawandels mit zunehmenden Wetterextremen und mit steigenden Fungizid-Resistenzen konfrontiert. Es werden nachhaltige biologische Lösungen zur Stabilisierung der Ernten benötigt. Dies liegt nicht zuletzt an der immer geringer werdenden Verfügbarkeit chemisch-synthetischer Pestizide, deren Einsatz im Rahmen des Green Deals der EU reduziert werden soll. Im Gerstenanbau ist der Echte Mehltau (*Blumeria graminis* f.sp. *hordei*) eine der größten Bedrohungen für den Gesamtertrag und daher ein zentraler Punkt in der Sortenwahl und Züchtung. Um das Wirt-Pathogen-System zu verstehen und vielversprechende Anbaustrategien zu entwickeln, sind Methoden zur künstlichen Inokulation ebenso wichtig wie das Wissen über die Anfälligkeit einzelner Genotypen und ihre Ansprechbarkeit auf biologische Alternativprodukte.

Daher wird in dieser Studie ein Inokulationsprotokoll vorgestellt, das einen einfach zu bauenden, kostengünstigen Inokulationsturm umfasst, dessen Merkmale statistisch validiert wurden. Die Methode wurde angewendet, um 40 Sommergerstengenotypen auf ihre Anfälligkeit gegenüber der Rasse A6 von *Blumeria graminis* f.sp. *hordei* zu untersuchen. Dabei wurde ein Anfälligkeitskontinuum von resistenten bis sehr anfälligen Genotypen gefunden, wobei 18 als sehr resistent eingestuft wurden. Die Ergebnisse ergänzen teilweise die Beschreibungen früherer Studien, lösen aber auch die bisher unbeantwortete Frage der Resistenz für neun Genotypen.

Eine systematische Untersuchung der Wirksamkeit biologischer Präparate gegen Echten Mehltau an mehreren Genotypen zeigte unterschiedliche Wirkungen, die von Genotyp-unabhängiger Symptomreduktion bis Genotyp-abhängiger Infektionsverstärkung reichten. Expressionsstudien ergaben nicht nur eine direkte expressionssteigernde Wirkung biologischer Präparate auf Pathogen-assoziierte Gene, sondern auch Priming-Effekte, was die Variabilität der gefundenen Reaktionen widerspiegelt. Dies erweitert das Wissen über die genetischen Grundlagen und die Wirkungsweisen biologischer Präparate. Die Möglichkeiten und Grenzen der Produkte waren auch bei gleichzeitigem Trockenstress divers. Zudem lieferten die Versuche Hinweise auf eine verstärkte Wirksamkeit nach wiederholter Applikation. Neben der Infektion beeinflussten die biologischen Präparate auch die oberirdische Trockenbiomasse, die Pflanzenhöhe, die Entwicklung sowie den Chlorophyllgehalt und den Stickstoffbilanzindex. Diese Merkmale zeigten eine große Variabilität in Abhängigkeit von Trockenheit, biologischen Produkten und dem Genotyp.

Die vorliegende Arbeit zeigt, dass die isolierte Betrachtung der mehltaureduzierenden Wirkung biologischer Produkte weder deren ganzes Potenzial noch deren Grenzen widerspiegelt. Daher sollten künftige Studien Kombinationen gleichzeitiger Stressoren, Untersuchungen physiologischer und wachstumsbezogener Parameter sowie Vergleiche verschiedener Genotypen umfassen.

List of abbreviations

Abbreviation	**Meaning**
AICc	Akaike Information Corrected Criterion
ALD	Alcohol dehydrogenase
AsPer1	Ascorbate peroxidase 1
BAX1	BAX inhibitor 1
Bgh	*Blumeria graminis* f. sp. *hordei*
Bgh A6	*Blumeria graminis* f. sp. *hordei* race A6
CLMM	Cumulative link mixed models
CoZiSuDi1	Copper-zinc superoxide dismutase 1
CT	Cycle Threshold
dai	Days after inoculation
das	Days after sowing
ERF1	Ethylene response factor 1
f.sp.	*Formae speciales*
GC-MS	Gas chromatograph-mass spectrometry
GluRed1	Glutathione reductase 1
GP	Golden Promise
g_{min}	Minimum conductance of a leaf to water
hpa	Hours post application
hpi	Hours post inoculation
HR	Hypersensitive response
HSP70	Heat shock protein 70
HSP90	Heat shock protein 90-1
LSD1	Lesion simulation disease 1
Mla	*Mildew locus a*
Mlo	*Mildew locus o*
MWHC	Maximum water holding capacity
NBI	Nitrogen balance index
NPR1	Non-expressor of pathogenesis-related genes 1

Abbreviation	**Meaning**
PAMPs	Pathogen-associated molecular patterns
PeLyLP	Pectin-lyase like Protein
Perox	Peroxidase 40
p-i	Post-inoculation
PR1	Pathogenesis-related protein 1
PR17b	Pathogenesis-related protein 17b
PR1b	Pathogenesis-related protein 1b
PR5	Pathogenesis-related protein 5
qPCR	Quantitative polymerase chain reaction
RBOH	Respiratory burst oxidase homologue
RBOHF2	Respiratory burst oxidase homologue F2
rh	Relative humidity
RWD	Relative water deficit
ROS	Reactive oxygen species
S.	Supplementary
SA	Salicylic acid
SAR	Systemic acquired resistance
Ubi	Ubiquitin
UHT	Ultra-high-temperature processing

1. Introduction

Global warming threatens harvests worldwide by increasing drought periods and weather extremes (Daryanto *et al.* 2017; Matiu *et al.* 2017). Thereby, yield losses of 50-80% depending on crop and region are reported (Zhang *et al.* 2018a). Crops are additionally damaged by the occurrence of associated infectious diseases such as powdery mildew. Resistance towards phytopathogens is one of the major goals in crop breeding and cultivar selection. This requires knowledge of the susceptibilities and resistances of genotypes. Additionally, chemical pesticides are often used to control infection, although these products may show negative environmental impacts, and the number of authorised products is continuously decreasing. For this purpose, biological control agents are required. They can be of animal or plant origin or contain living microorganisms or their metabolites. There are numerous terms, some of which cannot be clearly distinguished from one another, as they categorise the products according to different criteria. 'Biostimulants', for example improve among others plant nutrient use efficiency, tolerance to abiotic stress or quality traits via stimulation of nutrition processes (Feldmann *et al.* 2022) – an effect-based categorisation. In contrast, 'bioprotectants' is a general term unifying organisms, botanicals as well as semio-chemicals (Feldmann *et al.* 2022) – an origin-based categorisation. In addition, there is a regulatory categorisation of products according to which, in European legal framework for plant protection, plant protection products are distinguished from basic substances that are not primarily used for plant protection but are useful for the protection of plants (EC No 1107/2009). The term 'biologicals' is used here, to address botanicals (plant extracts), microorganisms-based products and basic substances. It is generally assumed that the negative influence of biologicals on biodiversity is lower than that of chemically synthesised products. Systematically collected data on their efficacy is required, including different genotypes and the combined occurrence of biotic and abiotic stresses. In addition to efficacy studies, knowledge about their mode of action is helpful for understanding their effects.

1.1 Barley

Since the species *Hordeum vulgare* L., colloquially known as barley, from the grass family Poaceae, the tribe Triticeae and the genus *Hordeum*, was domesticated by humans 10,000 years ago (Badr *et al.* 2000), this species has played an important role in the development of mankind. If the domestication was monophyletic in the Fertile Crescent (Zohary *et al.* 2012), or polyphyletic involving the Tibetan Plateau (Dai *et al.* 2012) is still under investigation. As one of the first domesticated plants in human history (Abbo *et al.* 2017), barley is grown today primarily as animal feed (International Grain Council 2021) but also for beer production and nutrition. Food made from barley grain can help against industrial diseases, because the grains are rich in soluble fiber, which reduces the risk for type II diabetes or cardiovascular diseases (Collins *et al.* 2010). Barley is grown as summer or winter barley, with summer barley being used mainly for malting and winter barley primarily as animal feed. The crop takes around 100 days to ripen in summer. In 2024 the barley world production was 142 million metric tonnes, with the European Union as major producer with 35% of the world production (USDA 2025). The ear type classifies the crop into two- and six-row barley (Le Gouis 1992). Six-row barley evolved from an ancestral two-row form (Pourkheirandish and Komatsuda 2007). Two-row barley lines contain e.g. more protein than six-row lines (Frégeau-Reid *et al.* 2001). However, six-row genotypes are more strongly stimulated in growth by increasing atmospheric CO_2 concentration, as predicted for the future, leading to increased yields (Mitterbauer *et al.* 2015). In addition to the agronomic significance of barley, it is also of great importance as a model organism in plant research (Stein and Muehlbauer 2018). Its almost worldwide migration (Singh *et al.* 2019), makes this crop to an adaptation specialist and to a model for the understanding of agricultural responses to climate change.

1.2 Powdery mildew

Some plant pathogens benefit from the changing climate, such as the powdery mildew fungi. Its airborne conidial concentration correlates positively with temperature and solar radiation and the amount of airborne conidia corresponds to disease index (Cao *et al.* 2012). Thereby, the combination of higher CO_2, and ozone concentration with higher temperature results in enhanced powdery mildew infection on barley (Mikkelsen *et al.* 2015). Powdery mildew is caused by a host-specific and obligate biotrophic ascomycete *Blumeria graminis*, infecting numerous grasses and cereals. It ranks on position six among the top ten fungal pathogens of great scientific and economic importance (Dean *et al.* 2012). It causes crop losses of up to 19% in wheat (Conner *et al.* 2003), up to 20% in barley (Czembor 2002) and 25-50% in pea (Fondevilla and Rubiales 2012). Taxonomically *Blumeria graminis* is divided into eight *formae speciales* (f.sp.), reflecting their host range. Each form infects a specific host and is not morphologically different from its closest relatives, which is why this classification is questioned (Troch*et al.* 2014). The high diversity of *Blumeria graminis* found today reflects the complex evolution including co-evolution between some f.sp and their host, fast radiation and host jumps (Menardo *et al.* 2017). The fungus causing powdery mildew on barley is *Blumeria graminis* (DC) E.O. Speer f.sp. *hordei* emend. É. J. Marchal (Bgh). It is one of the major threats for overall yield in barley production and therefore a focus in cultivar selection and breeding processes. The fungus has the potential to cause serious disease outbreaks and subsequent yield losses (Jørgensen *et al.* 2014; Czembor 2002). Additionally, Bgh is a model for host-pathogen interaction studies and phytopathological research (Collins *et al.* 2003; Schweizer and Stein 2011; Menardo *et al.* 2017; Bousset *et al.* 2002; Spanu *et al.* 2010). A few days after the initial contact of a plant with infectious conidia, each of them forms colonies (pustules) releasing countless numbers of asexual conidia, dispersing airbornely over long distances (Tucker *et al.* 2013) (Figure 1 A). In later stages of infection, a characteristic mealy coating becomes the main symptom (Both *et al.* 2005) (Figure 1 B). Effects of powdery mildew infection include a reduced number of fertile tillers and grains per head, as well as a decreased root growth as consequence of an early infection (Scott and Griffićths 1980). Moreover, Bgh favours lodging, a trait which in turn further reduces yields (Marzani *et al.* 2023). To infect its host, Bgh penetrates the cuticle and the cell wall. Thereby the asexual conidia attach to the leaf surface and recognise the substrate, possibly via taking up anionic low-molecular-weight materials (Nielsen *et al.* 2000). On its host, the conidium forms a primary germ tube within 30 to 120 min (Both *et al.* 2005; Heitefuss 2001; Edwards 2002; Zhang *et al.* 2005). It is assumed that the primary germ tube serves conidial adhesion, water uptake as well as the perception of leaf-derived signals (Edwards 2002; Heitefuss 2001). A secondary germ tube forms within four hours after the initial contact, which develops into an appressorium within the next hours. A penetration peg forms underneath the appressorium that pierces the epidermal cell wall. At the site of penetration, a cytoplasmic aggregation is formed developing a papilla - a cell wall apposition. Comparing resistant and susceptible genotypes, differences in papillae structure were found but none of them could be correlated to host reaction and compatible or incompatible host-pathogen combinations. Once the pathogen has entered the cell interior and formed a haustorium for nutrient uptake from the surrounding host cell, the infection is successful (Heitefuss 2001). The nutrients promote the branching of ectopic hyphae and sporulation (Zhang *et al.* 2005). It takes about 24 hours from the first contact of Bgh with its host to the successful establishment of a haustorium, three days until mycelium/colony formation on the leaf surface and five days until new conidial chains are formed from aerial conidiophores from which apical mature conidia detach (Both *et al.* 2005; Zhang *et al.* 2005). Although barley has a broad resistance diversity (Dreiseitl 2017), even small losses of resistance can cause an epidemic (Schwarczinger *et al.* 2021) as each Bgh colony releases 120,000 to 200,000 conidia during its lifetime (Moriura *et al.* 2006). In cleistothecia on aging leaves sexual spores are formed, allowing over-wintering or survival of harsh environmental conditions (Zhang *et al.* 2005). Resistance to powdery mildew is therefore crucial for cultivar selection and breeding processes. Thus, it is essential to characterise the susceptibility of genotypes towards Bgh.

Studies on powdery mildew require inoculation methods that mimic the natural airborne spread of the fungus. Inoculation towers are and have been used for this purpose. Their main features as partially enclosed systems are the distribution of conidia in a defined space and a reduced spore escape. The descriptions range from wooden constructions (Reifenschneider and Boiteaux 1988) to complex metal towers (Bell *et al.* 1952; Reuber *et al.* 1998). By incorporating additional features, the functionality of inoculation towers can be refined. Such features include fan heaters and heat distributors (Petersen 1959), the use of compressed air (Salinas *et al.* 1989), meshes (Reuber *et al.* 1998) or a rotating table inside the tower (Lück *et al.* 2020) to improve the homogeneity of the infection on experimental plants. Thereby, these features should mainly lead to the separation of conidia, contribute to the reduction of conidia clusters, and thus lead to a uniform infection. Nevertheless, how homogeneous conidia are distributed is not evident from every study using such towers. Most of the studies lack a detailed description of the tower characteristics or they describe technically complex and expensive towers.

Figure 1. Visual symptoms of powdery mildew on barley. A: symptoms seven days after inoculation; I infected leaves; II: pustules on a leaf; III: conidial chains (5-fold magnification). B: infection signs two weeks after inoculation.

1.3 Withstanding pathogens- Plant immune response

Plants interact with their environment, benefitting from symbiotic interactions and threatened by pathogenic interactions. Coevolution between attack mechanisms of phytopathogens and defence mechanisms of plants has led to the current diversity of host-pathogen interactions. To infect a plant, pathogens recognise their host, escape its defences, or switch them off and finally weaken their host. Conversely, plants recognise phytopathogens and evolve defence mechanisms. They can be divided in those that are constantly maintained or preformed, and those that are inducible. Preformed chemical barriers involve tannins, phenols, terpenes or alkaloids and preformed structural barriers include hairs, thorns, spines, borke, epidermis and cuticle (Lüttge *et al.* 2010, p. 858; Fernández *et al.* 2016). The cuticle is a lipid layer independent from the cell wall with a heterogeneous structure (Fernández *et al.* 2016) and mostly coated with epicuticular waxes (Müller and Riederer 2006). The cuticle can be seen as a milestone in the adaption to terrestrial plant lifestyle (Xue *et al.* 2017), as it marks the interface between aerial plant organs and their environment (Müller and Riederer 2006). When plants came ashore more than 450 million years ago (Graham 1993), they began to face the dilemma between protecting themselves from water loss and pathogen attack on the one hand and enabling gas exchange on the other. Thus, the cuticle fulfils the following key functions: limiting water loss, activating defence, attuning UV radiation and protecting against pathogens and insect attacks

(Fernández *et al.* 2016; Serrano *et al.* 2014). As the outer layer of leaves and stems, the cuticle is the first point of contact between most pathogens like the powdery mildew fungus and the plant and in this way the cuticle plays a decisive role in determining whether infection follows contact of a plant with a phytopathogen or not. Thereby, the chemical composition of the cuticle is of importance (Zabka *et al.* 2008; Ringelmann *et al.* 2009; Hansjakob *et al.* 2011). Hence, cuticle components can influence the infection course, for example the development of the powdery mildew fungus which is influenced by cuticular wax structure and composition (Hansjakob *et al.* 2010). In maize, very-long-chain aldehydes trigger the prepenetration process of powdery mildew (Hansjakob *et al.* 2011).

In addition to the aforementioned structural, preformed defence mechanisms, plants evolved inducible defence responses. These are triggered by contact with a pathogen such as Bgh. When the effects of induced mechanisms are local it is referred to as induced resistance. In the case of systemically active effects it is called systemic acquired resistance (SAR) (Métraux *et al.* 2002). Though, in preventing the immune response of barley from being triggered, conidia of Bgh evolved reconstruction and maintenance processes to conceal themselves from the host (Pham *et al.* 2019a). If Bgh managed to penetrate the interior of the plant without triggering an induced reaction, the infection is not necessarily successful as the receptors of plant's plasma membrane recognise pathogen-associated molecular patterns (PAMPs) when contacting invading pathogens. In this way a PAMP-triggered immunity is initiated, based on the recognition of conserved pathogen patterns and their categorisation as non-self from the plant (Hückelhoven 2005). To overcome or alter plant immune response at this stage of infection, pathogens evolved e.g. effector proteins. Fungal effectors are mostly small peptides enabling the fungus to establish a haustorium in the host cell, which itself can secrete effector proteins. Plants in turn have an effector-triggered immunity to limit microbial infection. Therefore, the concept of plant proteins recognising effectors, is referred to gene-for-gene resistance. If a rapid and local activation of programmed cell death, also known as hypersensitive response (HR) occurs as reaction to Bgh contact, the infection is incompatible (Schulze-Lefert and Vogel 2000). The so initiated host resistance is associated with H_2O_2 mediated local cell death, preventing nutrient uptake by the pathogen (Chisholm *et al.* 2006; Hückelhoven 2005; Lamb and Dixon 1997). In compatible host-pathogen interactions, besides H_2O_2 additionally antioxidative mechanisms like reactive oxygen species (ROS) are activated to face the pathogen and to induce signal transduction networks (El-Zahaby 1995; Saxena *et al.* 2016). Thereby, H_2O_2 has a signalling function and might be fungitoxic (Hückelhoven 2005). Stressed plants produce ROS, with the disadvantage that they can cause cell damage. ROS accumulation can oxidise lipids, proteins and nucleic acids and thus cause lasting cell damage. To protect themselves from this, antioxidant defence of plants can detoxify ROS - examples are the ascorbate-glutathione cycle in which ascorbate peroxidase catalyses the reaction of H_2O_2 into H_2O, regulated via biotic and abiotic stress (Caverzan *et al.* 2012), as well as the scavenging systems of glutathione reductase (Cruz de Carvalho 2008).

Additionally, the activation of genes with antimicrobial activity in presence of a pathogen is part of plant defence response. Their proteins are among others referred to as pathogenesis-related proteins (PR). Today, 19 families of *PR* genes are distinguished (Islam *et al.* 2023). Genes of a *PR* family have the following characteristics: 1) they can be found in many plant species of different families, 2) play a role in biotic stress response to at least two plant-pathogens combinations (or a single plant-pathogen combination confirmed in different laboratories) and 3) pathogen contact induces the accumulation also in tissue that not normally express the PR protein (van Loon and van Strien 1999). Pathogenesis-related proteins can interact directly with a pathogen and/or indirectly via signalling molecules stimulating other defence pathways (Islam *et al.* 2023). An example for such an indirect effect is PR1b. The protein is part of the penetration resistance to Bgh in barley, correlates with H_2O_2 production as a response to Bgh and is associated with HR, making the protein to a marker of Bgh attack and SAR (Schultheiss *et al.* 2003; Walters *et al.* 2014). The expression of *PR* genes can be linked to pathogen developmental stages like haustoria formation (Christensen *et al.* 2002; Mouradov *et al.* 1994). *Pathogenesis-related proteins 5*, also Bgh associated, encode thaumatin-like proteins (Wang *et al.* 2018) and a study on spruce revealed that they have antifungal activities (Liu *et al.* 2021). One barley isoform of PR5 attacks Bgh infection by preventing the formation of secondary hyphae (Lambertucci *et al.* 2019).

Under field conditions, stressors do not occur in isolation from other stressors and evidence was found for increased as well as decreased susceptibility under simultaneous stressor exposure (Ramegowda and Senthil-Kumar 2015). Abiotic stress often reduce susceptibility to biotrophic pathogens (Saijo and Loo 2020), for example, increasing salinity leading to salt stress increases the resistance of barley towards Bgh (Wiese *et al.* 2008). Drought stress combined with pathogen attack often occurs under field conditions. Thereby, the severity of the stressor determines plant ability to withstand both stresses (Ramegowda and Senthil-Kumar 2015). Interestingly, plants exposed to biotic and abiotic stress simultaneously or to several abiotic stressors, can exhibit a tailored response which is not expressed under the occurrence of just one of these stressors (Ramegowda and Senthil-Kumar 2015; Saijo and Loo 2020; Atienza *et al.* 2004). In addition, based on the redundancy of some signal molecules, the adaption to one stressor can affect via cross-tolerance the tolerance to another stressor (Tippmann *et al.* 2006). Phytohormones like abscisic acid, salicylic acid (SA), jasmonic acid and ethylene are involved in the crosstalk between biotic and abiotic stress responses, as well as ROS (Fujita *et al.* 2006). Therefore, the potential to reduce one stressor, even under the simultaneous occurrence of another stressor is a crucial point for the efficacy of crop protection products.

1.4 Biological plant protection

Since the use of domesticated plants, humans have experienced diseases of their agricultural crops. A document from 1750 BC already describes an animal pest on plants. In the Middle Ages, out of frustration over crop failures, pests were banned by the church or put on trial, such as the cockchafer in Avignon in year 1320. The basis of modern plant protection only emerged in the course of the 18th century through the work of Anton de Bary on the biology of rust fungi and potato late blight or through Julius Kühn's work on the causes and prevention of plant diseases (Börner 2008). For a long time, chemical pesticides were widely applied to prevent or overcome disease outbreaks, until their negative effects on biodiversity and human health became increasingly present. Today chemical pesticides are progressively discredited because of environmental concerns and their potential for increasing fungicide resistance, also in Bgh (Brown *et al.* 1992). This leads to even stricter regulation of their application. A fungicide with efficacy against powdery mildew and a multisite mode of action, reducing fungicide resistance, is the element sulphur which can be used in today's organic agriculture and has already been used as a pesticide by ancient Greeks 1000 B.C. (Onofre *et al.* 2021). Sulphur acts directly against conidia of powdery mildew as a vapour (Yarwood 1957). However, sulphur has an impact on beneficial arthropods and microorganisms (Prischmann *et al.* 2005), making its reduced application in organic farming desirable. In addition, The EU's Green Deal and the Farm to Fork strategy aim to reduce the use and risk of chemical pesticides by 50% in 2030 (European Union 2020). So, farmers are confronted with a reduced availability of chemical crop protection agents. This opens the stage for sustainable alternatives.

One of these are biologicals. These products can be used to stabilise crop yields and support plant health. They have the potential to reduce biotic and abiotic stress, to promote plant growth and stress tolerance and to support crop resilience (Nada Parađiković *et al.* 2019; Sharma *et al.* 2014; van Oosten *et al.* 2017; Daayf *et al.* 2000; Pylak *et al.* 2019). Furthermore, they can reduce dependencies on fertilisers and therefore play a key role in overcoming sustainability challenges (Chiaiese *et al.* 2018). Numerous studies prove the effectiveness of biologicals. For example seaweed extract has the power to improve plant growth and tolerance to drought, heat and salinity (van Oosten *et al.* 2017). In tobacco, *Bacillus amyloliquefaciens* YN201732 reduces powdery mildew severity (Jiao *et al.* 2020), while giant knotweed (*Fallopia sachalinensis* F. Schmidt) extract reduces powdery mildew infections in courgette (Margaritopoulou *et al.* 2020), cucumber (Daayf 1995) or tomato (Konstantinidou-Doltsinis *et al.* 2006), and horsetail herbal tea lead to reduced infestation with downy mildew and black rot in grapevine (Taylor *et al.* 2022). Besides, the combined use of biologicals with synthetic pesticides can reduce the quantity of chemical plant protection products applied (Iwaniuk *et al.* 2022). Thereby, the risk of resistance building decreases. Constraints associated with eco-friendly biologicals might be responsible for them having not taken over the agro-market

(Arora and Mishra 2016). Biologicals can either have a direct effect on the phytopathogen, such as growth inhibition or antibiosis, or they have an indirect effect on the pathogen via plant reactions. The latter one takes place via an overall strengthening of the plant or the induction of resistance genes. In this way, application of biologicals can boost plant immune responses and fasten immune reactions in a subsequent event of infection, e.g. with Bgh (Molitor *et al.* 2011). However, the current understanding of plant responses to biologicals, meaning their mode of action, is insufficient to explain the observed plant processes (Brown and Saa 2015) and often not discovered at all (Pylak *et al.* 2019). The understanding of biological's mode of action can help in product development and application. It is therefore necessary to improve the understanding of mechanisms behind biological's efficacy to make predictions on promising combinations for usage in agriculture (van Oosten *et al.* 2017). For some biological-plant interactions, however, there is knowledge about their intracellular effects. So, the combined application of *Ascophyllum nodosum* L. extract and chitosan activates the expression of the gene *Non-expressor of pathogenesis-related genes 1* (NPR1) and *pathogenesis-related protein 1* (PR1), the effect of which then contributes to suppress infection with powdery mildew in pea (Patel *et al.* 2020). In the case of PR1, it is known that it is strongly expressed in Bgh resistant barley genotypes but not in susceptible ones (Schultheiss *et al.* 2003). However, the exact function of PR1 is still unknown. Plant immune response is influenced by plant age. Due to changes in jasmonate-pathway, for example, older plants of *Arabidopsis thaliana* L. become more resistant to herbivore attacks (Mao *et al.* 2017). In barley, resistance develops in several genotypes against Bgh infection in aging plants (Gupta *et al.* 2015). Efficient protection against Bgh infection is therefore particularly important at early developmental stages and juvenile development.

1.5 Aims of this study

The general aim of this study was the characterisation of 40 spring barley genotypes regarding their powdery mildew susceptibility towards *Blumeria graminis* f.sp. *hordei* (Bgh) race A6 and the responses of selected genotypes to biologicals. Therefore, an inoculation tower and protocol were established enabling standardised studies on infection intensity. The responsiveness to biologicals, of genotypes, differing in their susceptibility, was studied to discuss opportunities and challenges in the context of biologicals. The effectiveness of the products against Bgh was initially investigated with preventive application. In addition, the effectiveness of some products was analysed in combination with abiotic stress (drought) for a systematic investigation of genotype-dependent effectiveness and off-target effects (biomass, chlorophyll content, nitrogen balance index). Therefore, products were tested for their preventive effectiveness against Bgh in the presence and absence of drought stress. Moreover, a further post-inoculation application was included, and the course of the disease was documented over four weeks.

In addition, this study aimed to answer the question of whether the preventive effectiveness against Bgh is based on induced resistance or whether the products directly stimulate the expression of individual genes. To this end, the mode of action of selected products was investigated by analysing the expression of pathogen-associated genes of one genotype. The cuticle composition of genotypes resistant or susceptible to Bgh race A6 was analysed to identify molecules associated with compatible and incompatible host-pathogen interactions. The minimum conductance of barley leaves to water was determined and discussed in relation to Bgh susceptibility.

This work aimed to contribute to the knowledge about the resistance spectrum of spring barley, to show possibilities and limits of biologicals against Bgh race A6 and finally to describe their mode of action.

2. Material and Methods

From October 2020 to January 2024 greenhouse trials were conducted at the Julius Kühn Institute, Institute for Biological Control (first in Darmstadt, then in Dossenheim) to study the susceptibility of seedlings of 40 spring barley genotypes and the response of two to four of them to a preventive application of biologicals and milk associated products against *Blumeria graminis* f.sp. *hordei* race A6 (Bgh A6), both with and without occurrence of drought stress. Plant height, developmental stage, physiological parameters (chlorophyll content, NBI) and the above-ground biomass were additionally evaluated. Therefore, an inoculation tower was constructed for airborne inoculation of barley seedlings with Bgh A6 and an inoculation and evaluation protocol were established. Quantitative polymerase chain reaction (qPCR) was used to study underlying gene expression patterns triggered by application of biologicals and/or Bgh A6 inoculation. The molecule composition of cuticle extracts was studied via gas chromatograph-mass spectrometry (GC-MS) and the minimum conductance of leaves to water was determined to conclude from these traits to Bgh A6 susceptibility.

2.1 Construction of an inoculation tower

A wooden tower was constructed for plant inoculation (Figure 2 A). For the woodwork, wood slats (3.8 x 2.4 cm cross section) with the individual length shown in Figure 2 A were screwed. The sides of the tower were covered with sturdy mesh foil, which was tacked to the roof battens, while the bottom remained open. The weight of 8.2 kg allowed manual lifting of the tower and placement over plants to be inoculated. The base area of 84 x 64 cm fitted exactly over two euro trays that can hold up to 48 pots (8 x 8 x 8.5 cm) in total. The lid was equipped with foil and Velcro fasteners for opening. A platform was installed at a height of 120 cm, enabling the placement of infected plants as inoculum source. The platform was made of coarse mesh (mesh 1.2 x 1.2 cm). Two USB-powered fans (Mini USB Fan (1.200 rpm, diameter 90 mm; Speedlink) were placed at the height of the platform at opposite corners to equally distribute the conidia. The positions of the pots under the tower were assigned to the position categories: border, middle, centre (Figure 2 B).

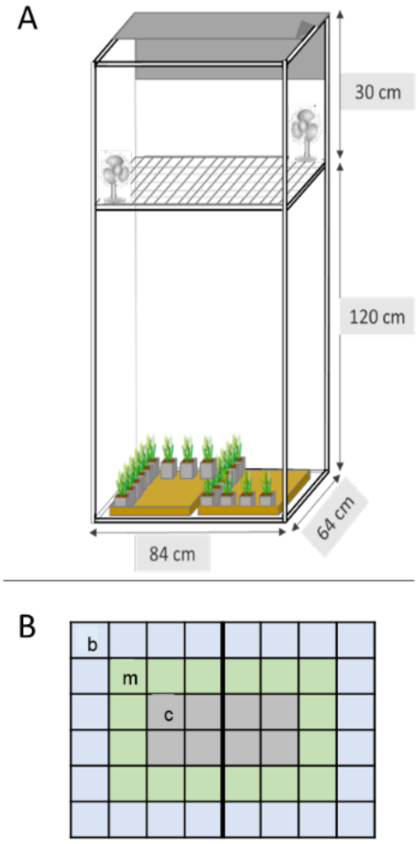


Figure 2. Schematic drawing of the inoculation tower and position categories. A: schematic illustration of the inoculation tower; B: position categories of the pots placed in the inoculation tower. b: border, m: middle, c: centre.

2.2 Permanent culture of Bgh A6

Blumeria graminis f. sp. *hordei* race A6 was cultivated in a permanent culture on susceptible genotype Golden Promise (GP) (Aguilar *et al.* 2016; Seeholzer *et al.* 2010) in an air-conditioned greenhouse (natural light, demand-based irrigation, temperature: 18.3-26.7 °C (mean 21.6 °C), humidity: 30.6% (mean)). Race A6 and GP were kindly provided from Justus Liebig University Gießen, Department of Phytopathology. The culture was rejuvenated weekly by blowing conidia over one-week old barley seedlings.

2.3 Culture of barley

Barley seeds were sown in pots (8 x 8 x 8.5 cm) with either Potground Proline (Klasmann-Deilmann, Geeste, Germany) or Fruhstorfer Erde type T (Hawita, Vechta, Germany) mixed 3:2 (v:v) with sand (Mauersand, Bauhaus, Mannheim, Germany) or in case of drought stress experiments (chapter 2.9) seeds were sown in soil:sand mixture (4:1). Plants for cuticle analysis were cultivated in Potground Proline mixed with sand 3:1 (v:v). If not otherwise declared, plants were grown in climate rooms at 16/8 h photoperiod, 18-22 °C (mean 20.5 °C) and 54% relative humidity (rh) (mean) for seven days or in an air-conditioned greenhouse (15°- 38.1 °C (mean 26.3 °C) at 47.8% rh (mean)). All inoculated plants were cultivated in an air-conditioned greenhouse at 16/8 h photoperiod (conditions as described for permanent culture of Bgh, chapter 2.2). Water management was demand-oriented, except for experiments with drought stress (chapter 2.9).

2.4 Determination of inoculation intensity and distribution in the inoculation tower

To validate conidia distribution in the inoculation tower and the associated distribution of infection on barley, *ad planta* experiments were conducted with seedlings of genotype GP. Four GP seeds were sown per pot. Three independent repetitions were conducted, each with 48 pots. They were placed in defined areas under the tower: border, middle or centre (Figure 2 B). For the evaluation of conidia distribution and infection success, the number of colonies/pustules on the first leaf was counted six days after inoculation (dai). In this way, 32 plants in the centre position, 64 in the middle position and 95 or 96 plants in the border position were examined per run.

Furthermore, 15 inoculation runs with GP and conidia masses from 0.5 mg to 8.9 mg were analysed to determine the correlation between conidia mass in the inoculation tower and infection symptoms on the first leaf.

2.5 Inoculation protocol

To ensure uniform and young age of conidia, colony-bearing plants were shaken one day before conidia harvest (Kuska *et al.* 2018). Plants to be inoculated were placed in the inoculation tower. Conidia were harvested from the permanent culture (chapter 2.2) immediately before inoculation by shaking and collecting in an aluminium funnel. Conidia were sprinkled into the centre of the inoculation tower at 120 cm height, while the fans were running for ten seconds. Subsequently, conidia were allowed to settle for 20 min. For the establishment of an inoculation and evaluation protocol the inoculum was weighed before sprinkling into the tower. For some experiments, the leaf area of the first leaf was determined prior to the inoculation seven days after sowing (das) ('genotype screening' (chapter 2.7)) or prior to the application of biologicals six das ('biological trial' (chapter 2.8), 'combined experiment' (chapter 2.10)). The measurement was done by use of Cl-202 portable laser leaf area meter (CID Bio-Science, Camas, United States), or by taking a picture or scanning and measuring via Adobe Photoshop (version 2022), using the magnetic lasso tool. In this way, it was taken into account that fast-growing genotypes had a larger leaf area at the time of inoculation and thus caught more conidia without being automatically more susceptible and that plants with a larger leaf area obtained more biologicals than plants with a smaller one. A preliminary trial in which the height of seedlings from 50 different genotypes was determined daily between the 4th and 7th das showed that there were differences in growth at seedling stage and that the measurement of the leaf area was necessary (Figure 3, statistical analysis chapter S 1, S Table 1). The seeds were provided from the Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance in Quedlinburg, Germany (further details Table 2).

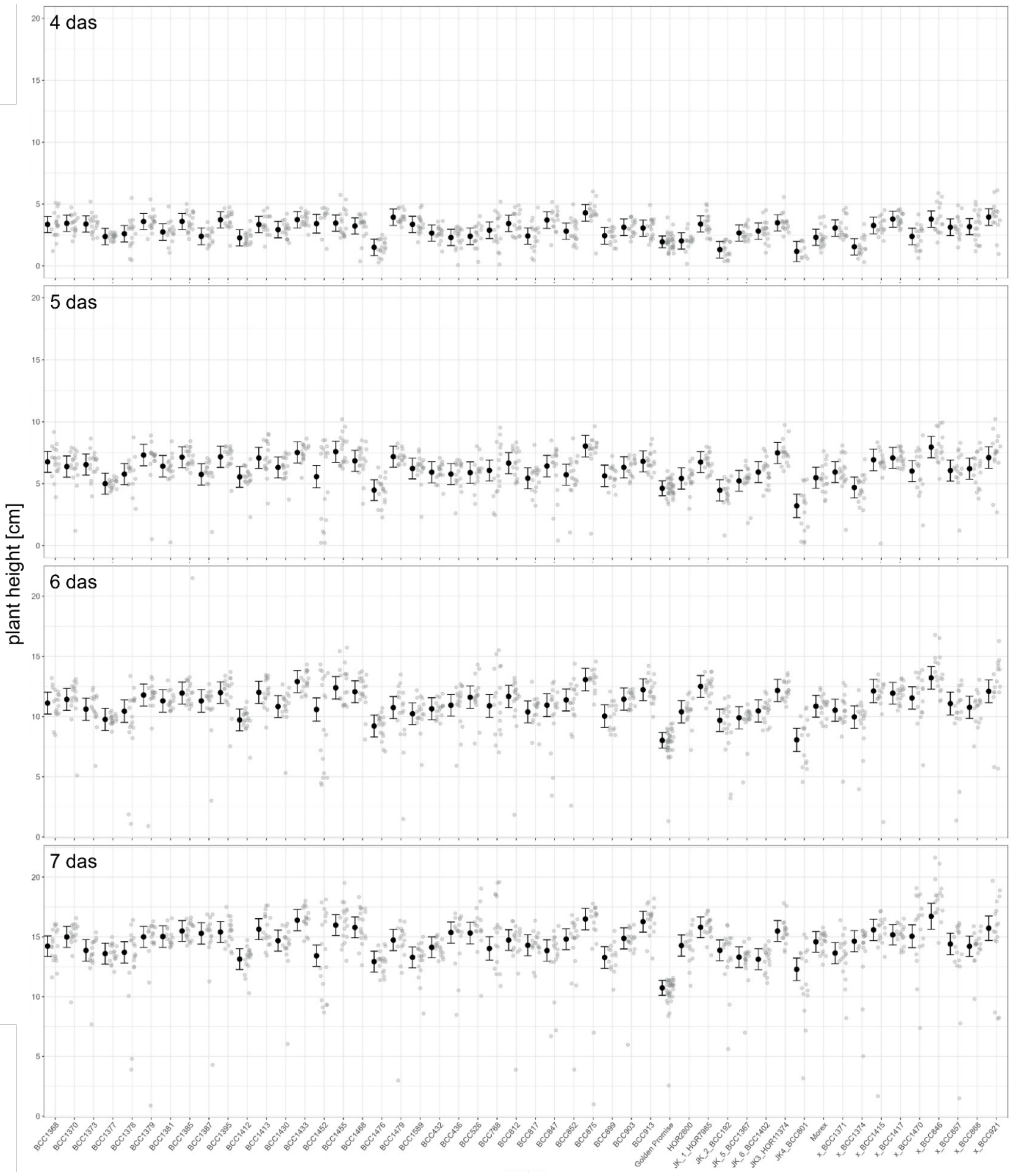


Figure 3. Seedling height development of 50 spring barley genotypes from four to seven days after sowing. For each genotype two pots, each with nine seeds were cultivated and the height of above-ground plant organs was monitored four, five, six and seven days after sowing (das), leading to 14-18 measured plants per genotype seven das. In case of genotype Golden Promise, 35 plants were studied. Genotypes named with JK_ were of unknown identity, genotypes named with x_ were afterwards found not to be genetically pure. Grey dots: raw data; black dots and error bars: adjusted mean and 95% confidence interval (details chapter S 1, S Table 1).

2.6 Preparation and application of biologicals

Based on a literature survey, 21 biologicals (Table 1) were selected for experiments on the preventive effect against Bgh A6 on barley. Biologicals from different types were included in the test panel: twelve botanicals, three microbials, two homeopathic agents, one animal-based and three further biologicals belonging to none of the mentioned types. The panel included 16 biologicals for foliar application as well as the chemical product Kumulus WG (active ingredient sulphur) as a positive control, three biologicals for seed treatment and two that were applied via watering. Apart from the seed treatments, the products were applied six das. Products for leaf application were applied via a glass laboratory sprayer (Carl Roth GmbH & Co., Karlsruhe, Germany). Plants of one pot were sprayed one by one until just before run off. The biologicals were prepared according to the manufacturer's protocol and scaled down where necessary. With the exception of burdock root and giant knotweed leaf extract, which were self-made, commercially available products were used. All dilutions were made with distilled water and prepared afresh before each usage.

Burdock root extract: root powder of *Arctium lappa* L available as food supplement (Vita Ideal, Enschede, Netherlands) was used for burdock root extract production. One gram of powder was mixed with 20 ml of 60 °C warm water and extracted for 40 min with continuous stirring. After cooling to room temperature, the extract was centrifuged, the supernatant decanted and the pellet extracted again as described. The supernatants were combined and filtered through a nylon net to prevent clogging of the laboratory sprayer. The filtrate was diluted 1:5 to adjust to a concentration of 0.5% (w/v).

Giant knotweed leaf extract: leaves of *F. sachalinensis* (harvested near Egelsbach, Germany) were air-dried and finely milled. The extract was prepared via ethanolic soxhlet extraction. Therefore, 20 g leaf powder were extracted in a Soxhlet with 150 ml 96% EtOH for four hours, the remainders in the flask were redissolved and the whole extract filtered using a vacuum pump (filter: thickness 180 µm, 11 µm particle retention). The pellet was rinsed with EtOH 96%. The filtrate was concentrated with a roto-evaporator (R-300 Rotavapor, Büchi, Flawil, Switzerland) and finally adjusted to 40 ml with EtOH (96%). This 50% (w/v) stock solution was stored at 7°C in the refrigerator and diluted to 1% for application.

Table 1 a-c. Biologicals and the chemical standard – product names and uses. Given are product or extract names, the use in this study (bt: biological trial; ce: combined experiment with Bgh and drought stress, mo: molecular studies, gmin: minimum conductance of leaves to water), the category of the product/extract, their mode of application (App; LT leaf treatment, W application via watering, ST seed treatment) as well as the applied concentration, the producer/company and additional information (based on producer information).

Product/Extract	**Use**	**Category**	**App**	**Concentration**	**Producer**	**Additional information/components**
			microbial			
RhizoVital®42	bt	soil additive	ST	7 µl RhizoVital 42 + 100 µl water + 18 seeds, incubation 2 h, shaking every 30 min	Biofa, Münsingen, Germany	spores of *Bacillus velezensis* (FZB42)
Serenade ASO	bt	plant protection product	LT	1.00%	Bayer, Leverkusen, Germany	spores of *Bacillus amyloliquefaciens* (QST 713)
T-Gro	bt	soil additive	ST	0.05%	Biofa, Münsingen, Germany	spores of *Trichoderma asperellum*
			animal based			
Raw milk	bt, ce, mo	basic substance	LT	10.00%, undiluted	Bauernhof Maas, Neubotzheim, Germany	
			homeopathic			
Biplantol agrar	bt	plant strengthener	W	0.02%	Bioplant, Konstanz, Germany	potentised form (D6-D100) of: potassium, calcium, iron, magnesium, phosphorus, boron, germanium, silicon, copper, manganese, uronic acids
Biplantol mycos V forte	bt	plant strengthener	W, LT	0.02%, 0.20%	Bioplant, Konstanz, Germany	potentised form (D6-D100) of: herbal plant extracts (sage, basil, malva), silica, gemstones, minerals

Table 1 b.

Product/Extract	Use	Category	App	Concentration	Producer	Additional information/components
botanical						
Ackerschachtelhalm Extrakt Compositum	bt	plant strengthener	LT	3.00%	Snoek, Rotenburg/Wümme, Germany	field horsetail, bracken, wormwood, humus extract
Alginure Agro Support F	bt	organic liquid fertiliser	LT	0.50%	Tilco-Alginure GmbH, Reinfeld, Germany	brown seaweed
AMN BioVit Konzentrat	bt	plant aid	LT	2.00%	Mack bio-agrar, Schorndorf, Germany	garlic, organic selenium, minerals, amino acids, alllicin
ASL Kombi Power	bt	plant strengthener	LT	0.50%	Snoek, Rotenburg/Wümme, Germany	silicic acid, sulphur, micronutrients, organic acids
Blattgrün	bt, ce, mo	plant aid	LT	undiluted	Snoek, Rotenburg/Wümme, Germany	Field horsetail, plant-based emulsifier, essential oils
Brennessel Extrakt Compositum	bt	plant strengthener	LT	3.00%	Snoek, Rotenburg/Wümme, Germany	nettle herb, cress
Burdock root extract	bt, ce	food supplement	LT	0.50%	Vita Ideal, Enschede, Netherlands	
CropCover 1000	bt, ce, gmin	adhesive	LT	5.00%	Amynova polymers, Zwenkau, Germany	adhesive, modified starch
Elot-Vis Green	bt, ce	plant strengthener	LT	5.00%	Akasia, Wittenberge, Germany	native plant extracts, salts, surface tension agents
Equisetum Plus	bt	for registration as a plant strengthener	LT	1.00%	Biofa, Münsingen, Germany	field horsetail
Giant Knotweed leaf extract	bt, ce mo, gmin	former plant strengthener	LT	1.00%	Own harvest	

Table 1 c.

Product/Extract	**Use**	**Category**	**App**	**Concentration**	**Producer**	**Additional information/components**
			botanical			
Tillecur	bt	plant strengthener	ST	7 mg Tillecur + 100 µl water, swelling for 60 min, addition of 18 seeds, 2 h of incubation, shaking every 30 min	Biofa, Münsingen, Germany	flours from native plants
			other			
Baking powder (Kaiser-Natron)	bt, ce	basic substance	LT	0.50% + 5 drops Tween80 (0.0125%)	Holste, Bielefeld, Germany	sodium bicarbonate
FytoSave	bt	plant protection product	LT	0.30%	Syngenta, Shanghai, China	chito-oligosaccharides (from crustaceans), oligo-galacturonic acid (from citrus fruit)
Kumulus WG	bt, ce	plant protection product	LT	3.00%	BASF, Ludwigshafen, Germany	sulphur
PottaSol	bt	plant strengthener	LT	0.50%	Biofa, Münsingen, Germany	sodium silicate

2.7 Resistance analysis of 40 spring barley genotypes towards Bgh A6

To characterise the susceptibility of 40 spring barley genotypes (Table 2) towards Bgh A6, each genotype was tested in three independent repetitions, each of them with three pots and six to nine seedlings per pot. The experimental setup of the ‘genotype screening’ resembled an alpha-design with each inoculation process representing one block. The investigated genotype panel included 27 advanced or improved cultivars and five traditional cultivars or landraces, two genotypes used for breeding and six genotypes of unknown identity, originating from 18 countries. Furthermore, two-rowed spring barley genotypes and six-rowed genotypes were included. Barley cultivation and inoculation followed the description in chapter 2.3 and 2.5. To determine the susceptibility of the genotypes, the number of colonies/pustules on the first leaf of each plant was counted six to seven dai. Additionally, a literature research on the findings on susceptibility and resistance of the genotypes towards powdery mildew was conducted to be able to contextualise the results obtained in the course of this work within the current state of knowledge (Table 2).

Table 2 a-e. Characteristics of 40 spring barley genotypes screened for their susceptibility towards *Blumeria graminis* f.sp. *hordei* race A6. Given are genotype names (named with JK_: unknown identity). Taxonomic description, biological status (according to FAO/IPGRI multi-crop passport description), accession name, country of origin (UN 3-letter country code), ear type and results from literature research (S: susceptible, R: resistant); Literature: [1]: Pogoda *et al.* 2020, [2]: Walters *et al.* 2002, [3]: Aguilar *et al.* 2016, [4] Matsuo *et al.* 2001, [5]: Balkema-Boomstra and Mastebroek 1995, [6]: Seeholzer *et al.* 2010, [7]: Jensen *et al.* 1992, [8]: Gatzke 2012.

Genotype	Taxonomic description	Biological status	Accession name	Country of origin	Ear type	Literature
BCC1368	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Aramier	NLD	2-row	R to A6 [7], S to Ty4 [7], S to D35/3, RiIII [1] S to AEV7532 [5]
BCC1370	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Beatrice	FRA	2-row	S to D35/3, RiIII [1]
BCC1373	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Blenheim	GBR	2-row	S to D35/3, RiIII [1]
BCC1377	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Ceres	FRA	2-row	S to D35/3, RiIII [1]
BCC1378	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Claret	GBR	2-row	S to A6 [7], S to D35/3, RiIII [1]
BCC1379	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Diamant	CZE	2-row	S to D35/3, RiIII [1]

Table 2 b.

Genotype	Taxonomic description	Biological status	Accession name	Country of origin	Ear type	Literature
BCC1381	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Georgie	GBR	2-row	R to A6[7], S to Ty4 [7], S to D35/3, RiIII[1]
BCC1385	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Gryf	POL	2-row	S to D35/3, RiIII [1]
BCC1387	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Hebe	NLD	2-row	
BCC1395	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Menuet	NLD	2-row	S to D35/3, RiIII [1]
BCC1412	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Sõrla	SWE	2-row	S to D35/3, RiIII [1]
BCC1413	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Sissy	DEU	2-row	
BCC1430	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Chevallier	FRA	6-row	
BCC1433	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Heils Franken	DEU	6-row	

Table 2 c.

Genotype	**Taxonomic description**	**Biological status**	**Accession name**	**Country of origin**	**Ear type**	**Literature**
BCC1452	*Hordeum vulgare* L. convar. *vulgare* var. *hybernum* Vib.	advanced/improved cultivar	Bigo	NLD	6-row	S to D35/3, RiIII [1]
BCC1455	*Hordeum vulgare* L. convar. *vulgare* var. *hybernum* Vib.	advanced/improved cultivar	Belogorskij	RUS	6-row	S to D35/3, RiIII [1]
BCC1468	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *medicum* Körn.	advanced/improved cultivar	Tselinij 213	KAZ	6-row	S to D35/3, RiIII [1]
BCC1476	*Hordeum vulgare* L. convar. *vulgare* var. *hybernum* Vib.	advanced/improved cultivar	Afrasiab	UZB	6-row	
BCC1479	*Hordeum vulgare* L. convar. *vulgare* var. *hybernum* Vib.	traditional cultivar/landrace	K 9338	RUS	6-row	S [8]
BCC1589	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	traditional cultivar/landrace	HOR 1555	ITA	2-row	
BCC432	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	breeding/research material	Fu 8	CHN	2-row	
BCC436	*Hordeum vulgare* L. convar. *vulgare* var. *hybernum* Vib.	advanced/improved cultivar	Vladivostok	CHN	6-row	S to D35/3, RiIII [1]
BCC526	*Hordeum vulgare* L. convar. *vulgare* var. *rikotense* Regel	breeding/research material	C-138	IND	2-row	S to D35/3, RiIII [1]
BCC768	*Hordeum vulgare* L. convar. *intermedium* (Körn.) Mansf. var. *haxtoni* Körn.	traditional cultivar/landrace	Ulleri 2	NPL	6-row	S to D35/5 [1]

Table 2 d.

Genotype	**Taxonomic description**	**Biological status**	**Accession name**	**Country of origin**	**Ear type**	**Literature**
BCC812	*Hordeum vulgare* L.	advanced/improved cultivar	Arupo	MEX	2-row	S to D35/3, RiIII [1]
BCC817	*Hordeum vulgare* L.	advanced/improved cultivar	Beecher	USA	2-row	S to D35/3, RiIII [1]
BCC847	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Compana	USA	2-row	S to D35/3, RiIII [1]
BCC852	*Hordeum vulgare* L. convar. *vulgare* var. *hybernum* Vib.	advanced/improved cultivar	Diamond	CAN	2-row	S to D35/3, RiIII [1]
BCC875	*Hordeum vulgare* L.	advanced/improved cultivar	Hazen	USA	2-row	S to D35/3, RiIII [1]
BCC899	*Hordeum vulgare* L. convar. *distichon* (L.) Alef. var. *nutans* (Rode) Alef.	advanced/improved cultivar	Libra	CHL	2-row	
BCC903	*Hordeum vulgare* L.	advanced/improved cultivar	Manley	CAN	2-row	
BCC913	*Hordeum vulgare* L.	advanced/improved cultivar	Otis	USA	2-row	
Golden Promise		Traditional cultivar		GBR	2-row	S to isolate CC220 [2], S to D35/3, RiIII [1,] S to A6[3,6], DH14 [3], K1[6]

Table 2 e.

Genotype	**Taxonomic description**	**Biological status**	**Accession name**	**Country of origin**	**Ear type**	**Literature**
JK_1_HOR7985	*Hordeum vulgare* L.				2-row	
JK_2_BCC192	*Hordeum vulgare* L.				2-row	
JK_3_HOR11374	*Hordeum vulgare* L.				6-row	
JK_4_BCC801	*Hordeum vulgare* L.				2-row	
JK_5_BCC1367	*Hordeum vulgare* L.				2-row	
JK_6_BCC1402	*Hordeum vulgare* L.				2-row	
Morex		traditional cultivar		USA	6-row	S to race Hh4 [4], S to D35/3, RiIII, CH4.8 [1]

2.8 Preventive efficiency of biologicals against Bgh A6

The preventive efficiency of 21 biologicals (Table 1) against Bgh A6 was tested on four barley genotypes (GP, Morex, BCC436 and JK1_HOR7985). This ‘biological trial’ was carried out with three repetitions. Thereby, three pots with one to six plants were examined per treatment, genotype and repetition, except for one repetition from BCC436, which was done with two pots per treatment. The experimental setup of the ‘biological trial’ resembled an alpha-design with each inoculation process representing one block. Sowing, inoculation, leaf area measurement and application were conducted as described in chapter 2.3, 2.5 and 2.6. No leaf area measurement was done for genotype BCC436, instead, six seeds per pot were sown and the seedling number was narrowed down to four plants of equal size prior to the application of the tested products. Distilled water was used as negative control. Kumulus WG (3%) was included as positive control. The number of pustules on the first leaf of each plant was counted six dai. Additionally, above-ground plant organs were harvested 14 das, dried (60-80 °C; seven days) and then weighed to measure their dry mass.

2.9 Milk compounds and milk associated products against Bgh A6

Plant trials were conducted with genotypes GP, Morex, BCC436 and JK1_HOR7985 to characterise the preventive mildew symptom reducing capacity of milk compounds and milk associated products. Plant trials for this ‘milk experiment’ were carried out two times independently with three pots per repetition. The experimental design followed an alpha-design with each inoculation process representing one block. For the efficacy analysis six seeds per pot were sown. The seedling number was narrowed down to four plants of equal size, prior to the application of the tested products (Table 3). Sowing, inoculation, and application were performed as described in chapter 2.3, 2.5 and 2.6. The concentration of milk compounds (lactoferrin, lactoperoxidase, lactose) was adjusted to the proportion in milk (Arnould *et al.* 2009; Gupta and Prakash 2017; Seifu *et al.* 2005; Da Alves Cunha *et al.* 2020). Lactose is a disaccharide in milk and an energy supplier as it is the source of carbohydrates in nursing period of *Mammalia* (Gambelli 2017). Lactoperoxidase, a heme peroxidase, belongs to an antimicrobial system in *Mammalia* (Kussendrager and van Hooijdonk 2000) and the transferrin protein lactoferrin is known to have antimicrobial activity in *Mammalia* (Giansanti *et al.* 2016).

Table 3. Milk compounds and milk associated products tested against *Blumeria graminis* f.sp. *hordei* race A6. Given are product name, used concentration and the producer. UHT-milk: ultra-high-temperature processing milk.

Product/Extract	**Concentration**	**Producer, country**
Lactoferrin	0.138%	Sigma-Aldrich, St. Louis, USA
Lactoperoxidase	0.003%	Sigma-Aldrich, St. Louis, USA
Lactose	4.600%	Peter Kölln GmbH & Co, Elmshorn, Germany
Milk Powder	13.000%	Carl Roth GmbH & Co., Karlsruhe, Germany
Pasteurized milk	undiluted	Alnatura, Darmstadt, Germany
Raw milk	undiluted	Bauernhof Maas, Neubotzheim, Germany
UHT-milk	undiluted	Alnatura, Darmstadt, Germany
Whey	undiluted	Untermühlbachhof in St. Georgen, Germany

2.10 Testing of biologicals under drought stress – the 'combined experiment'

Since drought stress and Bgh can occur simultaneously under field conditions, the preventive effect of nine selected biologicals against Bgh in the presence of drought stress was investigated (Table 1) in the 'combined experiment' for the following genotypes: GP, BCC436 and BCC1589. These genotypes differ in Bgh A6 susceptibility (chapter 3.2) and drought stress tolerance (GP: early-stage drought stress sensitive; BCC1589 and BCC436: tolerant towards early-stage drought stress (Töpfer unpublished)). The results of the 'biological trial' were used as a basis for the composition of the biological test panel for the 'combined experiment'. The set contained biologicals with genotype-dependent and -independent effects, products with negative and one without a significant effect on Bgh infection. In addition, botanicals, microorganism-based and homeopathic agents were included to form a diverse test set. Distilled water was used as negative control. Kumulus WG (3%) was included as positive control. Plants of the 'combined experiment' were germinated at 40% of the maximum water holding capacity (MWHC) for two days. Two water regimes were introduced as described by Wehner *et al.* (2015), with a drought (20% MWHC) group and a well-watered control (70% MWHC) (Figure 4). The two irrigation regimes were maintained for 26 days. The water status was determined gravimetrically and newly adjusted three times a week. Three pots with one to six plants were examined for each treatment and water regime of the three repetitions, except for baking powder, CropCover, burdock root extract and raw milk (10%, 100%) which were tested twice. The experimental setup resembled a split plot design. Cultivation, leaf area determination, inoculation and biological application were conducted as described, except of Biplantol mycos V forte which was applied via leaf spraying (chapter 2.3, 2.5 and 2.6). An additional spraying of biologicals was carried out seven days post-inoculation (p-i) to increase the concentration of possible active substances. In addition, this should provide initial indications on whether repeated application at the first symptoms of infection can mitigate or even terminate the course of the infection. The trial was terminated 28 das with the harvest of the above-ground plant organs to determine their dry mass (as described in chapter 2.8). After harvesting, photos of overturned pots of genotype GP and BCC1589 (only run one) were taken to get indications of possible changes in root growth due to the tested biologicals. Over the course of the experiment, physiological and morphological parameters were recorded weekly (chapter 2.10.1), and the level of infection was documented (chapter 2.10.2).

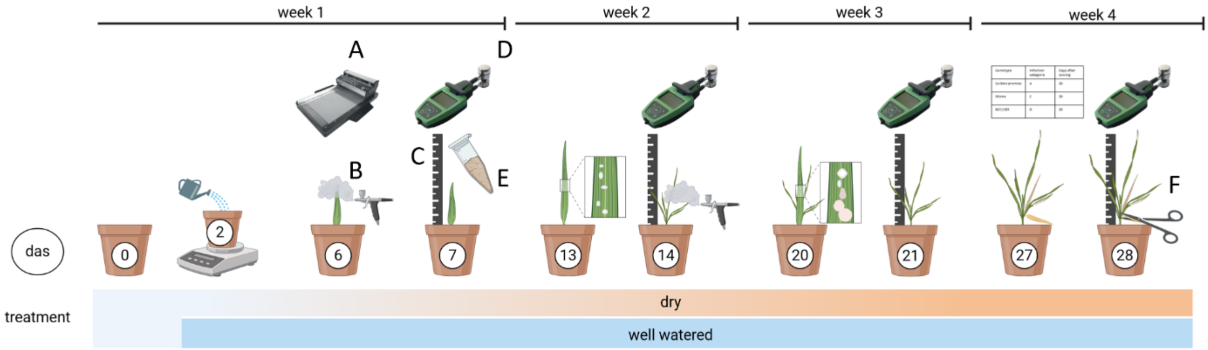


Figure 4. Experimental procedure to study the effectiveness of biologicals against *Blumeria graminis* f.sp. *hordei* race A6 on barley with and without simultaneous drought stress. Das: days after sowing. A: leaf area measurement via leaf area scanner; B: application of biologicals, C: measurement of plant height and 28 das also determination of BBCH; D: measurement of physiological parameters using Dualex Force A; E: inoculation with *Blumeria graminis* f.sp. *hordei* race A6; F: harvest of above-ground plant organs; at day 13, 20 and 27 after sowing infection signs were monitored. Figure was created using BioRender.

2.10.1 Determination of physiological and morphological parameters

Using Dualex (Force A, Paris, France) a sensor for clipping onto leaves, chlorophyll content and nitrogen balance index (NBI) were measured seven, 14, 21 and 28 das. Thereby, the youngest fully developed leaf was measured triple (leaf basis, middle, top), outside of infection sites. Notwithstanding this, the chlorophyll content was not determined for GP treated with burdock root extract, raw milk or CropCover 14 das. Additionally, plant height was measured seven, 14, 21 and 28 das. In addition, 28 das the developmental status of each plant was documented in the form of the BBCH stage (Brockerhoff 2015).

2.10.2 Evaluation of symptom intensity

The signs of infection were evaluated at three time points (Figure 4), (1) six dai by counting the number of pustules on the first leaf, (2) 13 dai by determining the percentage of infected leaf area and (3) 20 dai by allocating disease categories to each plant (from 1=healthy to 5=heavily infected; Table 4).

Table 4. Disease categories of powdery mildew on 28-day old barley plants.

Category	Description
1	No signs of infection with powdery mildew
2	Sporadic pustules (maximum 5 pustules) and/or a few old infection sites
3	Several pustules (>5) and in some cases also old infection sites
4	Many pustules and possibly a dried leaf due to a strong infection
5	Numerous pustules, mostly older leaves up to and including the third leaf, at least 10% dried out due to severe infection

2.11 Expression analysis of defence-related genes

Gene expression analysis was conducted to get more insight into the underling mechanisms of the effectiveness of selected biologicals. Cultivation and experiments took place in an air-conditioned greenhouse at 16/8 h photoperiod, 18.9-38.7 °C (mean 26.4 °C) at 62.7% rh (mean) and demand-adapted irrigation. First, the suitability of 23 primer pairs was evaluated via the assessment of their specificity by PCR and gel electrophoresis, which was also tested via melting curve in the Real-Time cycler (Table 5). Based on the results, 16 primer pairs and a reference gene were chosen for the experiment on the underling mechanisms of biological effectiveness. The expression of these 16 genes was analysed and based on the results, the experiment was repeated for ten genes (Table 5).

2.11.1 Experimental design

Four seeds of genotype GP per pot were sown as described in chapter 2.3. Three pots were prepared for each treatment and each sample time, except for control plants (six pots). The inoculation with Bgh A6 and the application of the tested preparations (raw milk (100%), Blattgrün, giant knotweed leaf extract, Table 1) were carried out as described in chapter 2.5 and 2.6. Distilled water was used as a control and a healthy control for each treatment was kept, isolated from the infected plants and cultivated under equal conditions in the greenhouse. For RNA extraction, all above-ground seedling organs were cut off. Three seedlings from different pots were combined for each sample. In case of the control plants, six plants were combined into one sample, three at the beginning of each sampling and three at the end, to compensate for possible time effects of the harvest. Sampling took place at six time points (Figure 5): (1) one hour, (2) five hours after application and (3) 24 hours after application. Time point three is immediately before inoculation to detect temporal changes in response to the application and to understand the initial situation before inoculation. Further samples were taken (4) one hour, (5) five hours and (6) 72 hours after inoculation. These time points were of particular interest because they are associated with the formation of the primary germ tube (4), the secondary germ tube (5) and haustoria formation as well as mycelium formation (6) of the pathogen (Both *et al.* 2005). All plants or nucleic acids were stored at -80 °C.

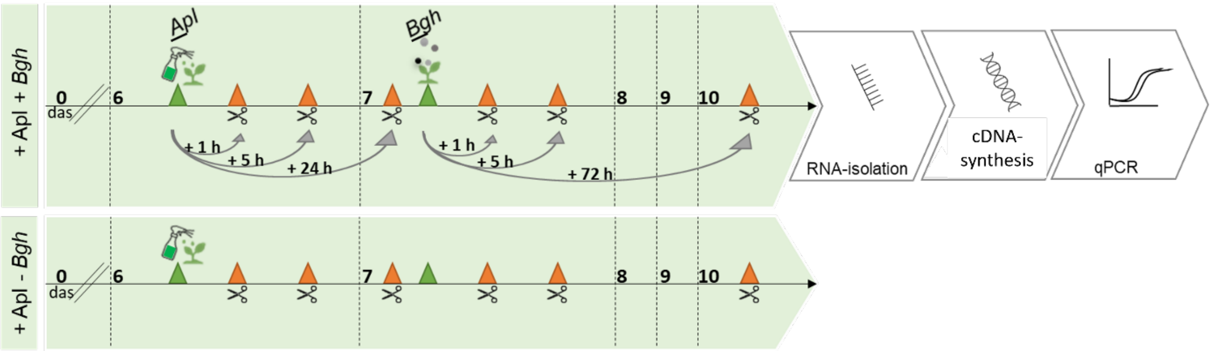


Figure 5. Schematic procedure of the experiment on the expression analysis of pathogen-associated genes by preventive application of biologicals and infection with *Blumeria graminis* f.sp. *hordei* race A6. das: days after sowing; +: with, -: without; Apl: application of biologicals; Bgh: inoculation with *Blumeria graminis* f.sp. *hordei*; scissors and orange rectangles: mark the time of harvesting of seedlings.

Table 5 a-b. Tested primer pairs with sequence and origin (reference). Rep: number of independent repetitions of the experiment; pt: pretest

Gene	Abbreviation	Rep	Sequence forward	Sequence reverse	Reference
ROS/ redox status associated					
Ascorbate peroxidase 1	*AsPer1*	2	CGGAGCTTTTGAGTGGTGACA	CCGCAGCATATTTCTCCACAA	McGrann and Brown 2018
Copper-zinc superoxide dismutase 1	*CoZiSuDi1*	1	ACCTCGGAAATGTGACAGC	ACCCTTGCCAAGATCATCAG	McGrann and Brown 2018
Glutathione reductase 1	*GluRed1*	2	GGGGCTATAGTGGTCGATGA	AATGCTCCACCTTCCATCAG	McGrann and Brown 2018
Respiratory burst oxidase homologue F2	*RBOHF2*	2	TGCTCGGTCAGCACTC	TCCGCAATAGAACACTCC	Kolozsváriné Nagy *et al.* 2022
chaperone					
Heat shock protein 90-1	*HSP90*	1	ACAAGAACGACAAGTCCGTCAA	GAGCATGCGGTGGATCCT	Kolozsváriné Nagy *et al.* 2022
Heat shock protein 70	*HSP70*	1	GGAGGTTGACTAAGCTGTTGC	ACATGACACGACGACAAACG	Molitor *et al.* 2011
cell-death associated					
BAX inhibitor 1	*BAX1*	1	GGCAGCTTCATGTTTGAGGT	AGGGCGTGCTTGATGTAGTC	McGrann and Brown 2018
BAX inhibitor 1	*BAX1-b*	pt	ATGTTCTCGGTGCCAGTCT	GGGCGTGCTTGATGTAGTC	Kolozsváriné Nagy *et al.* 2022
Lesion simulation disease 1	*LSD1*	2	TATCCACATGGAGCACCTTCTG	CGTCGACAGTCATAGGGTTCTC	Johrde 2009
metabolism					
Alcohol dehydrogenase	*ALD*	1	TCACCTACAACTCGGTCGAC	TTCAGCCCGTGGTACTTCAT	Li *et al.* 2019
pathogenesis-related					
Pathogenesis-related protein 1b	*PR1b*	2	GGACTACGACTACGGCTCCA	GGCTCGTAGTTGCAGGTGAT	Kolozsváriné Nagy *et al.* 2022
Pathogenesis-related protein 17b	*PR17b*	2	CGAGGTTCCTCGACTACTGC	ATCACATTCAGCCTCCGAAC	Shrestha *et al.* 2019
Pathogenesis-related protein 5	*PR5*	2	GCCGACCAACTACTCAATGT	AGGGCAGGTGAAGGTGCT	Molitor *et al.* 2011
Pathogenesis-related protein 2	*PR2*	pt	TACTTCGCGTACCGTGACAA	GTGTAGGTCAGCCCGTTGTT	Molitor *et al.* 2011
Non-expressor of pathogenesis-related genes 1	*NPR1*	2	CAGGTCGACAACCCTTTCAT	TAAATCCGGCAAGCAGTTTC	Kumar *et al.* 2021

Table 5b.

Gene	Abbreviation	Rep	Sequence forward	Sequence reverse	Reference
reference gene					
Ubiquitin	*Ubi*	2	ACCCTCGCCGACTACAACAT	CAGTAGTGGCGGTCGAAGTG	Kolozsváriné Nagy *et al.* 2022
Actin	*Actin*	pt	CCCAATTTACGAAGGTTTCTCTC	TCAGCGGTTGTGGAAAAAGT	Pennington *et al.* 2016
Glyceraldehyde-3-phospate dehydrogenase	*GAPDH*	pt	GGAGCCGAGTACATAGTAGAGT	GGAGGGTGCCGAAATGATAAC	Pennington *et al.* 2016
Ubiquitin	*Ubi-b*	pt	GGAGCCGAGTACATAGTAGAGT	GGAGGGTGCCGAAATGATAAC	Kolozsváriné Nagy *et al.* 2022
α-Tubulin	*TubA*	pt	GGTCACTACACTGTTGGTAAAGA	CCGAAGGAATGGAATACAAGAAAG	Pennington *et al.* 2016
other/unclear/diverse					
Pectin-lyase like Protein	*PeLyLP*	1	GCAATAACAATAGGGTAACTGGGAA	GCACCATGGAAGCCGAAATC	Johrde 2009
Peroxidase 40	*Perox*	2	GAGCAATGGAGAATATGGGAAAAAT	GGAGCCAGCCAGCCAAACAC	Johrde 2009
Germin-like protein 5	*GER5a*	pt	TAGCAAGCAAGCATTGACCA	CCCCTGTTTTGCTGGAAGT	Zimmermann *et al.* 2006
phytohormone associated					
Ethylene response factor 1	*ERF1*	2	GTCCGTCTTCGTACCGAGTG	CTGCTTGCCCCATCCTCATT	Own design, using Geneious Prime R11

2.11.2 RNA-extraction and cDNA-synthesis

RNA was isolated using the QIAGEN RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol including the optional DNase digestion. RNA quality and integrity was checked using a nanodrop spectrophotometer (NanoDrop 2000c, peqlab, Erlangen, Germany) and gel electrophoresis, respectively. The CT046342 Cycler from BioRad was used for reverse transcription with iScript (BioRad, Hercules/California, USA) according to the manufacturer's protocol, whereas 1000 ng of RNA were transcribed.

2.11.3 Quantitative PCR

Quantitative PCR was done using CFX96^{TM}Real-Time System (BioRad, Hercules/California, USA) with iTaq Universal SYBR Green Supermix (BioRad, Hercules/California, USA) according to the manufacturers's protocol. Gene expression was normalised to the frequently used reference gene *ubiquitin* (Zhang *et al.* 2018b; Kolozsváriné Nagy *et al.* 2022), because the cycle threshold (CT) comparison in response to Bgh A6 infection, biological application and over time showed very low variation (chapter S 2, S Table 2). If not otherwise declared, the experiment was carried out with two independent runs and three technical replicates each. Non-reverse-transcribed RNA, no template and an interrun control were included in the analyses. Table 5 indicates the used primers, based on literature research. Primers were first tested for specificity by PCR and gel electrophoresis, where all PCR-products showed one single band. This was confirmed by a melting curve analysis in the Real-Time cycler. To determine the primer efficiencies, a dilution series of cDNA was analysed with three technical replicates each (50 ng, 25 ng, 5 ng, 2.5 ng, 0.5 ng, 0.25 ng, 0.05 ng). The mean CT values of the technical replicates were plotted against the logarithmical cDNA quantity and the slope of the linear equilibrium line was used to calculate the efficiency according to Pfaffl (2001). For primer pairs, which have been repeatedly analysed, at least five data points in the linear range of the amplification were used for the equilibrium line of each primer pair, resulting in high linearity with $R^2 > 0.98$. Based on this, the primer efficiencies ranged from 1.9 to 2.1 for further tested primer pairs (chapter S 2, S Table 3). The relative expression ratio (here fold-change) of the target genes was calculated in accordance with Pfaffl (2001). To analyse the influence of Bgh A6 infection on expression, the CT values of water treated infected plants were compared with those of water treated non-infected plants according to Pfaffl (2001). To determine the influence of biological application, biological treated healthy plants were compared with water treated healthy plants. To illustrate the combined effects of biological application and Bgh infection, these values were compared with those of water treated infected plants. Visualisation was done using Microsoft Excel (version 2403).

2.12 Minimum conductance of leaves to water and leaf shrinkage

When stomata are closed e.g. in response to water deficit, water is lost through the leaf surface. This is partly due to insufficiently closed stomata and partly due to evaporation through the cuticle, which is referred to as the minimum conductance of a leave to water (g_{min}) (Duursma *et al.* 2019). The g_{min} was studied on healthy and inoculated barley leaves of nine genotypes (BCC1589, BCC1476, BCC812, GP, BCC1468, HOR7985, BCC1452, BCC1479, BCC436) differing in their Bgh A6 susceptibility (chapter 3.2) to find out whether g_{min} of resistant genotypes differ from that of susceptible genotypes. Additionally, g_{min} of CropCover or giant knotweed leaf extract treated GP leaves was studied to find out whether these biologicals influence g_{min}. The two biologicals both were applied as described in chapter 2.6 and 2.8. To consider possible temporal effects of the biological application, plants were treated seven or 14 das. Cultivation of uninfected plants took place in climate rooms, inoculated plants were cultivated in a greenhouse (chapter 2.3). Inoculation took place seven das by blowing conidia over the plants. To determine g_{min}, secondary leaves of five, 14-day old seedlings were studied per genotype and treatment (inoculation, biological treatment; except for inoculated HOR7985 and giant knotweed treated GP seven das n=4).

To calculate g_{min}, the weight loss of detached barley leaves over time was documented. In dehydration curves, the conductance was visualised as a function of relative water deficit, and g_{min} was determined by averaging the data points in the plateau phase (chapter S 3, S Figures 2-6). In this phase the stomata are assumed to be closed and the water loss is therefore due to insufficiently closed stomata as well as evaporation through the cuticle. The experiment and the calculation of g_{min} were performed in accordance with earlier studies (Messerschmid 2016; Simon 2019; Bleser 2020), but with differing weighing intervals (Table 6). The experiment was stopped after three days when about 20% of the leaf area were dried or shrivelled. The final drying of the leaves was done at 80 °C for two days.

Table 6. Measurement intervals for the determination of the minimum conductance of barley leaves to water.

Day of measurement	Duration	Measurement interval
1	For 40 min	10 min
	For 90 min	15 min
	For 120 min	20 min
	For 100 min	25 min
	For 150 min	30 min
2	For 10 min	10 min
	For 30 min	15 min
	For 90 min	30 min
	For 80 min	40 min
	For 240-300 min	50 – 70 min
3	For 120-240 min	120 min or 4 measurements every 2 hours

For the calculation of g_{min}, a function of leaf area changes during drying was generated as it is the basis for the transpiration rate needed for the calculation of g_{min}, as these parameters depend on the surface area. The leaf shrinkage process has been studied exemplarily for GP in accordance to earlier studies (Messerschmid 2016; Simon 2019; Bleser 2020). The function of leaf area changes during drying determined in this way was also used as the basis for the g_{min} calculations of the other genotypes, as their leaves hardly differed in shape or succulence from those of GP. Secondary leaves of eight, 14-days old GP seedlings were examined. Documentation of leaf area and leaf weight was done at increasing intervals: about half-hourly for the first two hours, then hourly for four hours, twice with 1.5 hours difference and the next day about every two hours and the third day twice. Between measurements leaves were kept at low humidity (Silica Gel Orange, Carl Roth, Karlsruhe, Germany) and in the dark and weighted down with 280 g (per 600 cm²) to prevent the leaves from curling. The final drying was at 80 °C for 24 hours, followed by the determining of the dry weight and the corresponding leaf area (scanning of leaf surface, measuring via Adobe Photoshop (version 2022)). Using Microsoft Excel, the relative water deficit (RWD) as well as the percentage of relative area loss were calculated for each leaf and time point (Messerschmid 2016). The RWD and the relative area loss of all eight investigated leaves were visualised in a scatterplot as a cumulatively summarised leaf shrinkage curve (Figure 6). As trend line a polynomial function three straight line was chosen, the function of which was used to calculate the area loss of the leaves during drying.

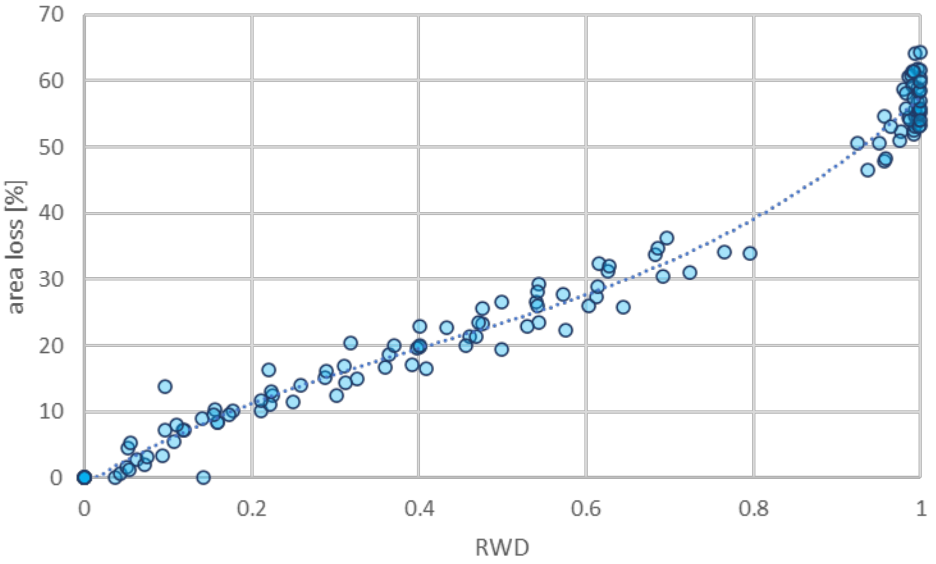


Figure 6. Leaf shrinkage curve of barley genotype Golden Promise. Plotted is the percentage of relative area loss and the relative water deficit (RWD) of eight secondary leaves from 14-day old barley leaves of Golden Promise plants. The trend line is a polynomic function three straight line and its formula $y = 74.814 * x^3 - 92.839 * x^2 + 76.576 * x - 0.9883$.

2.13 Searching for differences in cuticle composition related to Bgh A6 susceptibility

To search for cuticle compounds that can be attributed to Bgh A6 resistance or susceptibility, two resistant and two susceptible genotypes were studied in cooperation with Prof. Dr. Thines' work group at the Institute for Molecular Physiology at Johannes Gutenberg-University of Mainz. Therefore, six pots each with six seeds per genotype were prepared per run and cultivated for thirteen days in climate rooms (chapter 2.3). The experiment was conducted twice. Secondary leaves were harvested the day before extraction and rehydrated overnight. Handling was done with gloves and contact areas were kept to a minimum. The extraction day started with the removal of adhering dirt particles of the leaves by rinsing with distilled water. Leaves were carefully patted dry with soft paper towel. Used glassware was rinsed with solvent, either ethyl acetate or cyclohexane. Fifteen leaves were bundled for extraction and immersed in solvent-filled beakers (150-200 ml, HPLC grade). To achieve complete wetting of the leaf surfaces with solvent, the leaf bundles were swirled extensively for 90 sec and to avoid contamination with internal molecules, the leaf base remained above the liquid level. Solvents were evaporated at 45°C in rotary evaporator (RE 100D, Phoenix instrument, Garbsen, Germany), the precipitate redissolved in the corresponding solvent and transferred to a glass vial (Supelco) before drying in a SpeedVac (RVC 2-33 CDplus, Christ, Osterode am Harz, Germany). Precipitates were redissolved in the corresponding solvent and transferred to an Eppendorf vessel, dried in a SpeedVac (Concentrator plus, Eppendorf, Hamburg, Germany) and afterwards metabolites were derivatised for 90 min at 40°C (shaking) by adding 10 µl of a 60 mg/ml ethoxyamine hydrochloride solution in pyridine and 20 µl of pyridine. Subsequently, the extracts were silylated for 50 min at 40°C with 70 µl *N*-Methyl-*N*-trimethylsilyl-trifluoroacetamide. The final centrifugation (5 min, 13,000 rpm) allowed any undissolved molecules to pelletise. The supernatant was subjected to GC-MS analysis (Shimadzu GCMS-QP 2010S, Kyoto, Japan). Injection volume was one µl per sample by AOC-20i auto injector to an Optima 5 MS column (30 m x 250 µm x 0,25 µm film thickness, Macherey-Nagel, Düren, Germany) using split mode 1:10 and an injection temperature of 250 °C. The GC oven temperature was initially 70 °C for 5 min and increased with 10 °C per min up to 320°C holding the maximum for 10 min. Helium (GC grade) was used as carrier gas with a column flow rate of 1 ml/min. Mass spectra detection was achieved via electron impact mode and full scan monitoring mode (m/z 15-800). The ion source temperature was 200 °C and the interface 250 °C. For chromatogram analysis, the chromatography software GCsolution 2.72 (Shimadzu, Kyoto, Japan) was used and results were compared for identification with the NIST spectra library (webbook.nist.gov/chemistry/).

2.14 Data processing, statistical analysis and figures

For analysis of the experimental data, inference analyses were conducted using R (version 4.2.0; R core Team 2022) and descriptive analysis was done via Microsoft Excel (version 2403) or R (Table 6). For inference statistics, generalised linear mixed effect models from the *glmmTMB* package (Brooks *et al.* 2017) or cumulative link mixed models (CLMMs) out of the *ordinal* package (Christensen 2022) for disease categories were used. Linear models were calculated with *stats* package (R Core Team 2022). The correlation between variables was checked with *pairs.panels* of the package *psych* (Revelle 2023) or *cor* of the package *stats* (R Core Team 2022). Global models were tested for the best distributional fit between poisson, nbinom1, nbinom2 and genpois family, while model fit was visualised with the package *DHARMa* (Hartig 2022), e.g. for dispersion, outliers, simulated residuals and zero inflation. Best distributional fit was selected by suitable diagnostics and Akaike Information Corrected Criterion (AICc) out of the package *MuMIn* (Bartón 2022). If not otherwise declared, models were selected by the lowest AICc. For the visualisation of the final models, significant differences and the compact letter display were calculated with the package *emmean*s (Lenth 2022). Furthermore, model effects were calculated with package *effects* (Fox and Weisberg 2019) and visualised combined with the compact letter display and the original data via *ggplot2* (Wickham 2016). Figures were finished in Inkscape (version 0.92.4), Microsoft Power Point (version 2403) or Microsoft Excel (version 2403).

Table 7 a-d. Overview of data analysis as well as plotted data. Given are the experiment, the variable analysed, the mode of analysis (i: inference; d: descriptive) and the models; if several models were tested, the one underlined performed best; dai: days after infection; das: days after sowing; * models were dredged if more than one fixed variable was included, with the *MuMIn* package (Bartón 2022); global models were tested for the best distributional fit between poisson, nbinom1, nbinom2 and genpois family; if not otherwise declared, generalised linear mixed effect models of the *glmmTMB* package (Brooks *et al.* 2017) were used; GP: Golden Promise.

Experiment	**Variable**	**Statistics**	**Models/calculations**
Distribution of infection in the inoculation tower	Number of pustules on the first leaf	i	Three global models with (a) the interaction of the position in the tower and the conidia mass with run as random effect, (b) the interaction of the position in the tower and the conidia mass without run as random effect and (c) the interaction of the position in the tower and the run; models were also calculated without interactions*; model c without interaction performed best
Relationship between conidia mass and infection symptoms	Number of pustules on the first leaf	i	Linear model for the number of pustules on the first leaf in response to the conidia mass in the inoculation tower
Growth differences of 50 spring barley genotypes	Plant height	i	One model per time point with genotype as fixed effect and gaussian family
Resistance analysis of 40 spring barley genotypes	Number of pustules on the first leaf	i	Three global models were created, (a) only with the genotype as fixed effect and, (b) with the interaction of the genotype and the leaf area and (c) the genotype as fixed effect and with the pots nested in the tower, which were nested in the run as random effect and the leaf area in the offset.
Resistance analysis of 40 spring barley genotypes	Biological status	d	The proportion of different biological statuses of the 40 genotypes in the susceptibility groups was calculated
Biological trial	Number of pustules on the first leaf	i	For each genotype: one global model with the treatment (biological) as fixed effect and the pots nested in tower, which were nested in the run as random effect and the leaf area in the offset; divergent: genotype BCC436 without contribution of leaf area
Biological trial	Number of pustules on the first leaf of control plants	d	The mean and the sample size per genotype and biological were calculated

Table 7 b.

Experiment	Variable	Statistics	Models/calculations
Biological trial	Above-ground dry biomass	d and i	The mean of above-ground dry biomass per biological treatment and genotype was calculated, based on which the percentage deviation from the mean of control was calculated per treatment and per genotype; For each genotype: one model with treatment (biological) as fixed effect and the pots nested in tower, which nested in the run as random effect (gaussian family), outliers were eliminated
Milk compounds and milk associated products against Bgh A6	Number of pustules on the first leaf	i	For each genotype: one global model with the treatment (biological) as fixed effect and the pots nested in tower, which nested in the run as random effect; for JK_1_HOR7985 not the model with lowest AICc was chosen but the one with the second lowest AICc as the simulated residue fit was better in this model
Combined experiment	Infection development over time	d	The mean per biological treatment, water status, genotype and time point was calculated, based on which the percentage deviation from the mean of control was calculated per biological treatment, water status, genotype (GP, BCC436) and time point; for BCC1589 the mean intensity of symptoms was plotted (6 dai in number of pustules, 13 dai percentage of infected leaf area, 20 dai infection category minus one)
Combined experiment	Number of pustules on the first leaf	i	For each genotype: one global model with interaction of treatment (biological) and water status as fixed effects and pots nested in tower, which nested in the run as random effect and the leaf area in the offset
Combined experiment	Infection category of plants	i	For each genotype: one CLMM model with interaction of treatment (biological) and water status and conidia mass as fixed effects and pots nested in tower, which nested in the run as random effect
Combined experiment	Above-ground dry biomass	d and i	Descriptive analysis: mean values per treatment, genotype and water status were used to calculate the percentage deviations of these from the average biomass of the corresponding control plants per treatment, genotype and water status. For each genotype: one model with interaction of water content and treatment as fixed effect and pot nested in tower, which nested in run as random effect (gaussian family)

Table 7 c.

Experiment	Variable	Statistics	Models/calculations
Combined experiment	Plant height development	d	Mean value and standard error per treatment, genotype and water status were calculated for each time point
Combined experiment	Plant height 28 das	d and i	The percentage deviation of the afore calculated means from the corresponding mean of control was calculated per genotype, treatment and water status; based on raw data, for each genotype one model with interaction of biological treatment and water status as fixed effect and pot nested in tower, which nested in run as random effect (gaussian family)
Combined experiment	NBI and chlorophyll content development	d	Using R; The mean value of the three measurements carried out for each plant was used to analyse the chlorophyll content and the NBI The mean of chlorophyll content and NBI per genotype, treatment and water status was calculated as well as the standard error.
Combined experiment	NBI and chlorophyll content 28 das	d and i	Descriptive analysis using R: mean values per treatment, genotype and water status were used to calculate the percentage deviations of these from the average NBI and chlorophyll content of the corresponding control plants. For each genotype: one model with interaction of biological treatment and water content as fixed effect and pot nested in tower, which nested in run as random effect and gaussian family; data: mean of three measurements carried out per plant
Combined experiment	BBCH (28 das)	-	BBCH per genotype, treatment and water status was plotted in a dot plot using R
Combined experiment	BBCH, plant height and above-ground dry biomass (28 das)	i	Spearman rank correlation coefficient was calculated using R for the interaction of above-ground dry biomass, plant height and BBCH for each genotype

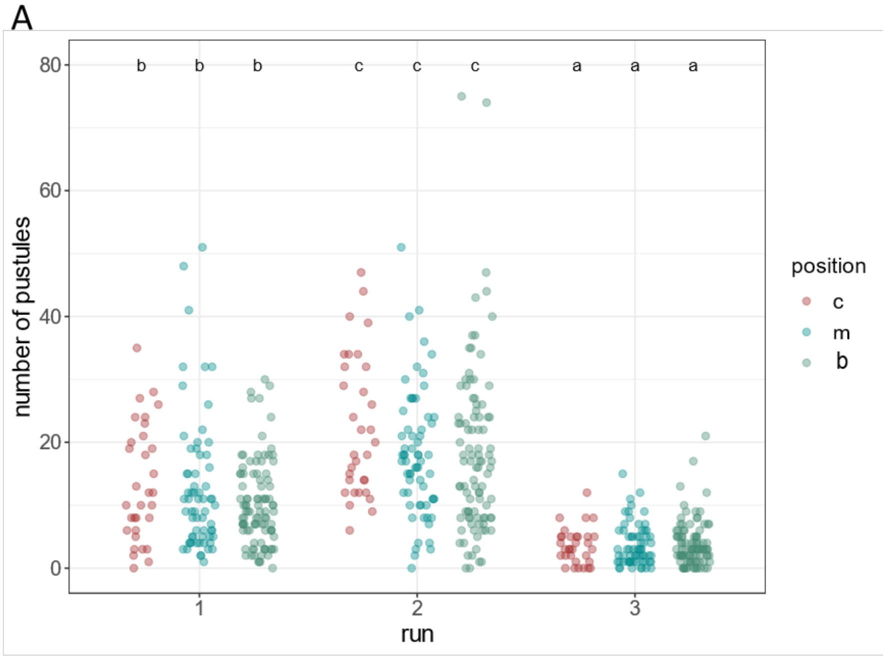
Table 7 d.

Experiment	**Variable**	**Statistics**	**Models/calculations**
Expression analysis of defence-related genes	Fold-change	-	Fold-change was plotted in bar charts using Microsoft Excel
Leaf shrinkage	Leaf area	-	Calculation and plotting in Microsoft Excel
Minimum conductance of a leaf to water	Minimum conductance	i	Calculation and plotting in Microsoft Excel; linear model for the minimum conductance in response to the genotype, to biological treatment or to Bgh infection; spearman rang correlation was calculated with *stats* package
Cuticle compounds	Percentage of molecules in the total extract	d	Descriptive analysis (mean, standard deviation) in Microsoft Excel

3. Results

3.1 Inoculation tower mediates uniform distribution of infection levels, the strength of which is influenced by the conidial mass

The evaluation of infection signs of *Blumeria graminis* f.sp. *hordei* race A6 (Bgh A6) inoculated Golden Promise (GP) plants showed that the position of the plants in central, middle or edge areas of the inoculation tower had no significant ($p<0.05$) influence on the infection (Figure 7). In contrast, the inoculation run significantly influenced the number of pustules on the first leaf. Additionally, it was shown that for the statistical analysis of such inoculation tower experiments the model with run as fixed effect and without interaction performed best (Δ AICc of 0.00) compared to the best fitted model with conidia mass as fixed effect (Δ AICc of 6.53).



B

contrast	estimate	se	t.ratio	p-value
center – middle	0.0873	0.0916	0.954	0.3406
center – border	0.1481	0.0865	1.712	0.0875
middle – border	0.0608	0.0692	0.878	0.3804

Figure 7. Characterisation of infection intensity and infection distribution of *Blumeria graminis* f.sp. *hordei* race A6 on barley genotype Golden Promise after inoculation via inoculation tower. A: Number of pustules on the first leaf for each position category. Three independent inoculations (runs) are shown. Positions: c: centre (red), n=32 plants per run; m: middle (petrol), n=64 plants per run; b: border (green), n=95 or 96 plants per run; pustule number was determined six to seven days after inoculation on thirteen- or fourteen-days old seedlings. Different letters indicate significant differences, confidence interval: 95%. B: Results of statistical analysis of the conidia distribution in the inoculation tower. For each comparison of tower position categories, the estimate, the standard error (se), the t-ratio (t.ratio) and the p-values are shown.

Since the different inoculation processes differ primarily in the mass of conidia used, the influence of the conidia mass on the expression of infection symptoms was examined (Figure 8). The mass of conidia has a significant ($p<0.001$) influence on the number of pustules on the first leaf. With a correlation coefficient of 0.390, the correlation can be categorised as weak (Papageorgiou 2022). If the correlation is calculated for conidia masses from 0.5 mg to 7.4 mg, it is moderate at a correlation coefficient of 0.514 (Papageorgiou 2022).

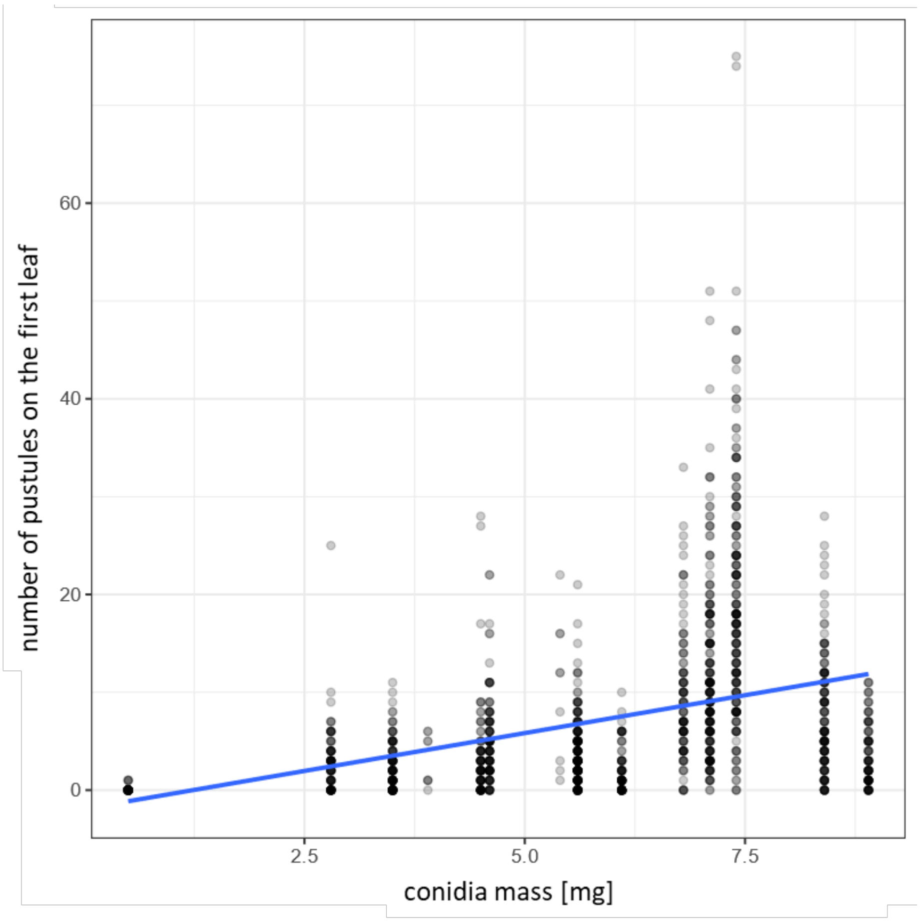


Figure 8. Correlation between conidia quantity for inoculation and symptom severity. The number of pustules on the first leaf was determined for 14 inoculations with different amounts of conidia and summarised in this plot, blue line: linear trend line; Sample size per conidia mass ranged from 84 plants (6.1 mg) to 191 plants (7.1 mg and 7.4 mg), except for 3.9 mg and 5.4 mg with eight or nine plants respectively.

3.2 Susceptibility continuum of 40 spring barley genotypes towards Bgh A6

The analysis of 40 spring barley genotypes revealed a susceptibility continuum towards Bgh A6 (Figure 9). Within this continuum, the genotypes were categorised into six groups according to the intensity of their susceptibility. Thereby, 18 genotypes were classified as Bgh A6 resistant (BCC1589, JK_2_BCC192, BCC899, JK_3_HOR11374, BCC1476, BCC1368, BCC1373, BCC1413, BCC1379, BCC1370, BCC1377, BCC1385, BCC1378, BCC1381, JK_5_BCC1367, BCC1387 BCC1395, BCC812), which made up the largest group with 45% of the tested genotypes. Most of these resistant genotypes were advanced or improved cultivars, one was a traditional cultivar/landrace and three were of unknown biological status (Figure 10). Genotypes BCC432 and BCC1433 had significantly ($p<0.05$) more pustules on the first leaf than the genotypes from the resistant group and were assigned to the 'very low susceptibility' category. Other than that, BCC817 and BCC1430 formed the group 'low susceptibility', a group consisting only of advanced or improved cultivars. Thirteen genotypes (35% of the tested genotypes) were part of the 'mid susceptibility' group (BCC1455, GP, Morex, BCC1412, BCC913, BCC526, BCC768, BCC852, JK_6_BCC1402, BCC1468, BCC847, JK_1_HOR7985, BCC903, JK_4_BCC801), unifying one breeding/research genotype, three traditional cultivars/landraces, seven advanced/improved cultivars, and three of unknown biological status. Five genotypes were of 'high susceptibility' (BCC1452, BCC1479, BCC875). The most susceptible genotype and the only one of the 'very high susceptibility' category was the advanced/improved cultivar BCC436.

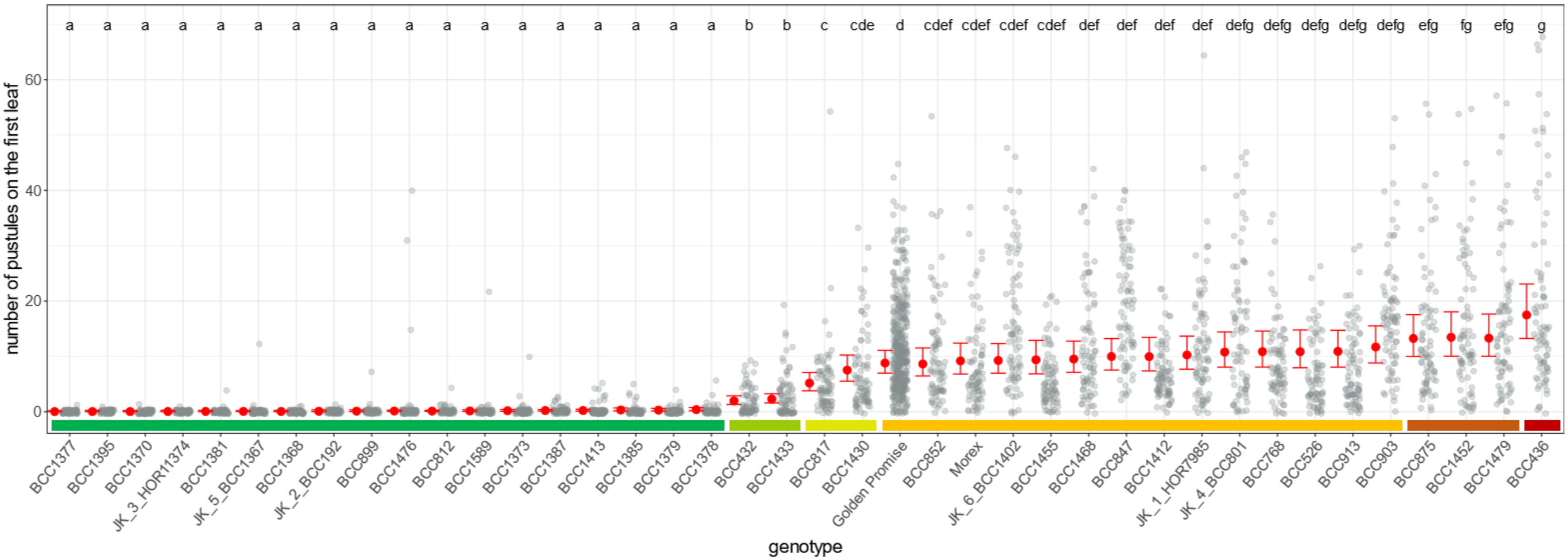


Figure 9. **Susceptibility continuum of 40 spring barley genotypes towards *Blumeria graminis* f.sp. *hordei* race A6.** Shown is the number of pustules on the first leaf, six days after inoculation. Grey dots: raw data, showing the number of pustules on the first leaf of each genotype; red dots/error bars: adjusted means with 95% confidence interval; number of evaluated plants per genotype 79-82 and 405 from genotype Golden Promise; different letters indicate significant differences ($p<0.05$). Coloured rectangles indicate the classification into susceptibility categories: dark green = resistant, green = very low susceptibility, yellow green = low susceptibility, orange = mid susceptibility, red = high and dark red = very high susceptibility.

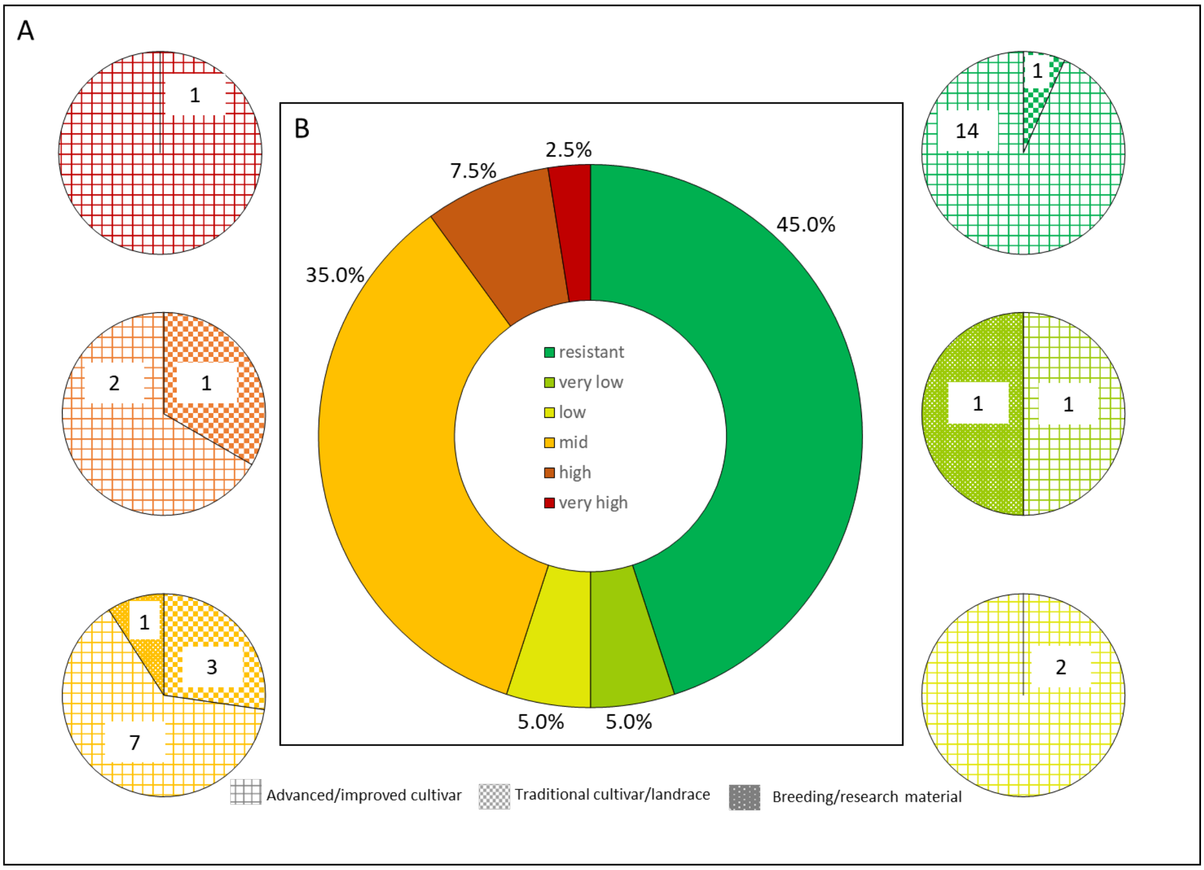


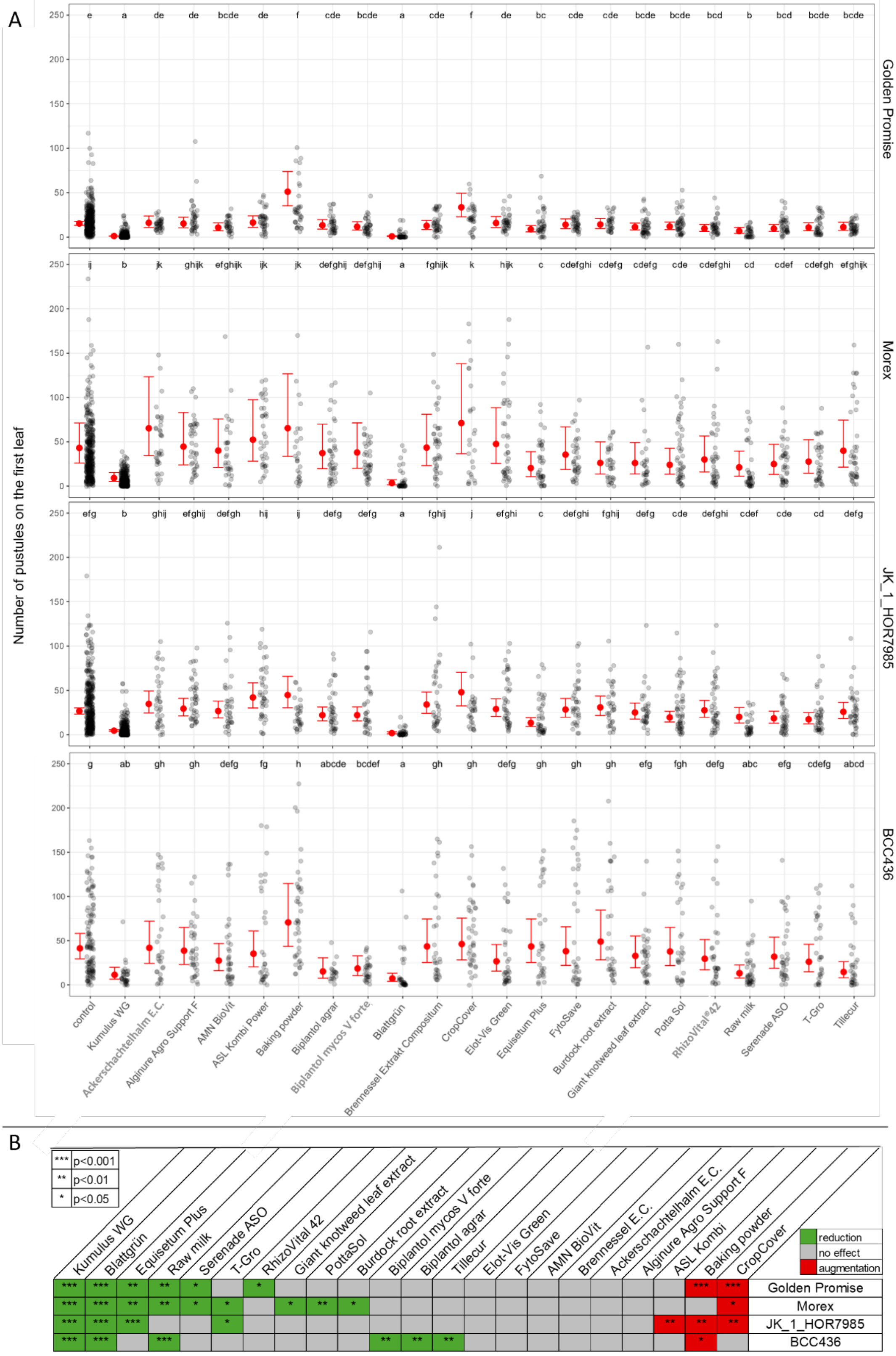
Figure 10. Powdery mildew susceptibility and biological status of 40 spring barley genotypes. A: composition of susceptibility categories regarding the biological status of the assigned genotypes; numbers = number of genotypes in this category; B: percentage of resistant, very low, low, medium, highly and very highly susceptible barley genotypes towards *Blumeria graminis* f.sp. *hordei* race A6 of 40 genotypes.

3.3 Genotypes have an impact on the effectiveness of biologicals against Bgh A6

To determine whether the preventive application of biologicals can protect against powdery mildew infection and whether their protective potential differs between genotypes, 21 biologicals and Kumulus WG were tested on four genotypes. It was found that the genotype had an impact on the effectiveness of biologicals against Bgh A6 (Figure 11) under the given infection pressure (mean number of pustules on the first leaf of control plants: 18.5 on GP and 51.9 on BCC436). The infection of GP seedlings with Bgh A6 was significantly reduced when Kumulus WG ($p<0.001$), Blattgrün ($p<0.001$), Equisetum Plus ($p=0.007$), raw milk ($p=0.001$), RhizoVital 42 ($p=0.022$) or Serenade ASO ($p=0.023$) were applied preventively (Figure 11 A). The use of baking powder and CropCover significantly aggravated the infection ($p<0.001$). In Morex, preventive treatments significantly reducing the number of pustules were: Kumulus WG ($p<0.001$), Blattgrün ($p<0.001$), burdock root extract ($p=0.027$), Equisetum Plus ($p=0.001$), giant knotweed leaf extract ($p=0.020$), Potta Sol ($p=0.006$), raw milk ($p=0.005$), Serenade ASO ($p=0.013$) and T-Gro ($p=0.045$). The use of CropCover significantly increased the number of Bgh pustules ($p=0.037$). The infection of genotype JK1_HOR7985 seedlings with Bgh A6 was significantly reduced when Kumulus WG (p<0.001), Blattgrün (p<0.001), Equisetum Plus (p<0.001) or T-Gro (p=0.024) were applied preventively. The use of ASL Kombi (p=0.009), baking powder (p=0.010) and CropCover (p=0.004) significantly aggravated the infection. The number of Bgh pustules on BCC436 seedlings was significantly reduced after preventive application of Kumulus WG (p<0.001), Blattgrün (p<0.001), Biplantol agrar (p=0.006), Biplantol mycos V forte (p=0.007), raw milk (p<0.001) or Tillecur (p=0.001). The use of baking powder significantly aggravated the infection (p=0.034). The comparison of the preventive effect of biologicals on Bgh A6 infection on four genotypes revealed an inconsistent pattern of effectiveness (Figure 11 B).

Fourteen of the 21 products tested showed a genotype-dependent effect meaning that the influence on the number of Bgh pustules differed between the four genotypes (Figure 11 B). Two products, Kumulus WG and and Blattgrün, significantly reduced (p<0.001) the number of pustules compared to water treated plants and independent of the tested genotype. Two further biologicals, Equisetum Plus and raw milk significantly reduced Bgh infection for three of the four genotypes – Serenade ASO and T-Gro for two genotypes. Seven biologicals (Biplantol agrar, Biplantol mycos V forte, burdock root extract, giant knotweed leaf extract, Potta Sol, RhizoVital 42, Tillecur) showed a significant reducing effect for at least one genotype. The application of six biologicals (Ackerschachtelhalm Extrakt Compositum, Alginure Agro Support F, AMN Biovit, Brennessel Extrakt Compositum, Elot-Vis Green and Fyto-Save) had no significant effect on the infection in neither of the investigated genotypes compared to the negative control, while three products (ASL Kombi, CropCover, baking powder) enhanced the number of pustules on at least one genotype.

Overall, the botanicals were found to have diverse effects on infection severity. The animal-based product (raw milk) had a genotype-dependent effect, as did the microbial and the homeopathic products. However, the latter were only effective on one genotype, the microbials on one to two and the animal-based product on three of the four genotypes.



***	p<0.001
**	p<0.01
*	p<0.05

🟩	reduction
⬜	no effect
🟥	augmentation

Kumulus WG	Blattgrün	Equisetum Plus	Raw milk	Serenade ASO	T-Gro	RhizoVital 42	Giant knotweed leaf extract	PottaSol	Burdock root extract	Biplantol mycos V forte	Biplantol agrar	Tillecur	Elot-Vis Green	FytoSave	AMN BioVit	Brennessel E.C.	Ackerschachtelhalm E.C.	Alginure Agro Support F	ASL Kombi	Baking powder	CropCover	
🟩 ***	🟩 ***	🟩 **	🟩 **	🟩 *	⬜	🟩 *	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥 ***	🟥 ***	Golden Promise
🟩 ***	🟩 ***	🟩 **	🟩 **	🟩 *	🟩 *	⬜	🟩 *	🟩 **	🟩 *	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥 *	Morex
🟩 ***	🟩 ***	🟩 ***	⬜	⬜	🟩 *	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥 **	🟥 **	🟥 **	JK_1_HOR7985
🟩 ***	🟩 ***	⬜	🟩 ***	⬜	⬜	⬜	⬜	⬜	⬜	🟩 **	🟩 **	🟩 **	⬜	⬜	⬜	⬜	⬜	⬜	⬜	🟥 *	⬜	BCC436

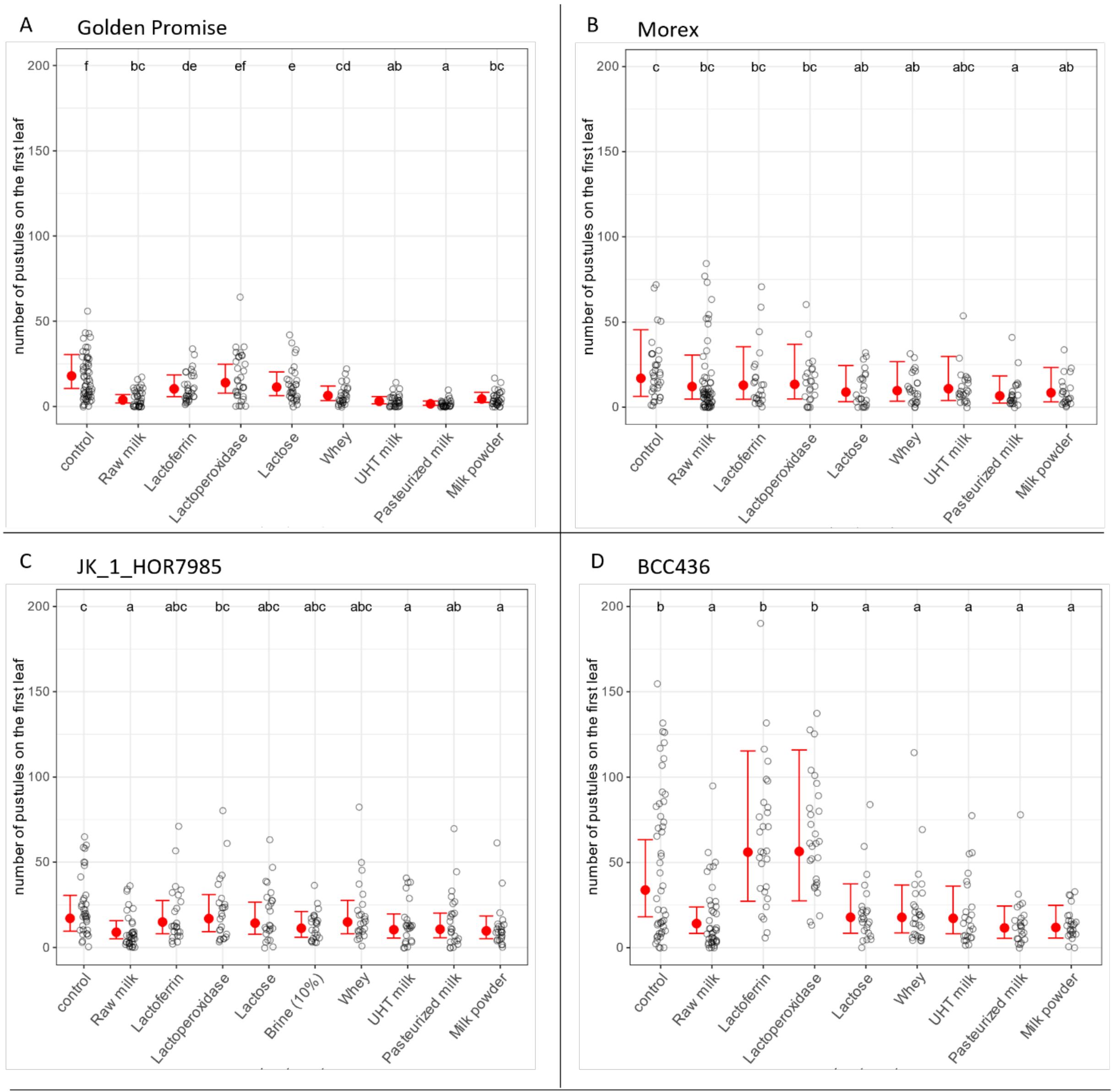
Figure 11. Preventive effect of biologicals and Kumulus WG on the infection with *Blumeria graminis* f.sp. *hordei* race A6 on susceptible barley genotypes. A: Number of pustules on the first leaf, six days after inoculation and after a preventive application of biologicals or Kumulus WG. Control: water treated; black dots: raw data; red dots/error bars: adjusted means with 95% confidence interval; number of evaluated plants per treatment and genotype 30-61 (36-398 for control, Kumulus WG; more details chapter S 4, S Table 4), different letters indicate significant differences (p<0.05); B: Comparison of biological effects on four different genotypes, compared to control; E.C: Extrakt Compositum.

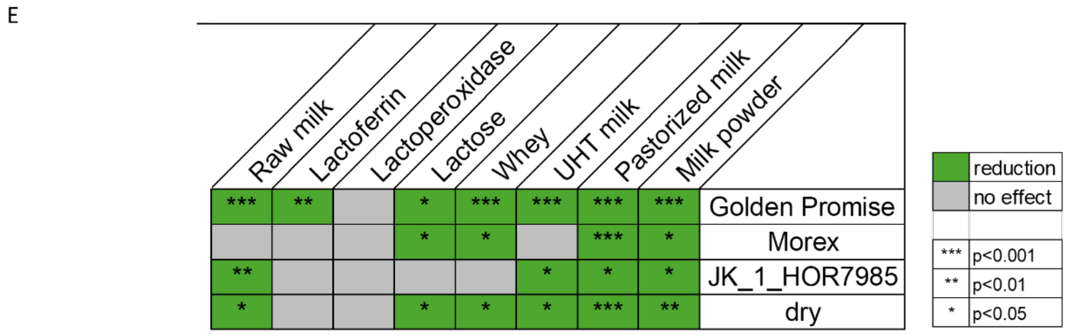
3.4 Preventively applied, basic substance milk and associated products reduce Bgh infection

Searching for active compounds leading to the observed efficiency of raw milk (Figure 11), different milk ingredients and milk associated products were tested for their preventive potential against powdery mildew (Figure 12). To include possible influences of the genotype on effectiveness, the experiment was carried out on four genotypes (GP, Morex, BCC436, JK1_HOR7985).

Genotype GP evolved significantly less pustules six days after inoculation (dai) than water treated control plants when preventively treated with raw milk ($p<0.001$), lactoferrin ($p=0.009$), lactose ($p=0.030$), whey ($p<0.001$), UHT milk ($p<0.001$), pasteurized milk ($p<0.001$) or milk powder ($p<0.001$). No significant reduction in the number of pustules was found after lactoperoxidase treatment (Figure 12 A). On genotye Morex preventive treatment with lactose ($p=0.017$), whey ($p=0.041$), pasteurized milk ($p=0.001$) and milk powder ($p=0.013$) reduced the infection significantly. No significant reductions in the number of pustules on Morex were found after raw milk, lactoferrin, lactoperoxidase and UHT milk treatment (Figure 12 B). On Genotype JK_1_HOR7985, the preventive application of raw milk ($p=0.009$), UHT milk ($p=0.033$), pasteurized milk ($p=0.042$) and milk powder ($p=0.016$) significantly reduced the number of pustules, while the application of lactoferrin, lactoperoxidase, lactose and whey did not (Figure 12 C). Genotype BCC436 evolved significantly less pustules six dai than water treated control plants when preventively treated with raw milk ($p=0.021$), lactose ($p=0.046$), whey ($p=0.036$), UHT milk ($p=0.035$), pasteurized milk ($p=0.001$) or milk powder ($p=0.001$). On BCC436 no significant reduction in the number of pustules were found after lactoperoxidase and lactoferrin treatment (Figure 12 D).

In summary, pasteurized milk and milk powder significantly reduced the number of pustules on all tested genotypes (Figure 12 E). Raw milk, lactose, whey and UHT milk were effective on three and lactoferrin on one genotype. Lactoperoxidase did not reduce the number of pustules significantly on any of the genotypes tested.




E

Raw milk	Lactoferrin	Lactoperoxidase	Lactose	Whey	UHT milk	Pastorized milk	Milk powder	
🟩 ***	🟩 **	▨	🟩 *	🟩 ***	🟩 ***	🟩 ***	🟩 ***	Golden Promise
▨	▨	▨	🟩 *	🟩 *	▨	🟩 ***	🟩 *	Morex
🟩 **	▨	▨	▨	▨	🟩 *	🟩 *	🟩 *	JK_1_HOR7985
🟩 *	▨	▨	🟩 *	🟩 *	🟩 *	🟩 ***	🟩 **	dry

🟩	reduction
▨	no effect
***	p<0.001
**	p<0.01
*	p<0.05

Figure 12. Preventive effectiveness of milk and milk associated products against *Blumeria graminis* f.sp. *hordei* race A6 on susceptible barley genotypes. A-D: Shown is the number of pustules on the first leaf, six days after inoculation and after a preventive treatment with milk or milk associated products. Control: water treated; non-filled dots: raw data; red dots/error bars: adjusted means with 95% confidence interval; different letters indicate significant differences (p<0.05) (detailed sample size information chapter S 4, S Table 5). A: Results of genotype Golden Promise, n=28-59; B: Results of genotype Morex, n=23-61; C: Results of JK1_HOR7985, n=24-36; D: Results of BCC436, n=24-48; E: Effect comparison of milk and milk associated products on four different genotypes in form of a heatmap.

3.5 Infection development under simultaneous drought stress and biological application

Ten biologicals and Kumulus WG were studied for their efficiency against Bgh and for off-target effects under simultaneous drought stress (20% maximum water holding capacity (MWHC)) over three weeks and compared with regular watering (70% MWHC) (chapter 3.5-3.13) as part of the 'combined experiment'. The investigation of the development of Bgh A6 infection over three weeks showed that 1) for genotype BCC1589, lower infection rates than for GP and BCC436 were observed throughout the trial (chapter S 5, S Table 6, S. Table 7), 2) well-watered control plants of GP and BCC436 showed more symptoms at each time point than dry cultivated control plants of the respective genotype (chapter S 5, S Table 6, S. Table 7), 3) well-watered control plants of BCC1589 had equally or slightly fewer infection symptoms than dry cultivated control plants (chapter S 5, S Table 6, S. Table 7), 4) the positive, i.e. mildew-reducing effect of most products decreases by 20 dai (Figure 13, Figure 14) and 5) the second application only led to an average enhanced reduction in mildew infection for a few products 13 dai. This was the case for GP under dry conditions treated with CropCover, baking powder, Elot-Vis Green, raw milk (100%), burdock root extract and Serenade ASO, as well as under dry conditions for BCC436 treated with CropCover or burdock. The signs of infection on BCC1589 remained very low during the experiment, whereby the slight increase in infection symptoms 20 dai in the dry cultivated control plants was also very low at 0.2 infection categories.

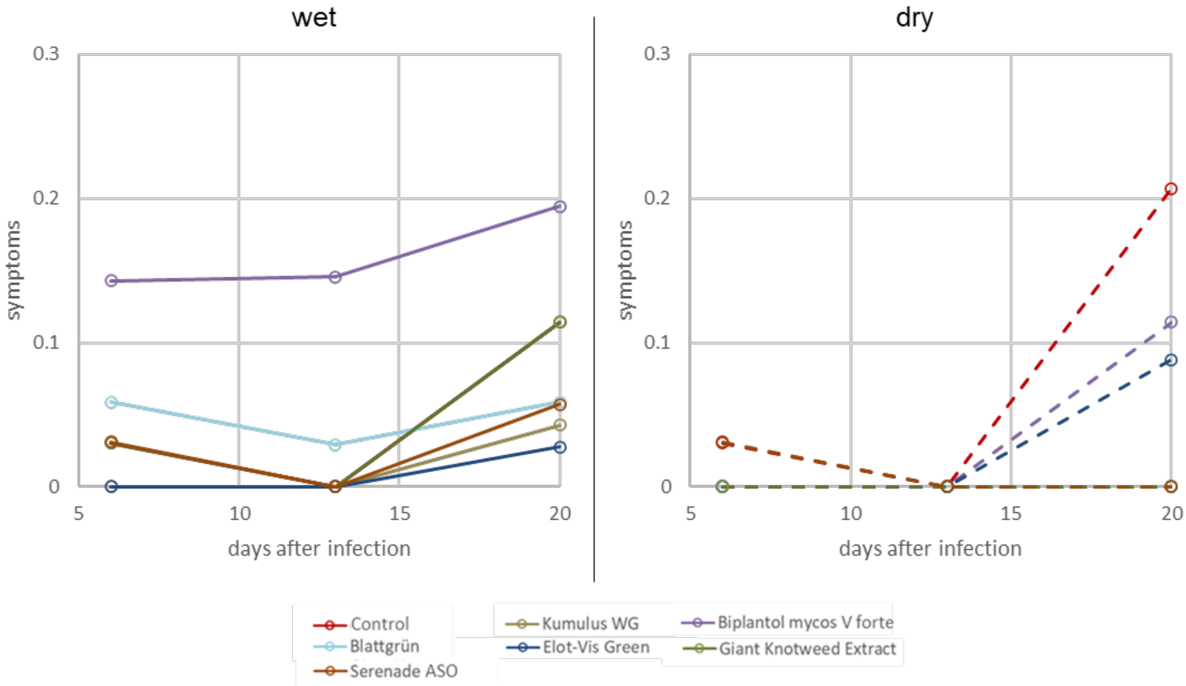


Figure 13: Infection development of *Blumeria graminis* f.sp. *hordei* race A6 on barley genotype BCC1589 in response to biologicals or Kumulus WG and drought stress at three time points. Plotted is the mean of symptoms, where symptoms include the number of pustules on the first leaf (six days after inoculation), the percentage of infected leaf area (13 days after inoculation) and the infection category minus one (20 days after inoculation); wet/dry: well-watered or dry cultivation conditions; mean of 29-72 plants per time point and biological (details chapter S 5, S Table 8).

Under well-watered conditions, plants treated with Biplantol mycos V forte had the most signs of infection, even if these were very low (Figure 13).

The variability in infection intensity of GP and BCC436 was smallest 20 dai (Figure 14). The infection trends of the biological treatments of well-watered GP plants and BCC436 of both water regimes were relatively similar. At six and thirteen dai, most products led to an infection reduction. The initially low infection levels of these treatments increased by 20 dai so that the deviations from the control became smaller. In addition to Kumulus WG, CropCover, burdock root extract and baking powder, these products also included raw milk (100%, 10%). In contrast, the variation in infection was greater in dry-cultivated GP at each time point and the application of burdock root extract led from an increase in infection of almost 150% six dai to a reduction of nearly 20% compared to the control 20 dai. A similar tendency was observed for the application of baking powder.

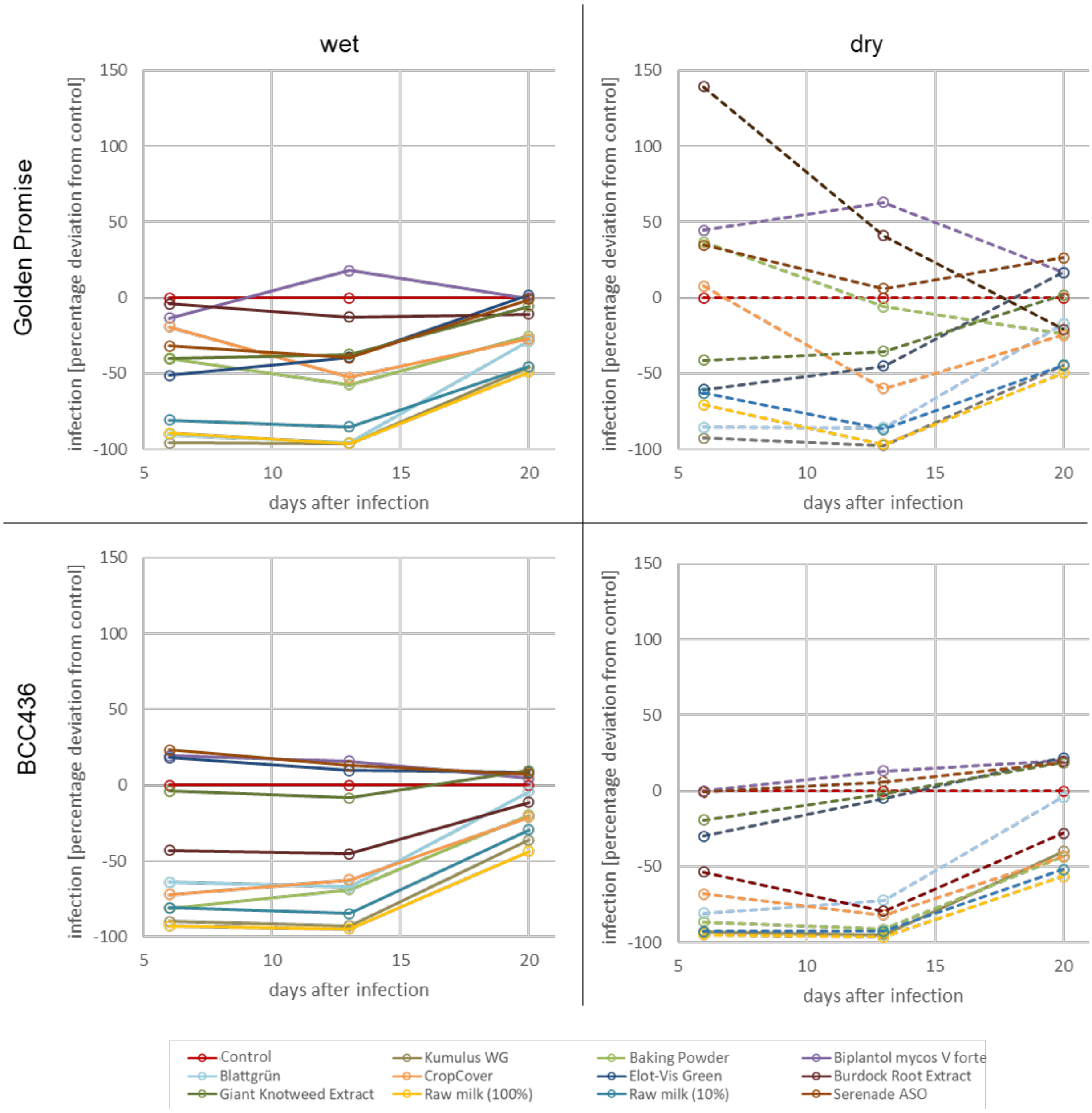
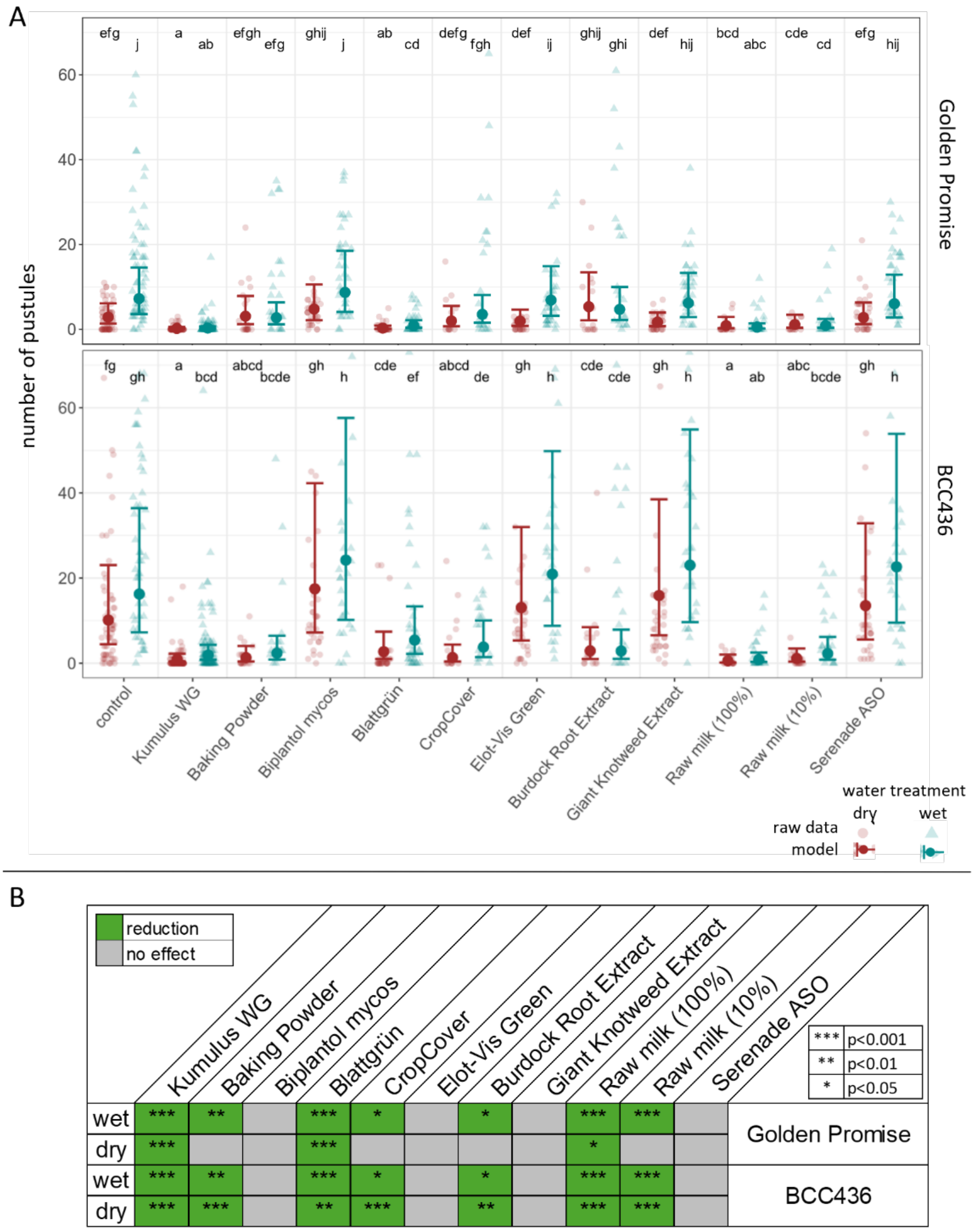


Figure 14: Infection development of *Blumeria graminis* f.sp. *hordei* race A6 on barley genotype Golden Promise and BCC436 in response to biologicals or Kumulus WG and drought stress at three time points. Plotted is the mean percentage deviation of the infection level from the mean of control (six days after inoculation (dai): based on the number of pustules on the first leaf, thirteen dai: based on the percentage of infected leaf area, 20 dai: based on infection categories); wet/dry: well-watered or dry cultivation conditions; BCC436 mean of 16-96 plants per time point and treatment; Golden Promise mean of 14-96 plants per time point and treatment (details in chapter S 5, S Table 8).

3.6 Some biologicals have a drought stress-independent effectiveness against Bgh

In addition to the assessment of the Bgh infection course under well-watered and dry conditions, a statistical analysis was carried out for the intensity of the infection symptoms six and 21 dai as part of the 'combined experiment' (2.10). Drought-stressed control plants of drought sensitive genotype GP had significantly (p<0.001) fewer pustules six dai than the well-watered GP control group (Figure 15 A). This was still the case three weeks after inoculation (Figure 16 A). For the drought stress tolerant genotype BCC436 no significant differences were found in infection signs of the control plants, although well-watered generally had more pustules than drought stressed plants (Figure 15 A, Figure 16 A). Preventive application of Kumulus WG, Blattgrün and raw milk (100%) significantly reduced powdery mildew infection on GP and BCC436 under both, well-watered and dry conditions compared to the respective control (p-values between 0.029 and <0.001). On BCC436, also treatments with baking powder, CropCover, burdock root extract and raw milk (10%) significantly reduced the number of Bgh pustules on the first leaf independent of the water status (p-values between 0.025 and <0.001). Whereas these products were water status-dependently effective on GP (ineffective on drought-stressed plants (Figure 15 A, B). When additionally treated with biologicals seven days post-inoculation (p-i), the water regime-dependent effect of these four biologicals was no longer observed and powdery mildew infection was significantly reduced under well-watered and dry conditions on GP (p-value between 0.019 and <0.001). In summary, the p-i sprays of Kumulus WG, baking powder, CropCover, and raw milk (10% and 100%) caused significant reductions of Bgh symptoms in both investigated barley genotypes under dry and well-watered conditions (p-value between 0.013 and <0.001) 21 dai. Elot-Vis Green significantly (p=0.034) aggravated the infection after p-i spraying under drought stress in GP and in both water regimes of BCC436 (p<0.001 under dry conditions, p=0.031 under well-watered conditions) whereas, Serenade ASO caused in both genotypes only under dry conditions an increased infection (GP: p=0.042, BCC436: p=0.009) and had no significant influences on well-watered plants. Biplantol mycos V forte remained ineffective with p-i spraying against Bgh infection on GP but significantly (p<0.001) increased the infection in drought-stressed plants of BCC436 (Figure 16). Under well-watered conditions, control plants of GP and BCC436 reached means of 16.529 and 39.103 Bgh pustules on the first leaf six dai, indicating a lower disease pressure than in the 'biological trial' (chapter S 4, S Table 4).



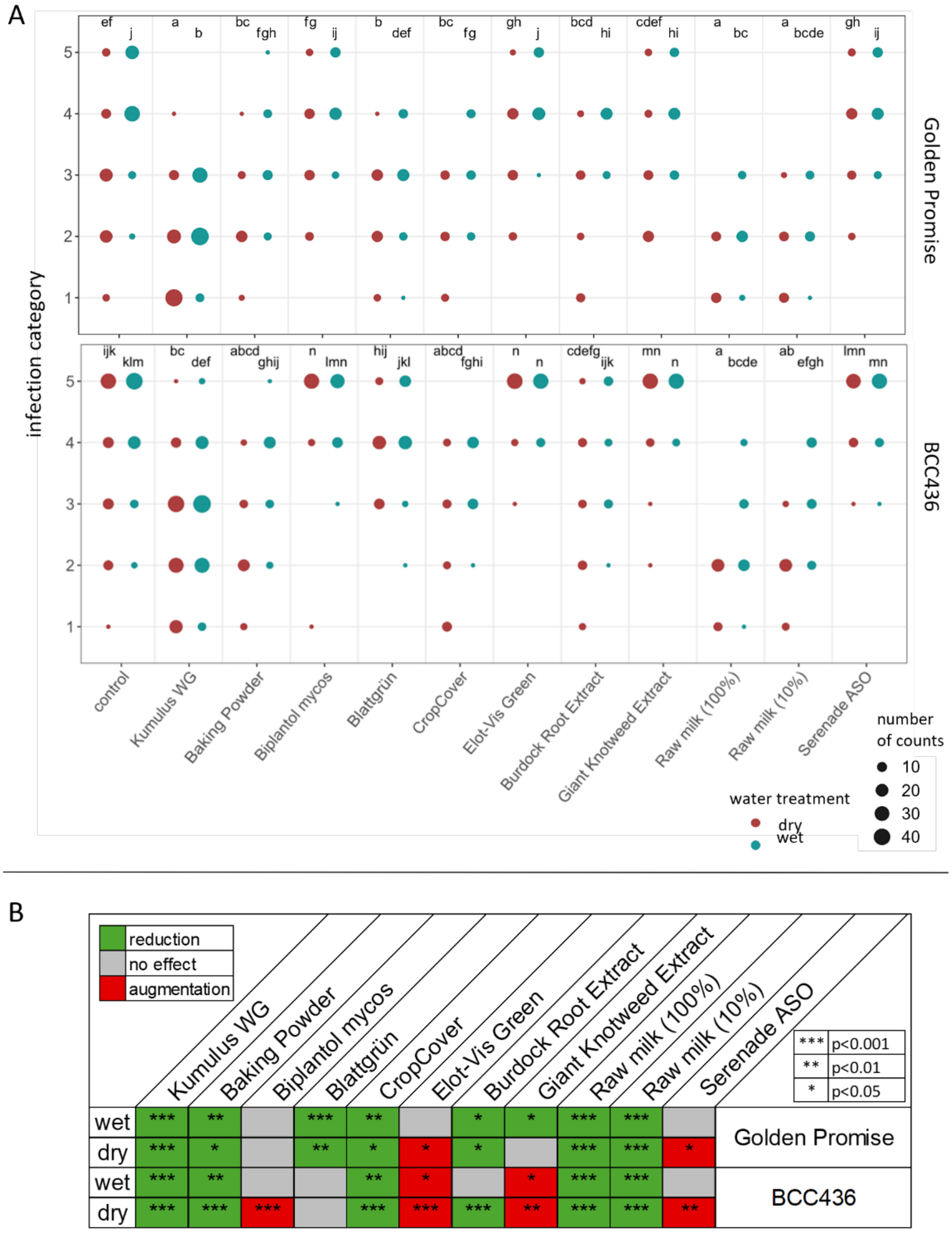
🟩 reduction

⬜ no effect

***	p<0.001
**	p<0.01
*	p<0.05

		Kumulus WG	Baking Powder	Biplantol mycos	Blattgrün	CropCover	Elot-Vis Green	Burdock Root Extract	Giant Knotweed Extract	Raw milk (100%)	Raw milk (10%)	Serenade ASO
Golden Promise	wet	🟩 ***	🟩 **	⬜	🟩 ***	🟩 *	⬜	🟩 *	⬜	🟩 ***	🟩 ***	⬜
	dry	🟩 ***	⬜	⬜	🟩 ***	⬜	⬜	⬜	⬜	🟩 *	⬜	⬜
BCC436	wet	🟩 ***	🟩 **	⬜	🟩 ***	🟩 *	⬜	🟩 *	⬜	🟩 ***	🟩 ***	⬜
	dry	🟩 ***	🟩 ***	⬜	🟩 **	🟩 ***	⬜	🟩 **	⬜	🟩 ***	🟩 ***	⬜

Figure 15. Preventive powdery mildew reducing capacity of biologicals and Kumulus WG under simultaneous drought stress on barley genotypes Golden Promise and BCC436 six days after inoculation. Biplantol mycos: Biplantol mycos V forte; wet/dry: well-watered or dry cultivation conditions; A: Number of pustules six days after inoculation; control: treated with water; little dots or triangles represent raw data, y-axis capped at 70; big dots/error bars: predicted means with 95% confidence interval; different letters indicate significant differences (p<0.05). Number of studied plants in total: 14-94 per treatment, watering and genotype; B: heatmap of preventive efficacy of biologicals and Kumulus WG.



🟩	reduction
⬜	no effect
🟥	augmentation

***	p<0.001
**	p<0.01
*	p<0.05

		Kumulus WG	Baking Powder	Biplantol mycos	Blattgrün	CropCover	Elot-Vis Green	Burdock Root Extract	Giant Knotweed Extract	Raw milk (100%)	Raw milk (10%)	Serenade ASO
Golden Promise	wet	🟩 ***	🟩 **	⬜	🟩 ***	🟩 **	⬜	🟩 *	🟩 *	🟩 ***	🟩 ***	⬜
	dry	🟩 ***	🟩 *	⬜	🟩 **	🟩 *	🟥 *	🟩 *	⬜	🟩 ***	🟩 ***	🟥 *
BCC436	wet	🟩 ***	🟩 **	⬜	⬜	🟩 **	🟥 *	⬜	🟥 *	🟩 ***	🟩 ***	⬜
	dry	🟩 ***	🟩 ***	🟥 ***	⬜	🟩 ***	🟥 ***	🟩 ***	🟥 **	🟩 ***	🟩 ***	🟥 **

Figure 16. Powdery mildew reducing capacity after preventive and additional post-inoculation spray of biologicals or Kumulus WG under simultaneous drought stress on barley genotypes Golden Promise and BCC436 21 days after inoculation. Biplantol mycos: Biplantol mycos V forte; wet/dry: well-watered or dry cultivation conditions; A: Frequency of infection categories three weeks after inoculation; ranging from high infection: category (5) to low or no infection: category (1); dot size represents the frequency of the respective category; control: water treated; different letters indicate significant differences (p<0.05); number of studied plants in total: 18-94 per treatment, watering and genotype. B: Heatmap of biological efficacy.

3.7 Drought stress does not change Bgh A6 resistance of genotype BCC1589

The effect of drought and biologicals on Bgh A6 inoculated plants of the resistant genotype BCC1589 were statistically analysed for the two time points six and 21 dai. Evidence was found that the Bgh A6 resistance of BCC1589 was altered neither under dry conditions nor by any of the tested biologicals six dai (Figure 17 A). Likewise, three weeks after infection, no significant difference (p=0.073) in infection intensity was found between well-watered and dry cultivated control plants (Figure 17 B). Similarly, the infection categories of the individual biological treatments did not differ between the two water regimes. However, a few significant differences were found when comparing treatments with control plants: under well-watered and dry conditions, the p-i application of Kumulus WG, Blattgrün and Serenade ASO significantly reduced infection (p<0.05).

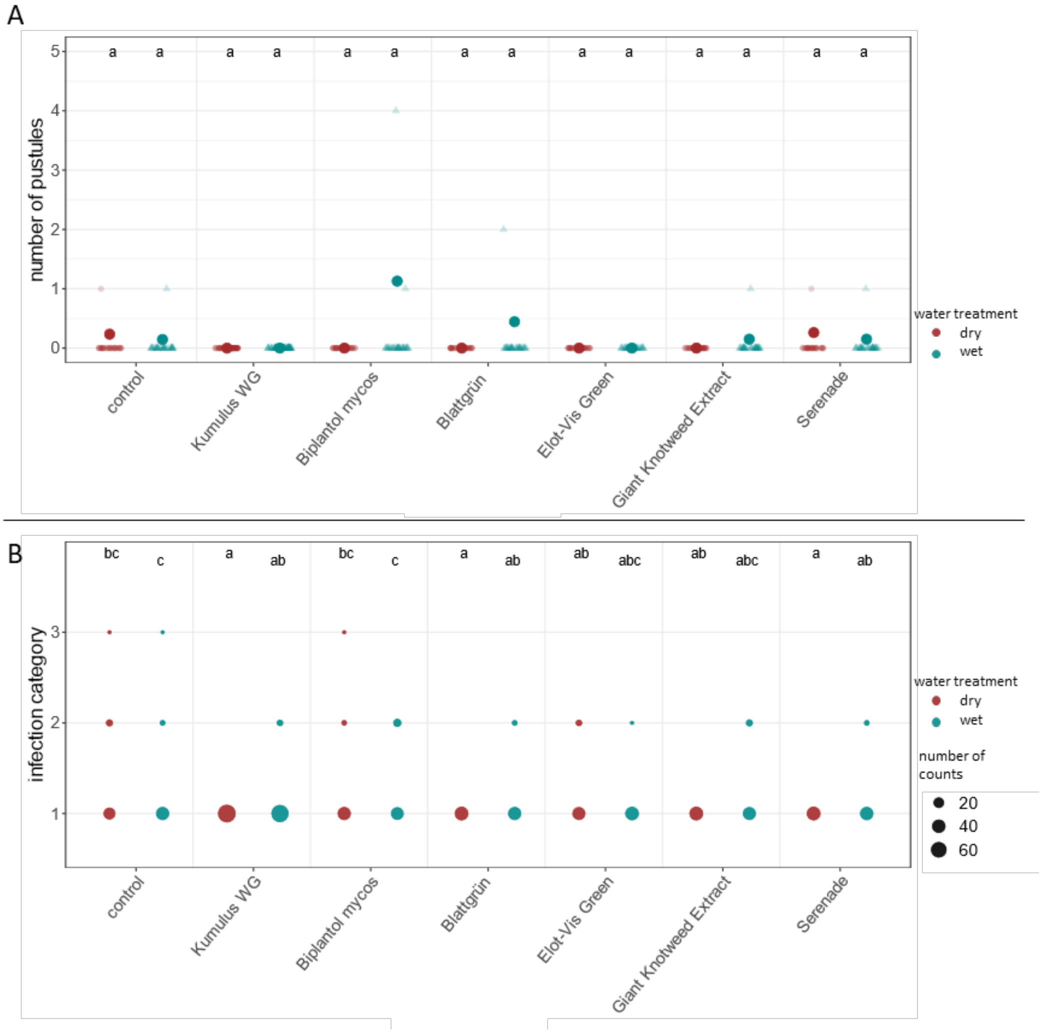


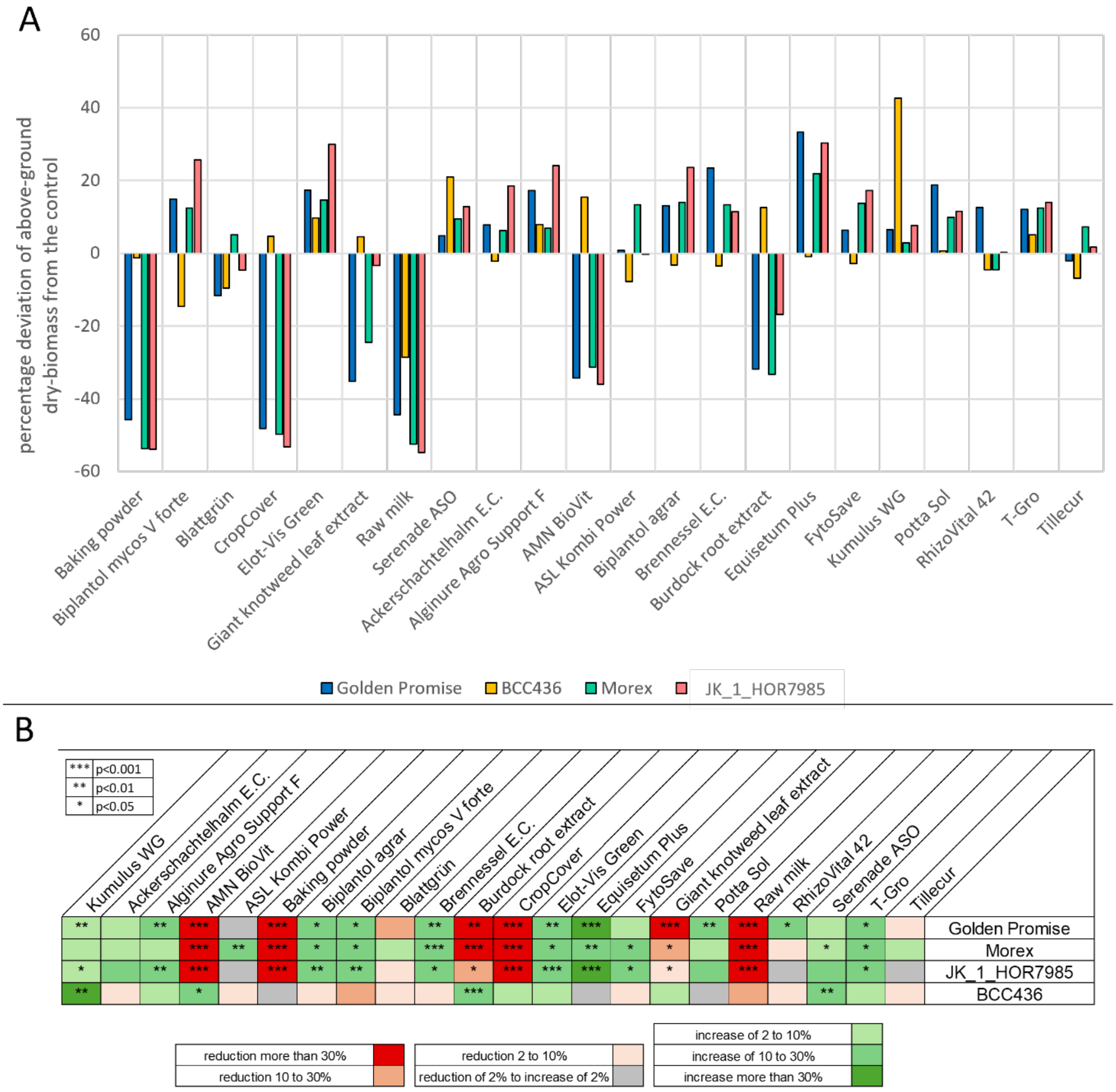
Figure 17. Powdery mildew infection after preventive and preventive plus additional post-inoculation spray of biologicals or Kumulus WG on barley genotype BCC1589 under simultaneous drought stress. Biplantol mycos: Biplantol mycos V forte; Serenade: Serenade ASO; wet/dry: well-watered or dry cultivation conditions; Control: water treated; A: Number of pustules on the first leaf six days after inoculation. little dots or triangles represent raw data; big dots: predicted means with 95% confidence interval; different letters indicate significant differences (p<0.05); number of plants per treatment and watering: 29-71; B: Infection category 21 days after inoculation. High infection: category 5, low or no infection: category 1; dot size represents the frequency of the respective category, axis capped at 4; detailed information on sample size chapter S 5, S Table 8.

3.8 Biologicals influence the above-ground biomass

To obtain information on whether the application of biologicals in the early developmental stage of barley influenced the growth of the plants, the above-ground dry biomass was determined 14 das, as part of the ‘biological trial’. It was found that water treated control plants of GP and BCC436 had on average a lower dry biomass than those of the genotypes Morex and JK1_HOR7985 (53.0 mg and 39.8 mg in the case of GP and BCC436, respectively, compared to 69.8 mg and 68.3 mg for Morex and JK1_HOR7985, respectively; chapter S 6, S Table 9). This was generally confirmed when plants were treated with biologicals. The above-ground dry biomass of Morex genotype varied by up to 22 mg from the water treated control due to the application of the biologicals, that of BCC436 by up to 28 mg. In contrast, the dry biomass of GP and JK1_HOR7985 varied from the control by 43 and 58 mg, respectively, meaning a stronger reaction to biologicals (chapter S 6, S Table 9).

For each genotype, the application of raw milk had a negative, so dry biomass reducing effect, compared to the control (Figure 18 A) which was not always significant (Figure 18 B). This effect was smallest for BCC436 with a mean dry biomass reduction of 28.7% compared to the control, and largest for JK1_HOR7985 with 54.8%. In three of the four genotypes, CropCover, baking powder and raw milk reduced the dry biomass by more than 40% (Figure 18 A). Moreover, AMN BioVit, burdock root extract and giant knotweed extract had a significant growth-reducing effect on three of the four genotypes (reduction of 2% to >30%) (Figure 18 B). A dry biomass increase of more than 10% in at least three genotypes, was achieved by applying Equisetum Plus, Elot-Vis Green, Biplantol agrar, Biplantol mycos V forte, Brennessel Extract Compositum and T-Gro. The highest dry biomass increase was found for Kumulus WG treated BCC436 with 42.7%.

The above-ground dry biomass of GP, Morex and JK1_HOR7985 was more often significantly influenced by biologicals than that of the BCC436 genotype 14 das (Figure 18 B). The above-ground dry biomass of BCC436 was significantly increased by Kumulus WG ($p=0.003$), burdock root extract ($p<0.001$), Serenade ASO ($p=0.007$) and AMN BioVit ($p=0.043$), a significant decrease was not observed. In contrast, in GP, Morex and JK1_HOR7985, above-ground dry biomass was significantly reduced by the application of AMN BioVit, baking powder, CropCover and raw milk (each $p<0.001$), as well as by burdock root extract (GP: $p=0.002$; Morex: $p<0.001$; JK1_HOR7985: $p=0.011$) and giant knotweed leaf extract (GP: $p<0.001$; Morex: $p=0.023$; JK1_HOR7985: $p=0.010$). With GP, a significant increase in dry biomass was observed with nine biologicals and Kumulus WG already seven days after application, as well as with JK1_HOR7985 and Morex even if the products that triggered this increase varied in part between the genotypes.



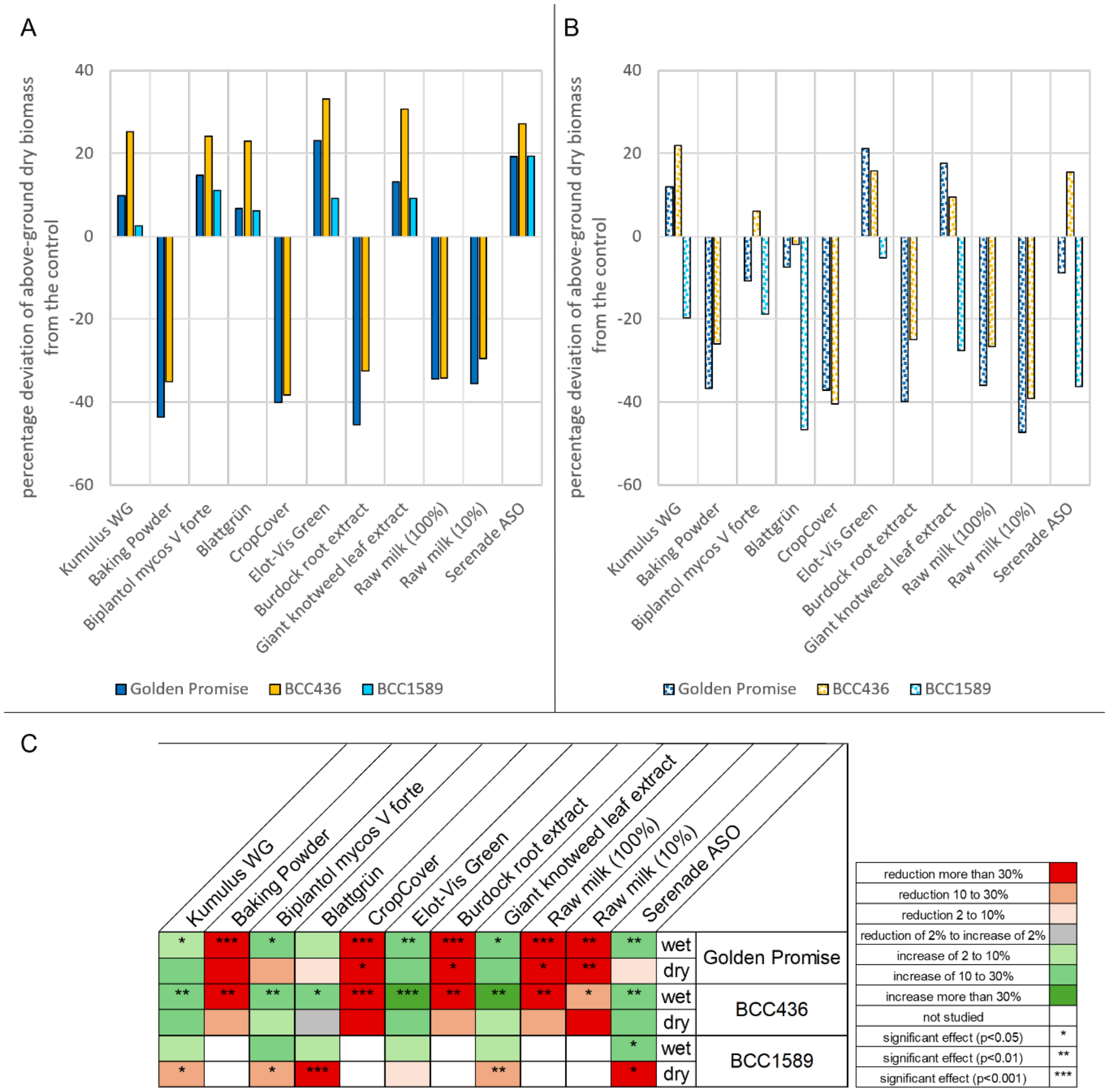
***	p<0.001
**	p<0.01
*	p<0.05

Kumulus WG	Ackerschachtelhalm E.C.	Alginure Agro Support F	AMN BioVit	ASL Kombi Power	Baking powder	Biplantol agrar	Biplantol mycos V forte	Blattgrün	Brennessel E.C.	Burdock root extract	CropCover	Elot-Vis Green	Equisetum Plus	FytoSave	Giant knotweed leaf extract	Potta Sol	Raw milk	RhizoVital 42	Serenade ASO	T-Gro	Tillecur	
🟢 **	🟢	🟩 **	🟥 ***	⬜	🟥 ***	🟩 *	🟩 *	🟧	🟩 **	🟥 **	🟥 ***	🟩 **	■ ***	🟢	🟥 ***	🟩 **	🟥 ***	🟩 *	🟢	🟩 *	🟨	Golden Promise
🟢	🟢	🟢	🟥 ***	🟩 **	🟥 ***	🟩 *	🟩 *	🟢	🟩 ***	🟥 ***	🟥 ***	🟩 *	🟩 **	🟩 *	🟧 *	🟢	🟥 ***	🟨	🟢 *	🟩 *	🟢	Morex
🟢 *	🟩	🟩 **	🟥 ***	⬜	🟥 ***	🟩 **	🟩 **	🟨	🟩 *	🟧 *	🟥 ***	🟩 ***	■ ***	🟩 *	🟨 *	🟩	🟥 ***	⬜	🟩	🟩 *	⬜	JK_1_HOR7985
■ **	🟨	🟢	🟩 *	🟨	⬜	🟨	🟧	🟨	🟨	🟩 ***	🟢	🟢	⬜	🟨	🟢	⬜	🟧	🟨	🟩 **	🟢	🟨	BCC436

reduction more than 30%	🟥
reduction 10 to 30%	🟧
reduction 2 to 10%	🟨
reduction of 2% to increase of 2%	⬜
increase of 2 to 10%	🟢
increase of 10 to 30%	🟩
increase more than 30%	■

Figure 18. Influence of biologicals and Kumulus WG on above-ground dry biomass at early developmental stage of four barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei* race A6. E.C.: Extrakt Compositum; A: Percentage deviation from mean of untreated control plants of above-ground dry biomass from biological or Kumulus WG treated plants. B: Heatmap of results of statistical analysis of biological influence on above-ground biomass 14 days after sowing. Heatmap colour code based on the percentage deviation of the means of above-ground dry biomass with all shades of red describing a reduction and all shades of green an increase, additionally indicated are significant differences based on raw data.

As part of the ‘combined experiment’, the above-ground dry biomass was determined 28 das in Bgh inoculated and well-watered or dry cultivated plants (Figure 19). Thereby, the comparison of well-watered control plants with their drought-stressed counterparts showed that the latter produced between 65% (BCC1589) and 75% (BCC436) less biomass, four weeks after sowing. At an average of 356.7 mg, the well-watered control plants of BCC1589 were heavier than those of BCC436 (255.0 mg) or GP (237.6 mg) (chapter S 6, S Table 10). Under well-watered conditions, the p-i application of Kumulus WG, Biplantol mycos V forte, Blattgrün, Elot-Vis Green, giant knotweed leaf extract and Serenade ASO augmented the above-ground dry biomass of all three genotypes (Figure 19 A). This biomass increase was significant (p-value between 0.017 and <0.001) in the case of BCC436 and GP for all the biologicals mentioned, except for Blattgrün in GP. In BCC1589, Serenade ASO increased the biomass significantly (p<0.05) (Figure 19 C). A significant reduction of biomass was observed after p-i spraying of burdock root extract, CropCover and raw milk (100%, 10%) on GP of both water regimes as well as on well-watered BCC436 plants (p-value between 0.033 and <0.001). Furthermore, baking powder reduced growth of well-watered plants of both genotypes significantly (GP: p<0.001; BCC436: p=0.002). Drought stressed plants of BCC1589 had in general a lower biomass production when treated with biologicals compared to control plants (Figure 19 B, C). The dry biomass of genotype BCC436 under dry conditions tended to be altered by the biologicals in the same direction as under well-watered conditions, but not to a significant extent. An exception was Blattgrün with a significant positive influence on growth under well-watered but no effect under dry conditions. On GP, Biplantol mycos V forte, Blattgrün and Serenade ASO showed differential responses under well-watered and dry conditions, while the reactions were in the same direction (reduction or increase in biomass) for the other biologicals tested.



Kumulus WG	Baking Powder	Biplantol mycos V forte	Blattgrün	CropCover	Elot-Vis Green	Burdock root extract	Giant knotweed leaf extract	Raw milk (100%)	Raw milk (10%)	Serenade ASO		
*	***	*		***	**	***	*	***	**	**	wet	Golden Promise
				*		*		*	**		dry	
**	**	**	*	***	***	**	**	**	*	**	wet	BCC436
											dry	
										*	wet	BCC1589
*		*	***				**			*	dry	

reduction more than 30%	🟥
reduction 10 to 30%	
reduction 2 to 10%	
reduction of 2% to increase of 2%	
increase of 2 to 10%	
increase of 10 to 30%	
increase more than 30%	🟩
not studied	
significant effect (p<0.05)	*
significant effect (p<0.01)	**
significant effect (p<0.001)	***

Figure 19. Effect of biologicals, Kumulus WG and drought stress on the above-ground dry biomass 28 days after sowing of barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Dry biomass measured 28 days after sowing after preventive plus post-inoculation treatments with biologicals or Kumulus WG. A: percentage deviation of above-ground dry biomass of well-watered plants. B: percentage deviation of above-ground dry biomass of dry cultivated plants. C: Treatment effects on above-ground dry biomass. Calculation of significance based on raw data; colour coding based on calculated percentage deviation from control with all shades of red describing a reduction and all shades of green an increase in above-ground dry mass; wet/dry: well-watered or dry cultivation conditions.

3.9 The development of plant height is more influenced by drought than by biologicals

During the experiment on the combined occurrence of drought stress and Bgh A6 with biological application, the height of the plants was determined weekly to record possible growth-inhibiting or growth-promoting effects of the treatments (biologicals and drought) over time. The development of plant height was visualised via plotting the means per genotype, treatment, water status and time point (Figure 20). The plant height was bigger for plants from the well-watered groups of genotype GP, BCC436 and BCC1589 than was the height of the groups with dry cultivation conditions – independent of biological applications. Well-watered plants of BCC1589 were between 40.2 cm (control) and 42.3 cm (Serenade ASO) tall 28 das, plants of BCC436 between 35.0 cm (raw milk 100%) and 37.4 cm (Elot-Vis Green) and those of GP between 30.1 cm (burdock root extract) and 34.0 cm (Elot-Vis Green) (Figure 20, chapter S 7, S Table 11). Plants of genotype BCC1589 from the drought regime were between 24.0 cm (Blattgrün) and 32.6 cm (control) tall, those of BCC436 between 19.8 cm (CropCover) and 24.0 cm (Kumulus WG), those of GP 17.8 cm (Serenade ASO) and 21.6 cm (Kumulus WG). Overall, the height development of the plants hardly varied within the water regimes. The greatest variation was observed under dry conditions at BCC1589 with a difference of 8.6 cm between the largest and smallest mean value per biological.

Additionally, to the development of the plant height, the height at the end of the combined experiment (28 das) was analysed statistically (Figure 21). Six products (Elot-Vis Green: 4.3-6.6%, Serenade ASO: 2.5-5.3%, giant knotweed extract: 1.2-3.4%, Biplantol mycos V forte: 0.3-3.1%, Blattgrün: 0.7-1.2%, Kumulus WG: 0.7-5.7%) led to an increase in growth in all three genotypes under well-watered conditions, although not significantly ($p>0.05$) while none of the products increased plant height of all three genotypes under dry conditions (Figure 21 A, B). Elot-Vis Green, which led to growth increases from 4.3% (BCC1589) to 6.6% (GP) in the well-watered regime, increased the growth of GP under dry conditions by 9.7% compared to the control, but reduced it by 12.4% in BCC1589. Overall, only seven of the 28 combinations of genotypes and biologicals tested under dry conditions showed an increase in shoot height, which was only significant in the case of BCC436 and Serenade ASO ($p=0.005$) (Figure 21 C). Height reductions ranged from less than 1% (baking powder in BCC436) to a reduction of 28% compared to the control in Blattgrün treated BCC1589. In the case of this genotype most significant height reductions (six out of eight reductions) were found under dry conditions (p-values of <0.001 to 0.038). Overall, the height of drought-stressed plants was stronger influenced by biologicals than that of well-watered plants.

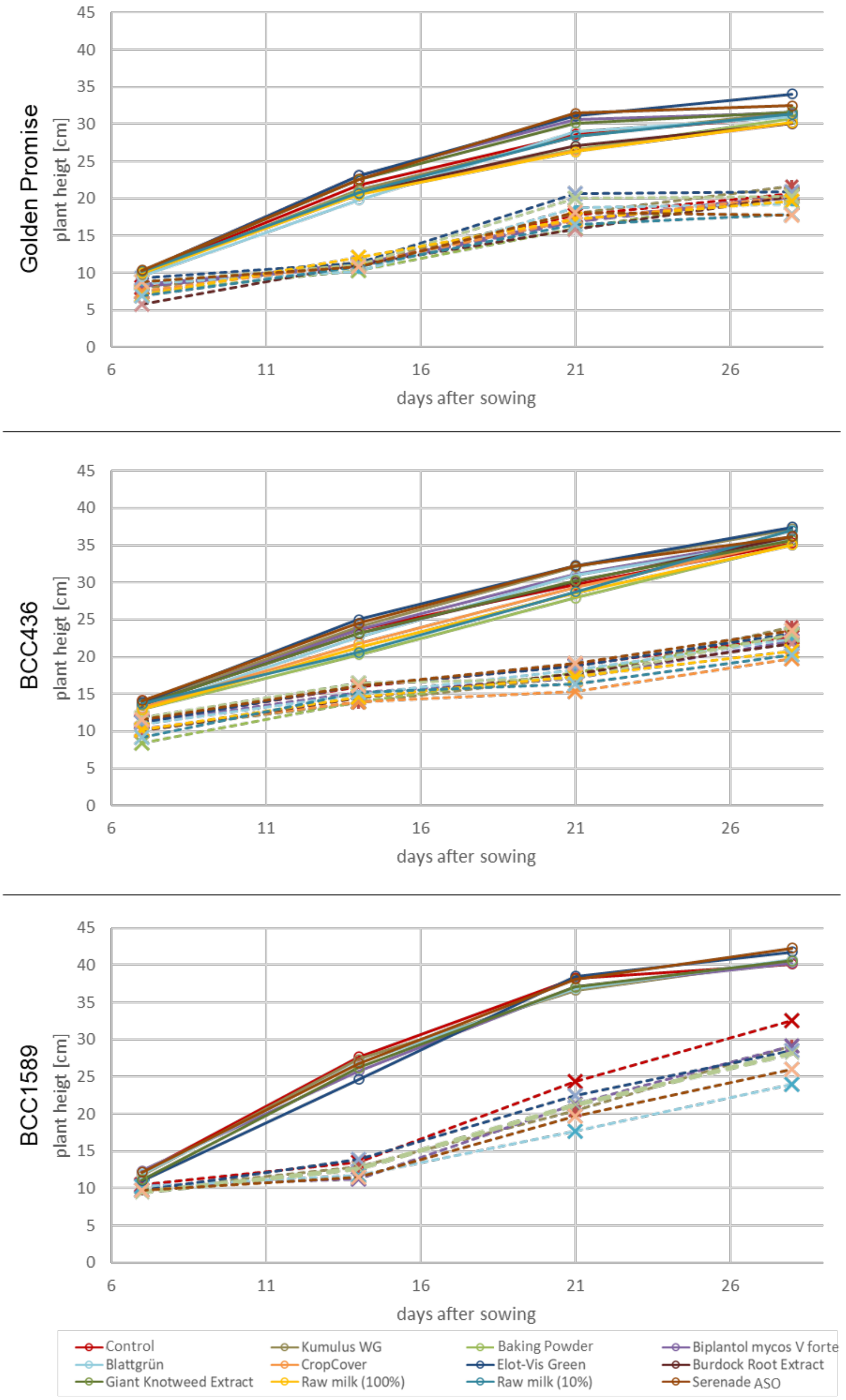


Figure 20. Development of plant height under well-watered and dry conditions for three barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei* race A6 and treated with different biologicals or Kumulus WG. Continuous line: well-watered cultivation conditions; dotted lines: dry cultivation conditions; control: water treated; shown is the mean per genotype, treatment and time point (sample size and standard error (always <1.5) chapter S 7, S Table 11).

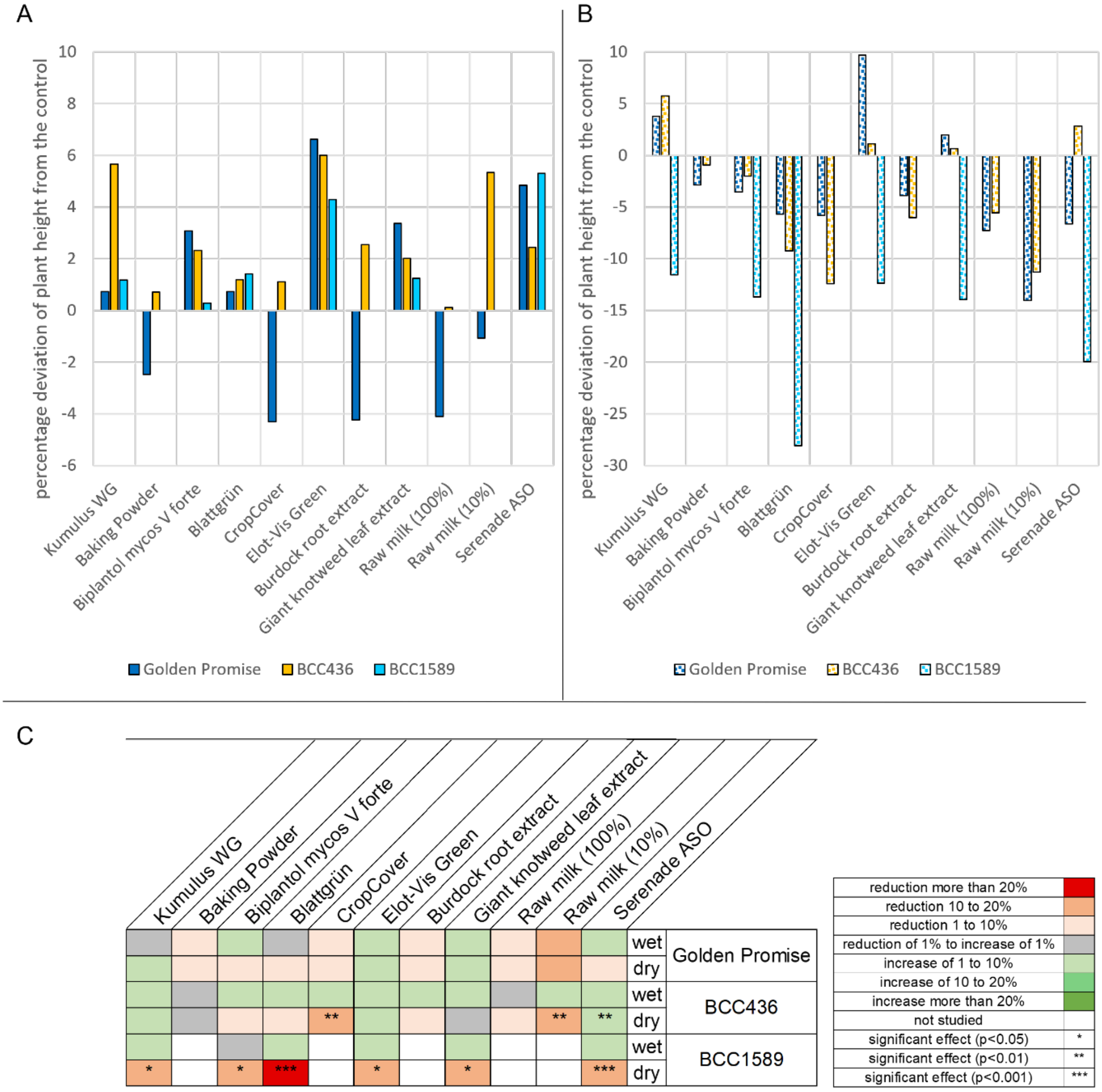


Figure 21. **Effect of biologicals, Kumulus WG and drought stress on plant height of *Blumeria graminis* f.sp. *hordei* race A6 inoculated barley genotypes 28 days after sowing.** Percentage deviation from the water control was calculated with the mean of each treatment; sample size chapter S 7, S Table 11; A: Percentage deviation of plant height under well-watered conditions; B: Percentage deviation of plant height under dry conditions; C: Heatmap of treatment effects on plant height; colour code based on the percentage deviation of the plant height with all shades of red describing a reduction and all shades of green an increase in plant height, additionally indicated are significant differences; wet/dry: well-watered or dry cultivation conditions.

3.10 BBCH is influenced by drought but also by genotype and biological application

Additionally, to plant height the BBCH of the plants was determined 28 das as part of the ‘combined experiment’. It was found that plants from the well-watered group were in general further developed than plants from the dry regime (Figure 22). However, genotype- and biological-dependent differences were found. Control plants of the well-water regime from genotype GP and BCC436 were nearly half-half allocated to BCCH below 15 and equal or above 21 with BCC436 having more plants with BBCH above 21 than GP. Well-watered control plants of BCC1589 were, except for one plant, in the tillering phase (BBCH>20). Dry cultivated BCC1589 plants were 28 das mostly in leaf developmental phase (BBCH 10-15), as were plants from BCC436 and GP. Baking Powder, CropCover, burdock root extract and raw milk (100%, 10%) applied on GP or BCC436 of both water regimes, caused a retardation in development as most of the plants were in the leaf development phase. In contrast, Serenade ASO on well-watered GP resulted for all plants in the tillering phase 28 das. Likewise, higher BBCH values than for the control plants were assigned for BCC1589 after Serenade ASO application.

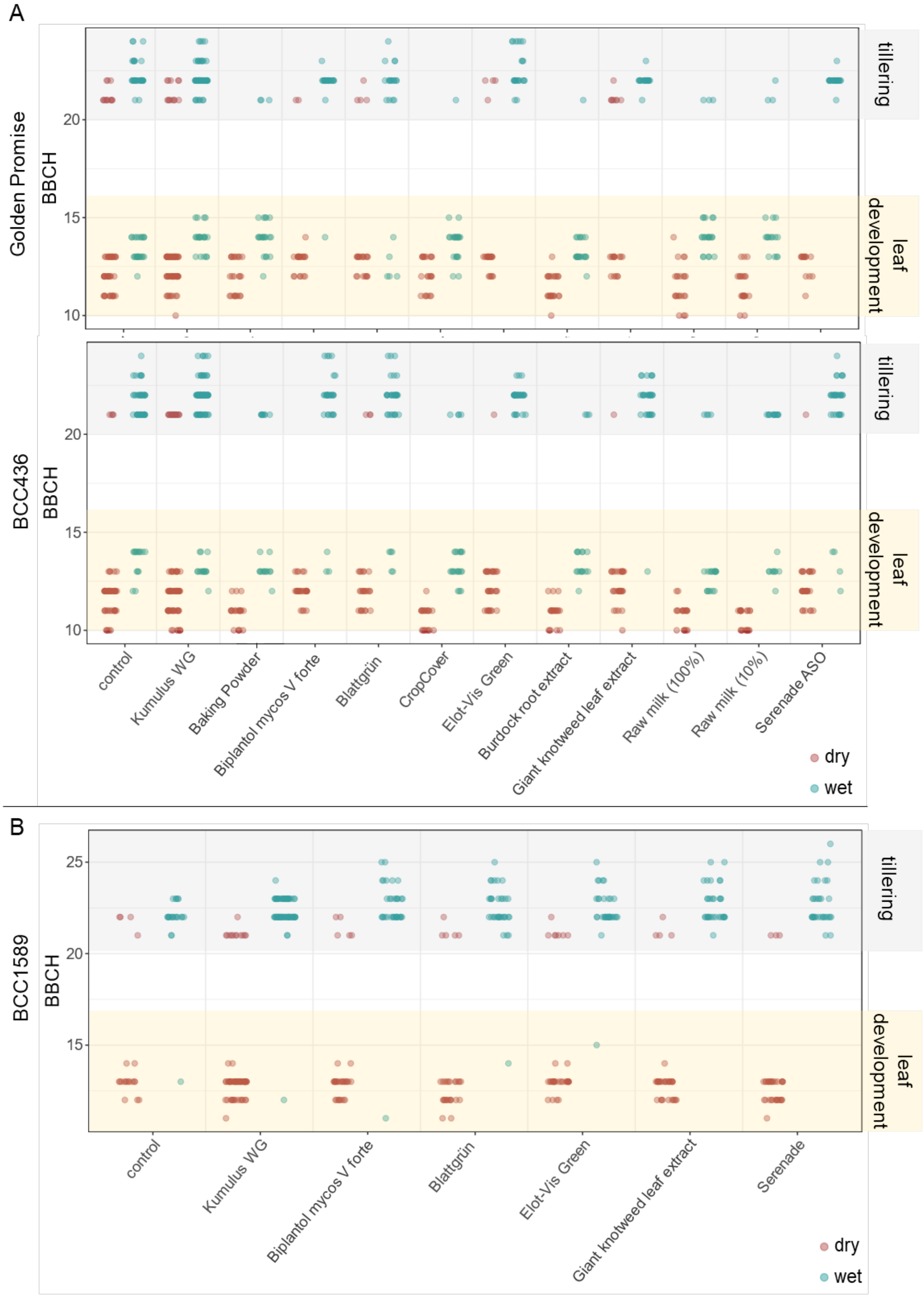
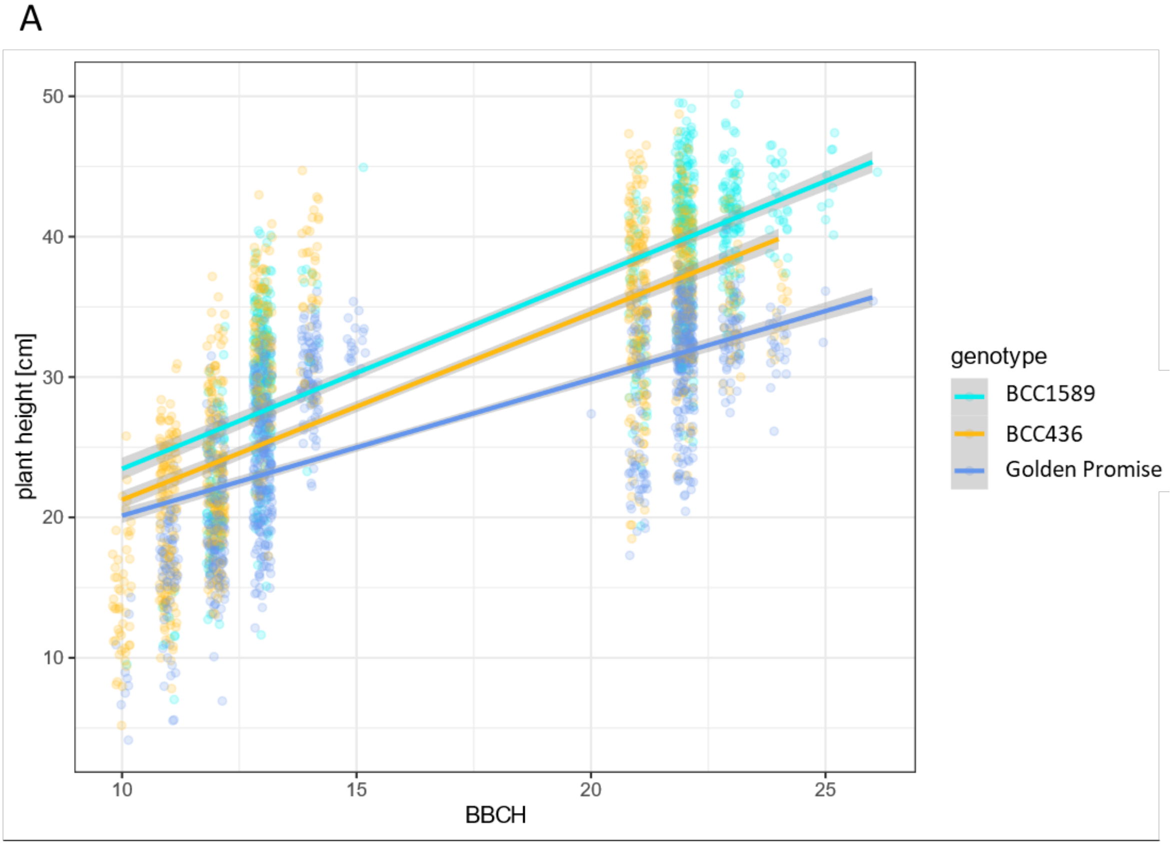


Figure 22. Developmental stages of biological or Kumulus WG treated and with *Blumeria graminis* f.sp. *hordei* race A6 inoculated plants of three barley genotypes under dry and well-watered conditions, 28 days after sowing. Wet/dry: well-watered or dry cultivation conditions; sample size per biological, water management and genotype: 14-36 plants; Kumulus WG and control (water treated) with sample size of 20-92 (detailed sample size is equal to plant height sample size 28 days after sowing (chapter S 7, S Table 11); A. Developmental stages of genotype Golden Promise and BCC436. B: Developmental stages of genotype BCC1589.

3.11 Above-ground dry biomass, plant height and BBCH correlate 28 days after sowing

It was shown that in all three genotypes a higher developmental stage correlated positively with plant height and above-ground dry biomass over all treatments, biological applications and water regimes (p<0.001) (Figure 23). The comparison between the genotypes revealed that GP was smaller at the same BBCH than the other genotypes, while BCC1589 was the tallest genotype (Figure 23 A). The correlations were with values between 0.87 and 0.93 very strong for above-ground dry biomass and BBCH as well as for plant height and BBCH of BCC1589, and strong for the plant height and BCCH of GP and BCC436 with values of 0.78 (Figure 23 B) (Papageorgiou 2022).



B

Golden Promise			BCC436			BCC1589		
dry weight	0.87	0.91	dry weight	0.88	0.93	dry weight	0.88	0.90
***	height	0.78	***	height	0.78	***	height	0.82
***	***	BBCH	***	***	BBCH	***	***	BBCH

Figure 23. Relationship between above-ground dry biomass, plant height and stage of development in *Blumeria graminis* f.sp. *hordei* race A6 inoculated barley genotypes. A: Plotted are the plant height and the BBCH of barley genotype BCC1589, BCC436 and Golden Promise 28 days after sowing of all treatments. Given is the linear regression line and the respective standard error in grey for each genotype (detailed sample size is equal to plant height sample size 28 days after sowing; chapter S 7, S Table 11); B: Spearman rank correlation coefficient and its significance (***: p<0.001) of above-ground dry biomass, plant height and developmental stage (BCCH) for genotype Golden Promise, BCC436 and BCC1589.

3.12 Drought stress influences root formation more than biological application

At the end of the experiment on the combined occurrence of drought stress and Bgh A6 in biological treated plants, the root development was recorded photographically by inverting the pots of GP and BCC1589 (run one) to obtain information on the root growth influencing properties of the products (Figure 24).

It was shown that many roots were formed in the pots of the well-watered regime. In contrast, less root structure was formed under dry conditions. When dry pots were turned upside down, some of the soil came out loose. The comparison of roots from biological treated pots showed no clear differences within each water regime. However, in one of the three dry GP control pots, more root structure was observed than in the other two pots and also more than in the other dry samples. Similar observations were made in the case of BCC1589 treated with Blattgrün.

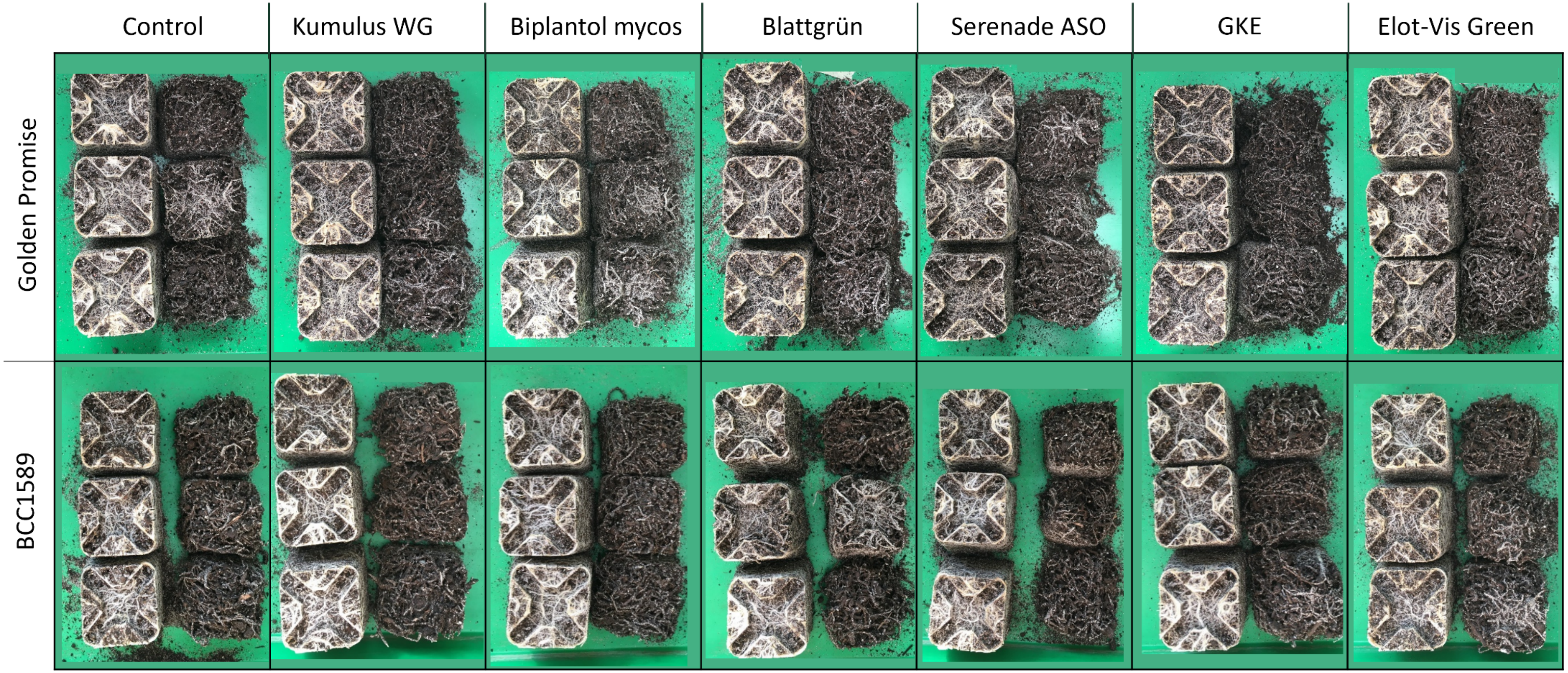


Figure 24. Root development of barley genotypes Golden Promise and BCC1589 28 days after sowing with and without drought and the use of biologicals, or Kumulus WG on *Blumeria graminis* f.sp. *hordei* race A6 inoculated plants. After harvest of run one, the pots of genotype Golden Promise and BCC1589 were inverted, and picture of root structure were taken. In each field, the well-watered pots are shown on the left and the pots of the drought regime on the right. Control: water treated; The three replicates of one run are shown; Biplantol mycos: Biplantol mycos V forte; GKE: giant knotweed leaf extract.

3.13 Several factors influence the variation of chlorophyll content and nitrogen balance index

Over the course of the experiment with simultaneous drought stress and Bgh A6 on biological treated plants, chlorophyll content and nitrogen balance index (NBI) were measured weakly via Dualex. The mean per biological and water regime of each time point was calculated for both parameters and plotted over time to get insights into the development of these physiological parameters (Figure 25, Figure 26).

The courses of the mean chlorophyll content of the three tested genotypes were similar both, within the well-watered and the dry group (Figure 25), except for BCC436 under well-watered conditions. In the well-watered group of GP and BCC1589, the mean chlorophyll content of the plants after biological treatments hardly changed between seven and 14 das. By 21 das, however, the mean chlorophyll content increased and dropped back to about the baseline level by 28 das for most biologicals. Mean chlorophyll content development under dry conditions for GP, BCC436 and BCC1589 was dominated by an increase between seven and 14 das and a subsequent decrease to 21 das and a renewed increase 28 das, but not above the level of 14 das. Well-watered BCC436 treated with CropCover, raw milk (100%), baking powder or burdock root extract showed almost identical mean values between seven and 21 das followed by an increase towards the last measurement (28 das), which was also the case for BCC436 of the dry regime for raw milk (10%).

Regarding the course of NBI of well-watered GP and BCC1589 plants, both were characterised by a decline in NBI during the trial (Figure 26). The NBI drop was shown from seven das for BCC1589 or from 14 das for GP. In contrast, the averaged NBI of Kumulus WG treated plants developed differently in all three genotypes, fluctuating slightly up to 21 das and dropping at the last measurement time point. The development of the average NBI per biological under dry conditions was like that of the respective genotype under well-watered conditions. However, was noticeable that seven das in dry cultivated BCC1589 the mean NBI was at a comparable level as 28 das, whereas under well-watered conditions a significant reduction in NBI was observed over the course of the experiment. For GP, the differences between the mean values of the biologicals were greater than for BCC436 at most measuring points. For BCC1589, the differences between the averaged NBI per biological were smaller than those of GP at most time points and similar or slightly larger than those of BCC436.

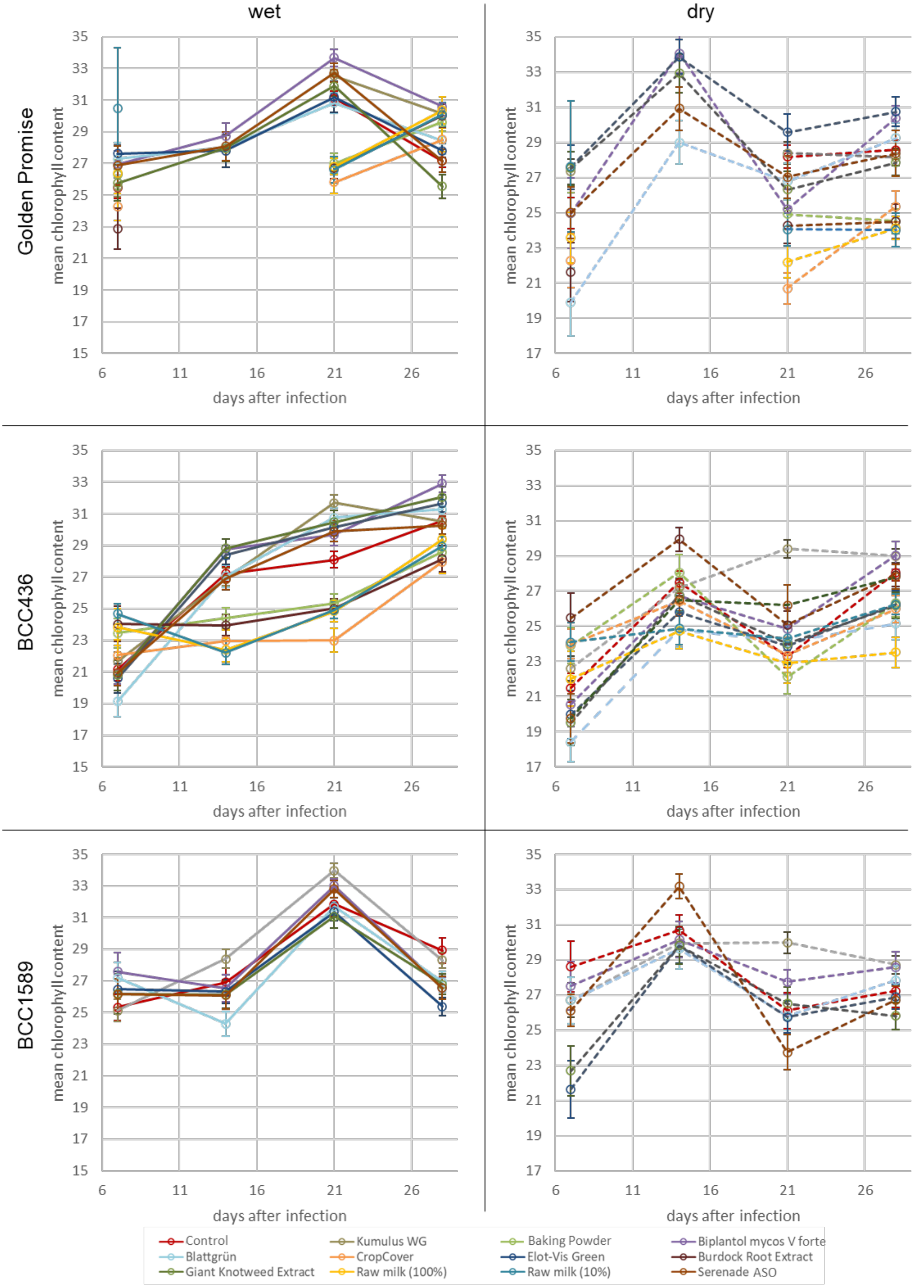


Figure 25. Development of chlorophyll content over four weeks under well-watered and dry conditions of three barley genotypes treated with different biologicals or Kumulus WG and inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Shown are mean values and standard errors per genotype, water regime, treatment and time point. Control: water treated; wet/dry: well-watered or dry cultivation conditions; n=12-108 for biologicals, 21-277 for control and Kumulus WG, details on sample size chapter S 8, S Table 12.

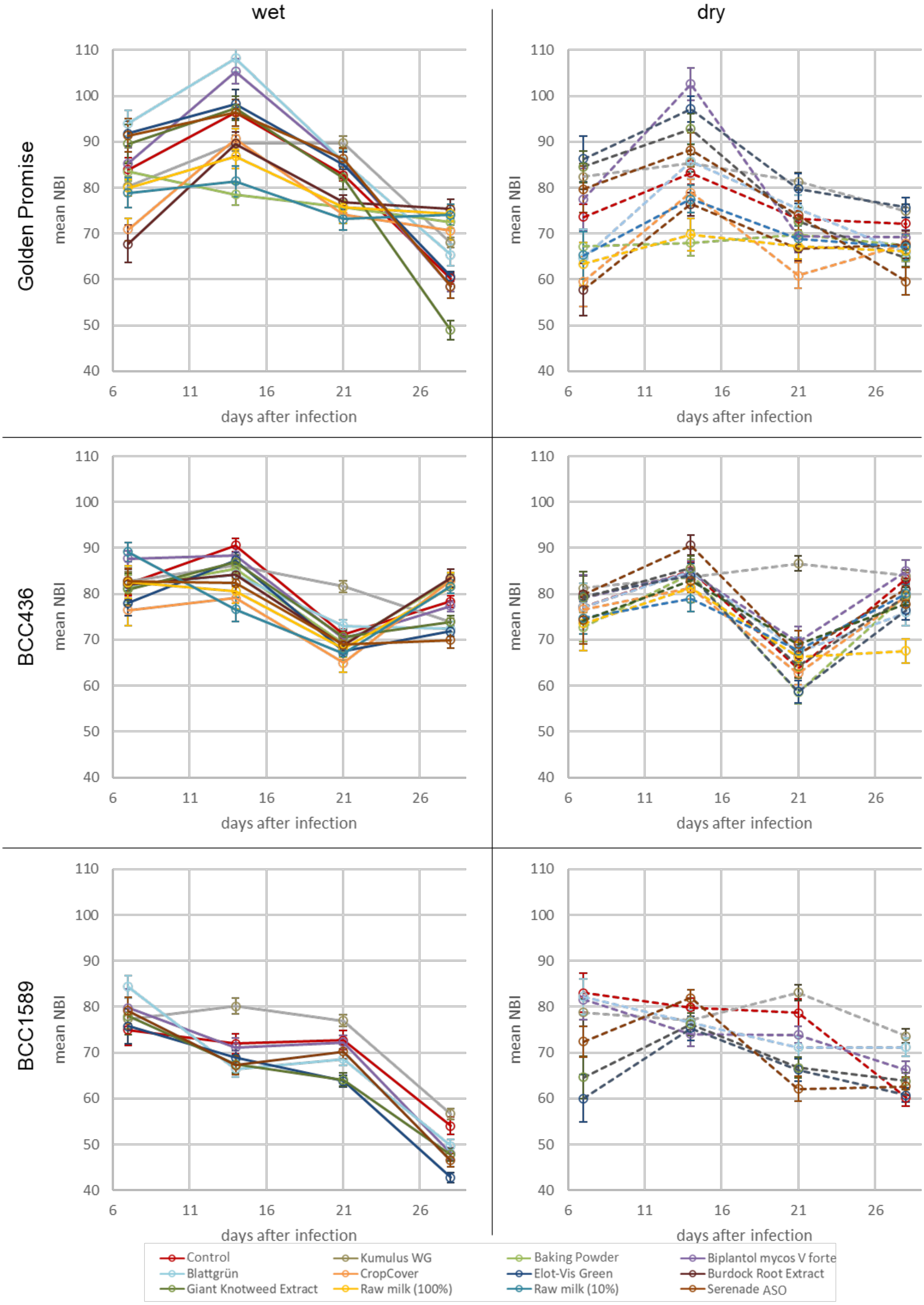


Figure 26. Development of nitrogen balance index (NBI) over four weeks under well-watered and dry conditions for three barley genotypes treated with different biologicals or Kumulus WG and inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Shown are mean values and standard errors per genotype, water regime, treatment and time point. Control: water treated; wet/dry: well-watered or dry cultivation conditions; n=12-108 for biologicals, 21-277 for control and Kumulus WG, details on sample size chapter S 8, S Table 12.

Additionally, to the development of chlorophyll content and NBI, a statistical endpoint analysis was done for the data 28 das. It was found that the chlorophyll content and NBI varied depending on biological application, drought stress and genotype (Figure 27). The chlorophyll content of control plants varied significantly (p=0.009) between the two water regimes in genotype BCC436, with well-watered plants having a higher chlorophyll content then drought stressed plants (chapter S 8, S Figure 7). Similar observations were made for BCC1589, although not significant. Control plants of GP from the dry regime had a higher chlorophyll content than those in the well-watered group (chapter S 8, S Figure 7).

Of the 56 genotype-water-regime-biological cases analysed, there was in three cases a significant increase in chlorophyll content after p-i application of a biological compared to water treatment, and a significant reduction in nine cases (Figure 27 A). Although a significant effect was only found in a few cases, drought stress and biological application clearly influenced the chlorophyll content. Reductions or increases in average chlorophyll content per genotype, water status and biological treatment of over 10% compared to the water-sprayed control were observed thirteen times. Only in five cases the influence (increase or decrease) of the biologicals on the chlorophyll content was less than 1%.

A significant increase in chlorophyll content was achieved in GP under well-watered conditions by the p-i application of Kumulus WG (p=0.005) and Biplantol mycos V forte (p=0.044). No significant increase in chlorophyll content was found in the GP dry group. Significant reductions were observed after p-i application of baking powder (p=0.009), CropCover (p=0.046), burdock root extract (p=0.009) and raw milk (100% and 10%: p=0.004) under dry conditions. Blattgrün and raw milk (100%) induced a chlorophyll reduction in plants of the dry regime of genotype BCC436 (p=0.034 and p=0.015 respectively), as did CropCover in the well-watered regime (p=0.037). Whereas Biplantol mycos V forte significantly (p=0.036) augmented the chlorophyll content in well-watered plants. The only significant change in chlorophyll content of BCC1589 was a reduction (p=0.005) by Elot-Vis Green, in the well-watered regime.

The NBI of drought stressed GP control plants was significantly (p=0.004) higher than that of well-watered GP control plants. The same ratio was observed for NBI values of BCC436 and BCC1589 genotypes, but the differences were not significant (chapter S 8, S Figure 8).

A significant augmentation of NBI was observed on well-watered GP after p-i application of baking powder (p=0.015)), burdock root extract (p=0.005), CropCover (p=0.037) and raw milk (100%, 10%, p=0.007 and p=0.013 respectively), as well as on dry cultivated plants of BCC1589 treated with Kumulus WG (p=0.016) (Figure 27 B). In four cases, a significant reduction of NBI was found in both water regimes, involving Blattgrün, Elot-Vis Green and Serenade ASO on BCC436 as well as giant knotweed leaf extract on GP. Besides, Serenade ASO significantly (p=0.022) reduced NBI on dry cultivated GP and raw milk (100%) treated BCC436 from the same water regime (p<0.001), while a significant reduction under well-watered conditions was found for Kumulus WG on BCC436 (p=0.048), and Elot-Vis Green on BCC1589 (p=0.033).

Drought stress and biological application clearly influenced the NBI. Reductions or increases in average NBI per genotype, water status and biological treatment of over 20% compared to the water-sprayed control were observed eight times. Only in four cases the influence (increase or decrease) of the biologicals on NBI was less than 2%.

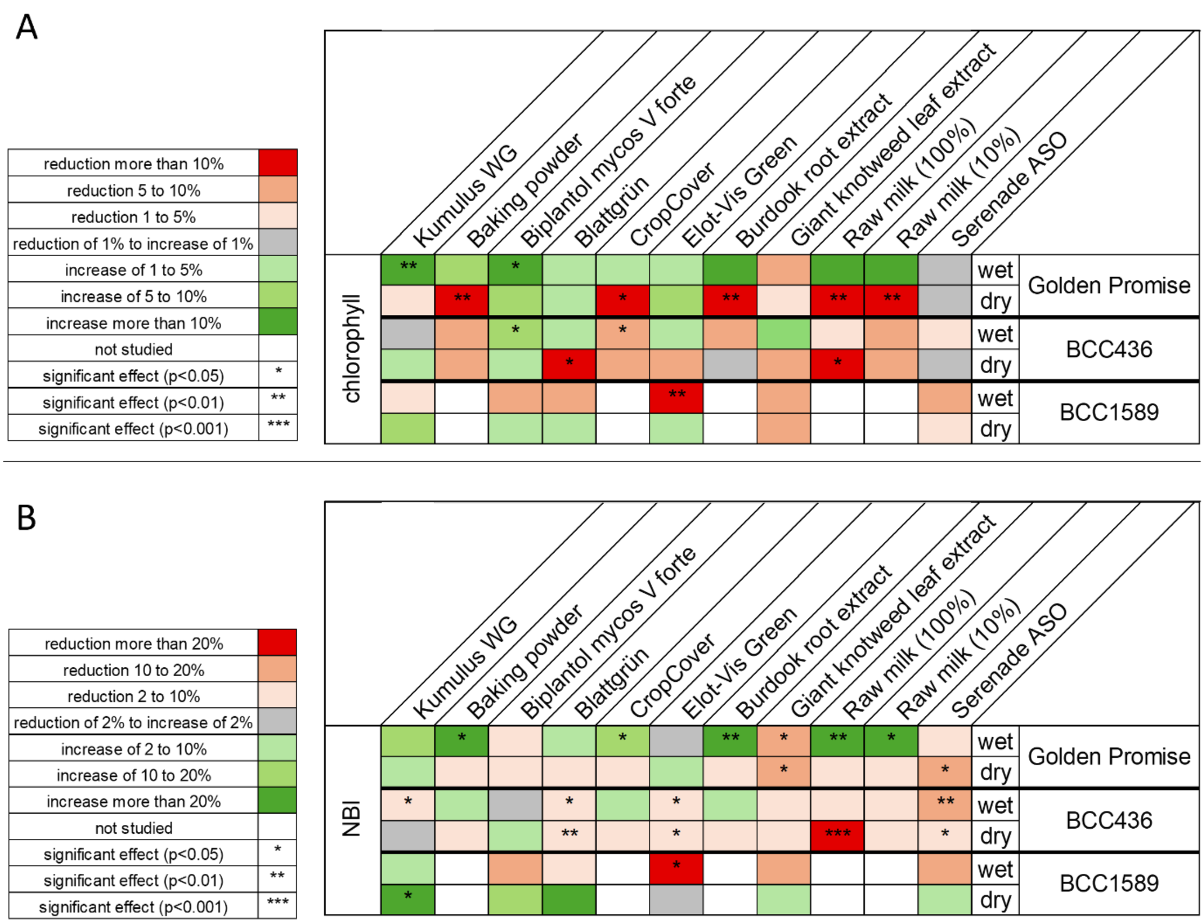


Figure 27. Influence of biologicals, Kumulus WG and dry cultivation conditions on chlorophyll content and nitrogen balance index of three barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei* race A6 28 days after sowing. Wet/dry: well-watered or dry cultivation conditions; information on sample size chapter S 8, S Table 12; A: Heatmap of treatment effects on chlorophyll content. colour code based on the percentage deviation from the respective mean of control with all shades of red describing a reduction and all shades of green an increase, additionally indicated are significant differences. B: Heatmap of treatment effects on nitrogen balance index (NBI). colour code based on the percentage deviation from the respective mean of control of the NBI content with all shades of red describing a reduction and all shades of green an increase, additionally indicated are significant differences.

3.14 Summarised responsiveness of the genotypes and overview of supportive biologicals

To get an overview of the responsiveness of the genotypes to the biologicals, experiments were summarised (Table 8, Table 9, Figure 28, Figure 29). Negative effects were distinguished from supportive ones. Supportive effects are favourable trends on a parameter, such as the significant reduction of infection symptoms, the significant increase in NBI or a significantly increased above-ground dry biomass – negative effects are the contrasting (at least $p<0.05$).

For the four genotypes of the 'biological trial' the study found more often supportive effects for Morex (17) than for GP (14), JK1_HOR7985 (eleven) and BCC436 (eight) (Table 8). However, only one negative effect was found for BCC436, seven for Morex, eight for GP and nine for JK1_HOR7985. In this trial, biomass was more often significantly influenced (positive and negative) by biological treatment than infection severity.

Table 8. Number of significant supportive and negative effects of biologicals on two parameters of the 'biological trial'. The number indicates the total number of significant effects per genotype and parameter (at least $p<0.05$); supportive: infection reduction or biomass increase (negative: vice versa); dai: days after inoculation; biomass: above-ground dry biomass.

		GP	BCC436	Morex	JK1_HOR7985	sum (supportive)	GP	BCC436	Morex	JK1_HOR7985	sum (negative)
	time	supportive					negative				
infection	6 dai	5	5	8	3	21	2	1	1	3	7
biomass	7 dai	9	3	9	8	29	6	0	6	6	18
sum		14	8	17	11	50	8	1	7	9	25

The comparison of the results of both Bgh A6 susceptible genotypes of the 'combined trial' (Table 9) showed that GP was positively affected more often (32 times) than BCC436 (28 times) and at the same time less often negatively (GP: 19 times; BCC436: 23 times). This also applies to the results under well-watered conditions: positive effects were observed more frequently (23 times) and at the same time less frequently negative effects (six) were found with GP than with BCC436 (positive: 16; negative: eleven). The results of the dry water regime showed a different picture: GP was affected positively less often (nine times) than BCC436 (twelve times) and negatively more often than BCC436 (thirteen and twelve respectively). It was further analysed on which of the investigated parameters (infection symptoms, above-ground dry biomass, height, chlorophyll content, NBI) the treatments most frequently had a significant influence. While the infection intensity was positively influenced 20 and 22 times respectively after preventive and p-i treatment and thus most frequently, most negative effects were observed on the above-ground dry biomass (14 times) and on the NBI (ten times). These two parameters, as well as chlorophyll content and plant height, were negatively influenced more often than positively.

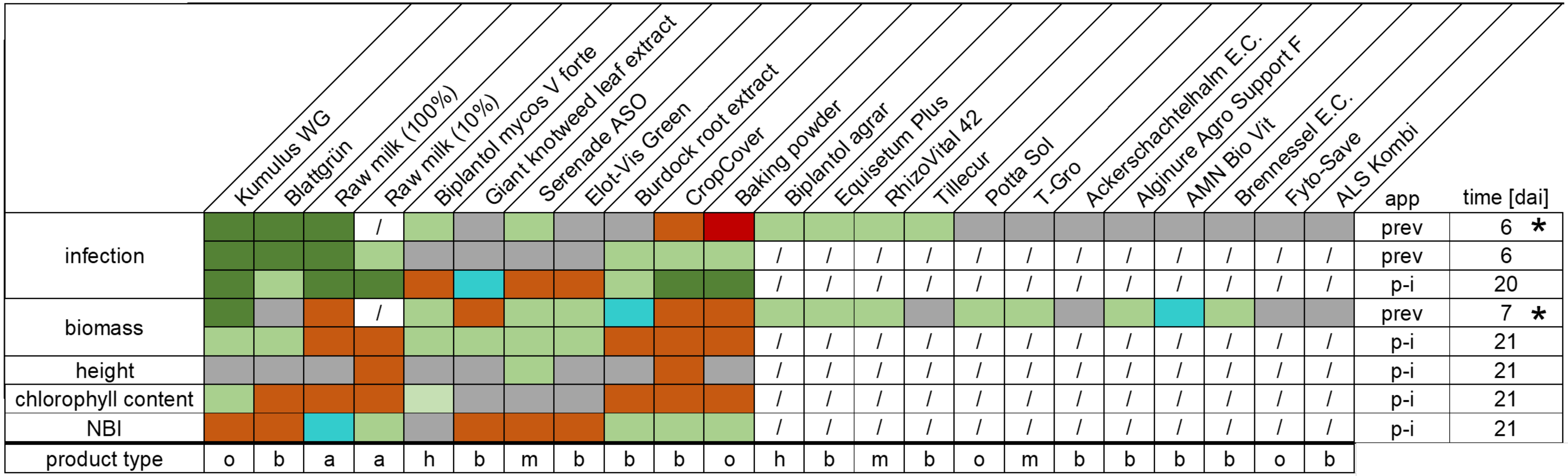
Table 9. Number of significant supportive and negative effects of biologicals on parameters of two barley genotypes also under the influence of drought. The number indicates the total number of significant effects (at least $p<0.05$); supportive: infection reduction or biomass, chlorophyll content or nitrogen balance index increase (negative: vice versa); treat: kind of application with 'prev' meaning preventive application and 'p-I', preventive plus post-inoculation application; dai: days after inoculation; biomass: above-ground dry biomass; NBI: nitrogen balance index.

			GP	GP	sum (GP)	BCC436	BCC436	sum (BCC436)	GP	GP	sum (GP)	BCC436	BCC436	sum (BCC436)
			supportive						negative					
	treat	time	wet	dry		wet	dry		wet	dry		wet	dry	
infection	prev	6 dai	6	2	8	6	6	12	0	0	0	0	0	0
	p-i	20 dai	7	6	13	4	5	9	0	2	2	2	4	6
biomass	p-i	21 dai	4	0	4	5	0	5	5	4	9	5	0	5
height	p-i	21 dai	0	0	0	0	1	1	0	0	0	0	2	2
chlorophyll content	p-i	21 dai	1	0	1	1	0	1	0	5	5	1	2	3
NBI	p-i	21 dai	5	1	6	0	0	0	1	2	3	3	4	7
sum			23	9	32	16	12	28	6	13	19	11	12	23

In addition, the question arose which products (biologicals and Kumulus WG) had the greatest influence and most supportive effects in relation to the analysed parameters (infection symptoms, above-ground dry biomass, plant height, chlorophyll content, NBI). To answer this question, only the results of GP and BCC436 were considered, as unlike the genotypes Morex and JK1_HOR7985, they were also tested under simultaneous drought stress. Thus, the effect of the products on Bgh infection could be described on the basis of their Bgh A6 susceptibility (unlike BCC1589, a resistant genotype) (Figure 28).

Blattgrün and raw milk (100%) performed best with a genotype-independent infection reduction in the 'biological trial' and a genotype- and water management-independent infection reduction after a preventive application in the 'combined experiment'. Additionally, they reduced the infection after p-i treatment depending on genotype and/or water status (Blattgrün), or independent of genotype and water status (raw milk 100%). The other biologicals only brought about significant improvements in a maximum of one experiment and often had significant negative effects on several parameters. Raw milk (100%) negatively influeced the biomass in both experiments and led in a reduced chlorophyll content, both depending on genotype and/or water status. Blattgrün also had negative effects on chlorophyll content and NBI. During the early leaf developmental phase (seven dai), Blattgrün had no significant effect on above-ground dry biomass, which changed to a significant biomass increase at the transition between leaf development and tillering phase (21 dai) depending on genotype and water status. Similarly, giant knotweed extract on GP led to a significant biomass reduction seven dai, but to a significant increase 21 dai. The use of Biplantol mycos V forte did not lead to a significant genotype- or genotype and water management-independent improvement in any of the parameters or experiments. However, the product had no more than one significant negative effect, which was not the case with any of the other ten selected biologicals in the 'combined experiment' – not even in the case of Blattgrün or raw milk (100%), as these products negatively influenced more than one parameter.

Kumulus WG led to a genotype-independent efficiency against Bgh A6 in all trials and was also effective under dry conditions. Its influence on the above-ground dry biomass was at seedling stage (seven dai) genotype-independently positive, i.e. biomass-promoting, which was 21 dai still the case for well-watered GP and BCC436, meaning water regime-dependent. The biomass-promoting effect under dry condition was also positive although not significant. The chlorophyll content was genotype- and/or water status dependently positively influenced. Sole, the NBI was genotype- and/or water status-dependent negatively influenced by Kumulus WG.



	Kumulus WG	Blattgrün	Raw milk (100%)	Raw milk (10%)	Biplantol mycos V forte	Giant knotweed leaf extract	Serenade ASO	Elot-Vis Green	Burdock root extract	CropCover	Baking powder	Biplantol agrar	Equisetum Plus	RhizoVital 42	Tillecur	Potta Sol	T-Gro	Ackerschachtelhalm E.C.	Alginure Agro Support F	AMN Bio Vit	Brennessel E.C.	Fyto-Save	ALS Kombi	app	time [dai]
infection	🟩	🟩	🟩	/	🟢	▒	🟢	▒	▒	🟧	🟥	🟢	🟢	🟢	🟢	▒	▒	▒	▒	▒	▒	▒	▒	prev	6 *
	🟩	🟩	🟩	🟢	▒	▒	▒	▒	🟢	🟢	🟢	/	/	/	/	/	/	/	/	/	/	/	/	prev	6
	🟩	🟢	🟩	🟩	🟧	🟦	🟧	🟧	🟢	🟩	🟩	/	/	/	/	/	/	/	/	/	/	/	/	p-i	20
biomass	🟩	▒	🟧	/	🟢	🟧	🟢	🟢	🟦	🟧	🟧	🟢	🟢	🟢	▒	🟢	🟢	▒	🟢	🟦	🟢	▒	▒	prev	7 *
	🟢	🟢	🟧	🟧	🟢	🟢	🟢	🟢	🟧	🟧	🟧	/	/	/	/	/	/	/	/	/	/	/	/	p-i	21
height	▒	▒	▒	🟧	▒	▒	🟢	▒	▒	🟧	▒	/	/	/	/	/	/	/	/	/	/	/	/	p-i	21
chlorophyll content	🟢	🟧	🟧	🟧	◌	▒	▒	▒	🟧	🟧	🟧	/	/	/	/	/	/	/	/	/	/	/	/	p-i	21
NBI	🟧	🟧	🟦	🟢	▒	🟧	🟧	🟧	🟢	🟢	🟢	/	/	/	/	/	/	/	/	/	/	/	/	p-i	21
product type	o	b	a	a	h	b	m	b	b	b	o	h	b	m	b	o	m	b	b	b	b	o	b		

Figure 28. Heatmap of significant effects of biologicals and Kumulus WG on barley genotype Golden Promise and BCC436. App = application; prev = preventive; p-i= additional post-inoculation spraying to the preventive one; dai= days after inoculation; *: part of the 'biological trial', others part of the 'combined experiment'; the colouring of the cells indicates significant (at least p<0.05) effects; dark green: positive effect for both genotypes and in case of p-i also for both water regimes; light green: positive effect but depending on genotype, water status or both; petrol: opposite effects on genotypes; grey: no effect on any genotype; orange: negative effect depending on genotype, irrigation or both; red: negative on both genotypes; line: not studied; product types: a=animal-based, b=botanical, h=homeopathic, m=microbial, o=other.

Of the total 110 product-, genotype- and parameter-combinations summarised (Figure 28), a genotype- and/or water status-independent positive effect was found in twelve cases. In eleven out of these twelve, the positive effects were due to a reduction in infection, and in one case due to an increase in above-ground dry biomass. A positive effect depending on genotype and/or water status was found in 35 cases. No significant effect on neither GP nor BCC436 on any of the tested parameters was found 32 times, whereas a negative effect depending on genotype and/or water status was found 26 times. Less frequent were genotype- and/or water status-independent negative effects (once) or contradictory effects depending on genotype and/or water status (four times).

At the level of product assignment to the groups botanicals, microbials, homeopathics, animal-based or others (Figure 29), it was shown that botanicals mostly (37.0%) had no significant effect on GP and BCC436. In about 25% of botanical applications, genotype and/or water status-dependent effects occurred (supportive: 27.8% and negative: 24.1%). Microbials, on the other hand, led to genotype and/or water status-dependent positive effects in more than half of their applications (58.3%), to no significant effects in a quarter and to genotype and/or water status-dependent negative effects less frequently (16.7%). Similar ratios were observed for homeopathics: 60.0% of the applications resulted in genotype and/or water status-dependent positive effects, 30.0% in the absence of significant effects and 10.0% in genotype and/or water status-dependent negative effects. Less frequently, the animal-based product led to genotype- and/or water status-independent positive effects (28.6%), whereas negative effects were more often (42.9%).

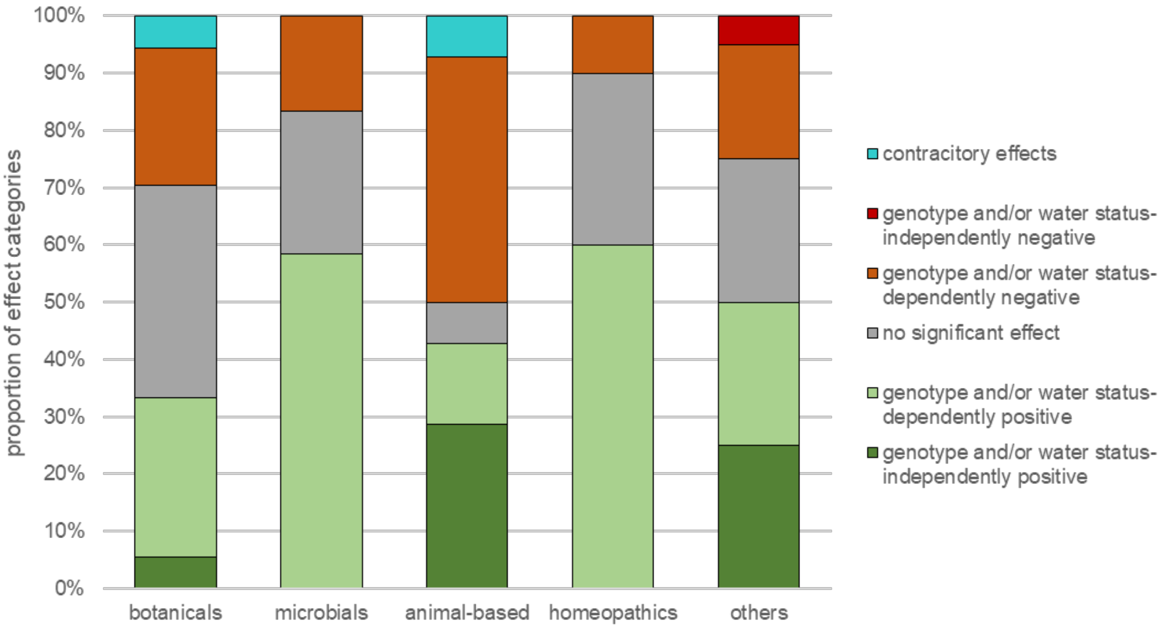


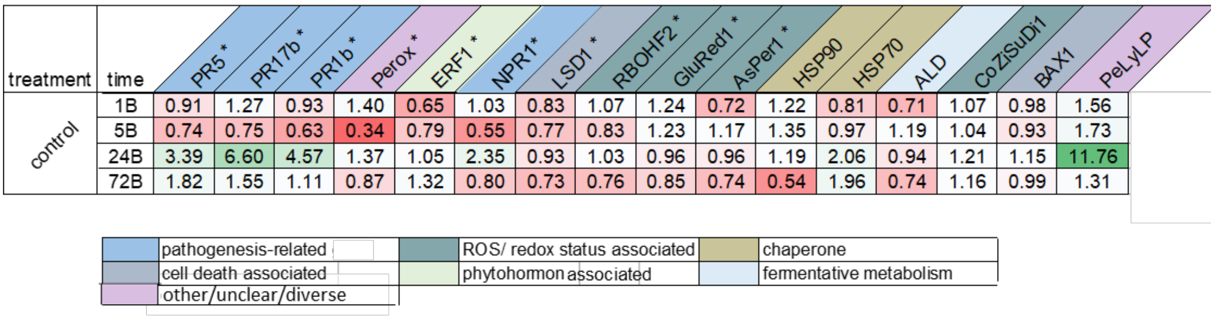
Figure 29. Bar char of the proportion of effects of biologicals and Kumulus WG on barley genotypes Golden Promise and BCC436. Values are based on the number of respective effects observed for GP and BCC436 in the 'biological trial' and the 'combined experiment'.

3.15 Effects of Bgh A6 and biologicals on gene expression

To get insights into possible gene expression changes due to biological application, to Bgh infection as well as the combined occurrence of Bgh inoculation and biological treatment, the expression of defence-related genes was studied on Bgh A6 susceptible and biological-responsive genotype GP (chapter 3.2, chapter 3.14). The experiments were carried out on a selection of genes with two replicates (chapter 3.15.1), while others were only examined once, which is why these results can be found in the supplementary data (chapter S 2; S Figure 1).

3.15.1 Selection of candidate genes for qPCR analysis

Based on a literature research, genes were selected for which a reaction was described in connection to infections or biologicals. The set comprises genes involved in different pathways (Figure 30). In a first trial the expression of 16 of these genes in response to Bgh A6 was evaluated for four time points (one, five, 24, 72 hours post infection (hpi) plants were water sprayed). With a fold-change of 11.76, the greatest increase in expression of these Bgh infected plants was 24 hpi for *pectin-lyase like protein* (*PeLyLP*), the lowest with 0.34 five hpi for *peroxidase 40* (*Perox*) (Figure 30). The infection with Bgh had an inducing effect on the expression of *pathogenesis-related protein* (*PR*) *17b* (fold-change: 6.60), *PR1b* (fold-change: 4.57), *PR5* (fold-change 3.39) 24 hpi. Based on these results, ten primers were selected, and the experiment was repeated with them. The selection was made so that genes with different functions and variable fold-change were included. The expression of the ten selected genes is described below in response to Bgh (chapter 3.15.2), biological treatment (chapter 3.15.3) and combined occurrence of Bgh and biological spraying (chapter 3.15.4).



treatment	time	PR5 *	PR17b *	PR1b *	Perox *	ERF1 *	NPR1 *	LSD1 *	RBOHF2 *	GluRed1 *	AsPer1 *	HSP90	HSP70	ALD	CoZiSuDi1	BAX1	PeLyLP
control	1B	0.91	1.27	0.93	1.40	0.65	1.03	0.83	1.07	1.24	0.72	1.22	0.81	0.71	1.07	0.98	1.56
	5B	0.74	0.75	0.63	0.34	0.79	0.55	0.77	0.83	1.23	1.17	1.35	0.97	1.19	1.04	0.93	1.73
	24B	3.39	6.60	4.57	1.37	1.05	2.35	0.93	1.03	0.96	0.96	1.19	2.06	0.94	1.21	1.15	11.76
	72B	1.82	1.55	1.11	0.87	1.32	0.80	0.73	0.76	0.85	0.74	0.54	1.96	0.74	1.16	0.99	1.31

pathogenesis-related	ROS/ redox status associated	chaperone
cell death associated	phytohormon associated	fermentative metabolism
other/unclear/diverse		

Figure 30. Fold-change of gene expression over time of 16 genes in water treated plants of barley genotype Golden Promise. * Primers selected for repetition; time B = hours post infection with *Blumeria graminis* f.sp. *hordei* race A6; colour of cell represents fold-change: greenish >1 and reddish <1; the stronger the fold-change, the darker the colours of the cell. PR5, PR17b, PR1b: *pathogenesis-related protein 5*, *17b* and *1b* respectively; Perox: *peroxidase 40*; ERF1: *ethylene response factor 1*; NPR1: *non-expressor of pathogenesis-related genes 1*; LSD1: *lesion simulation disease 1*; RBHOF2: *respiratory burst oxidase homologue F2*; GluRed1: *glutathione reductase 1*; AsPer1: *ascorbate peroxidase 1*; HSP90, HSP70: *heat shock protein 90* or *70* respectively; ALD: *alcohol dehydrogenase*; CoZiSuDi1: *copper-zinc superoxide dismutase 1*; BAX1: *BAX inhibitor 1*; PeLyLP: *pectin-lyase like protein*.

3.15.2 Expression of some defence-related genes is influenced by Bgh infection

A gene expression peak of all analysed *PR* genes was found in both repetitions 24 hpi. A mean fold-change of run one and two of 4.2 (± 1.1) was found for *PR5*, of 12.6 (± 8.5) for *PR17b* and of 7.3 (± 3.9) for *PR1b*. An increased expression, albeit lower, was also found 24 hpi for *Perox* (1.8 ± 0.7), *ethylene response factor 1* (*ERF1*, 1.3 ± 0.5) and *non-expressor of pathogenesis-related genes 1* (*NPR1*, 2.0 ± 0.6). The expression of *ERF1*, *lesion simulation disease 1* (*LSD1*), *respiratory burst oxidase homologue F2* (*RBOHF2*), *glutathione reductase 1* (*GluRed1*) and *ascorbate peroxidase 1* (*AsPer1*) did not exceed a fold-change of 2.0 in any repetition but *LSD1*, *RBOHF2*, *GluRed1* and *AsPer1* were slightly down regulated (fold-change: 0.7 - 0.9) in infected compared to healthy plants 72 hpi (Figure 31).

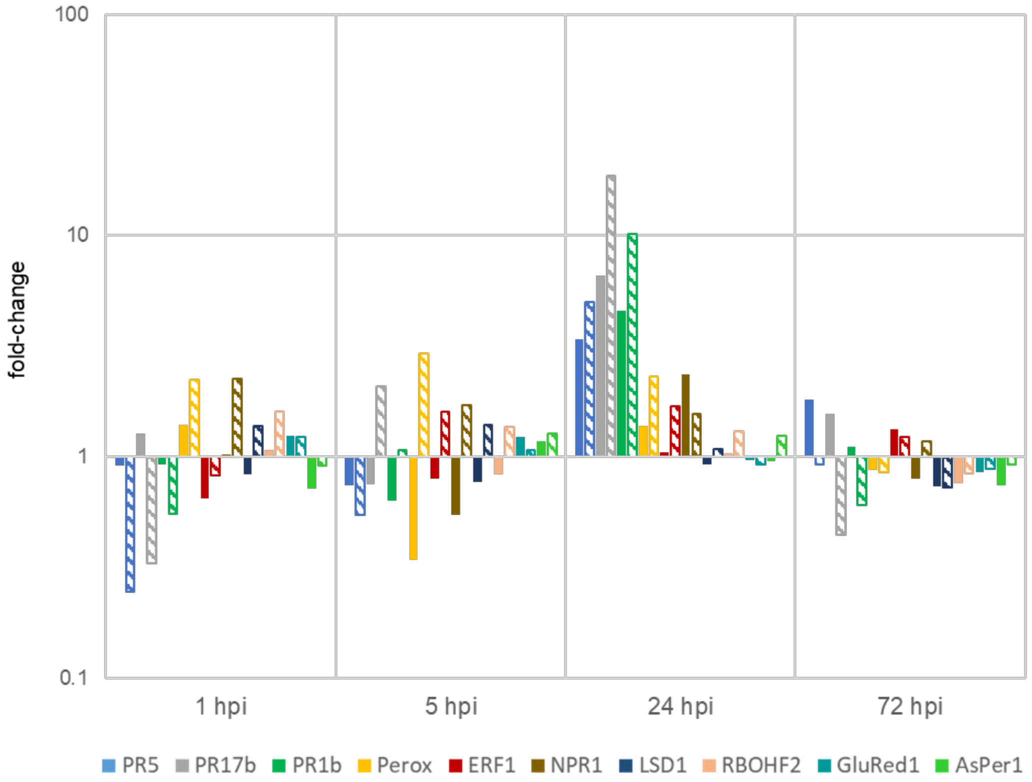


Figure 31. Fold-change of gene expression in barley genotype Golden Promise in response to *Blumeria graminis* f.sp. *hordei* race A6 inoculation over 72 hours. Fully coloured bars show the fold-change of the first run, dashed bars show the repetition. hpi: hours post inoculation; PR5, PR17b, PR1b: *pathogenesis-related protein 5*, *17b* and *1b* respectively; Perox: *peroxidase 40*; ERF1: *ethylene response factor 1*; NPR1: *non-expressor of pathogenesis-related genes 1*; LSD1: *lesion simulation disease 1*; RBHOF2: *respiratory burst oxidase homologue F2*; GluRed1: *glutathione reductase 1*; AsPer1: *ascorbate peroxidase 1*.

3.15.3 Biologicals modulate pathogen associated gene expression

The influence of three biologicals on gene expression of ten defence related genes was analysed on GP at seven post application time points (Figure 32). Overall, the increase in expression of *Perox* dominated the gene expression pattern, in some cases as early as one hour post application (hpa). The expression of *Perox* clearly increased after raw milk and Blattgrün treatment from five hpa up to and including 48 hpa (fold-change between 0.5 and 33.4, mostly in both replicates over 2.0 or 4.0). A treatment with giant knotweed leaf extract, on the other hand, led to a *Perox* peak 24 hpa only in one repetition. Depending on the treatment, the expression of *PR* genes also increased 48 hpa of Blattgrün and raw milk and in knotweed treated plants 24 hpa. While *ERF1*, *NPR1*, *LSD1*, *RBOHF2*, *GluRed1* and *AsPer1* achieved a fold-change of more than 2.0 in at least one replicate 28 hpa after knotweed treatment, no noticeable expression change induced by Blattgrün or raw milk was observed over the course of the experiment for these genes, except for *AsPer1*. Overall, the two repetitions did not present a uniform pattern.

Figure 32. Fold-change of gene expression in barley genotype Golden Promise in response to biological application and over 96 hours. The applied biologicals were Blattgrün (top), raw milk (100%) (middle) and giant knotweed leaf extract (bottom). The y-axis is capped at 100. Fully coloured bars show the fold-change of the first run, dashed bars show the repeat run; hpa: hours post application; PR5, PR17b, PR1b: *pathogenesis-related protein 5*, *17b* and *1b* respectively; Perox: *peroxidase 40*; ERF1: *ethylene response factor 1*; NPR1: *non-expressor of pathogenesis-related genes 1*; LSD1: *lesion simulation disease 1*; RBHOF2: *respiratory burst oxidase homologue F2*; GluRed1: *glutathione reductase 1*; AsPer1: *ascorbate peroxidase 1*.

3.15.4 Biological treated and Bgh infected plants upregulate PR gene expression

The influence of three biologicals on gene expression of ten defence related genes on Bgh infected GP was analysed at four time points (Figure 33). One hpi, an increase in expression of *PR5* and *Perox* was observed in plants pre-treated with Blattgrün (fold-change *PR5*: 5.3, 3.3; *Perox*: 11, 4.8), and albeit to a lesser extent in raw milk-treated plants (fold-change *PR5*: 1.7, 1.3; *Perox*: 8.9, 1.3). By giant knotweed leaf extract only *PR5* expression was stimulated in both repetitions one hpi (fold-change 3.2, 3.1). Five hpi all treatments led to an increase in gene expression of *PR1b*, *PR5* and *PR17b*, with the fold-change being highest in Blattgrün-treated plants (max: 42.4) and lowest in knotweed-treated plants (min: 1.5). Blattgrün and raw milk also induced a persistent expression of *Perox* five hpi and 24 hpi. The expression of *PR* genes decreased until 72 hpi, at which time it was lower in Blattgrün treated plants than in water treated plants (fold-change 0.6 to 0.2). The results of the two repetitions showed partly contrary expressions, especially in giant knotweed leaf extract treated plants.

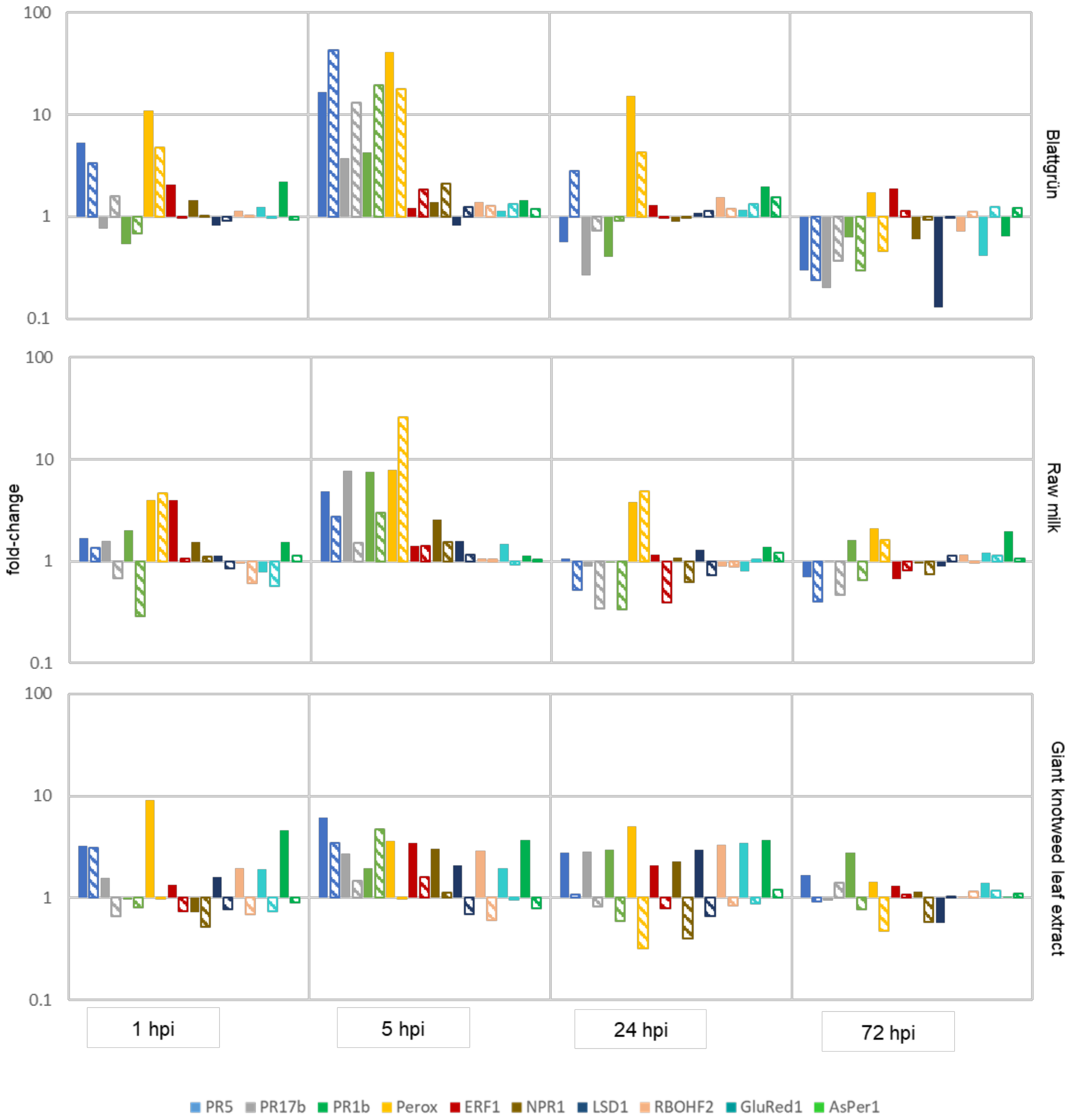


Figure 33. Fold-change of gene expression in barley genotype Golden Promise in response to biological application and inoculation with *Blumeria graminis* f.sp. *hordei* race A6 over a 72 hours-period. The applied biologicals were Blattgrün (top), raw milk (100%) (middle) and giant knotweed leaf extract (bottom). Fully coloured bars show the fold-change of the first run, dashed bars show the repetition; hpi: hours post inoculation; PR5, PR17b, PR1b: *pathogenesis-related protein 5*, *17b* and *1b* respectively; Perox: *peroxidase 40*; ERF1: *ethylene response factor 1*; NPR1: *non-expressor of pathogenesis-related genes 1*; LSD1: *lesion simulation disease 1*; RBHOF2: *respiratory burst oxidase homologue F2*; GluRed1: *glutathione reductase 1*; AsPer1: *ascorbate peroxidase 1*.

3.16 The minimum conductance of leaves to water varies between spring barley genotypes and is influenced by Bgh inoculation

The minimum conductance (g_{min}) of leaves to water was studied for nine genotypes of different Bgh A6 susceptibility. The g_{min} of non-inoculated leaves was significantly ($p<0.05$) influenced by genotype (Figure 34 A). Likewise, in inoculated barley leaves the genotype has a significant ($p<0.05$) influence on g_{min} (Figure 34 B). The values of inoculated and non-inoculated leaves both showed great variability and fluctuated in a comparable numerical range (non-inoculated: $8.5*10^{-5}$ – $2.4*10^{-4}$ m/s; inoculated: $7.9*10^{-5}$ – $2.0*10^{-4}$ m/s). However, g_{min} did neither correlate significantly ($p>0.05$) with Bgh susceptibility within the non-inoculated group (spearman rank correlation: -0.093) nor within the inoculated group (spearman rank correlation: 0.132). Nevertheless, the direct comparison of g_{min} with and without inoculation of selected genotypes showed that Bgh A6 can influence g_{min}. Inoculated leaves of BCC812 had a higher g_{min} than non-inoculated plants (Figure 35 A), although not significantly ($p=0.065$). In the case of the genotype BCC1476, inoculated leaves had a significantly ($p<0.001$) higher g_{min} than the non-inoculated leaves (Figure 35 B).

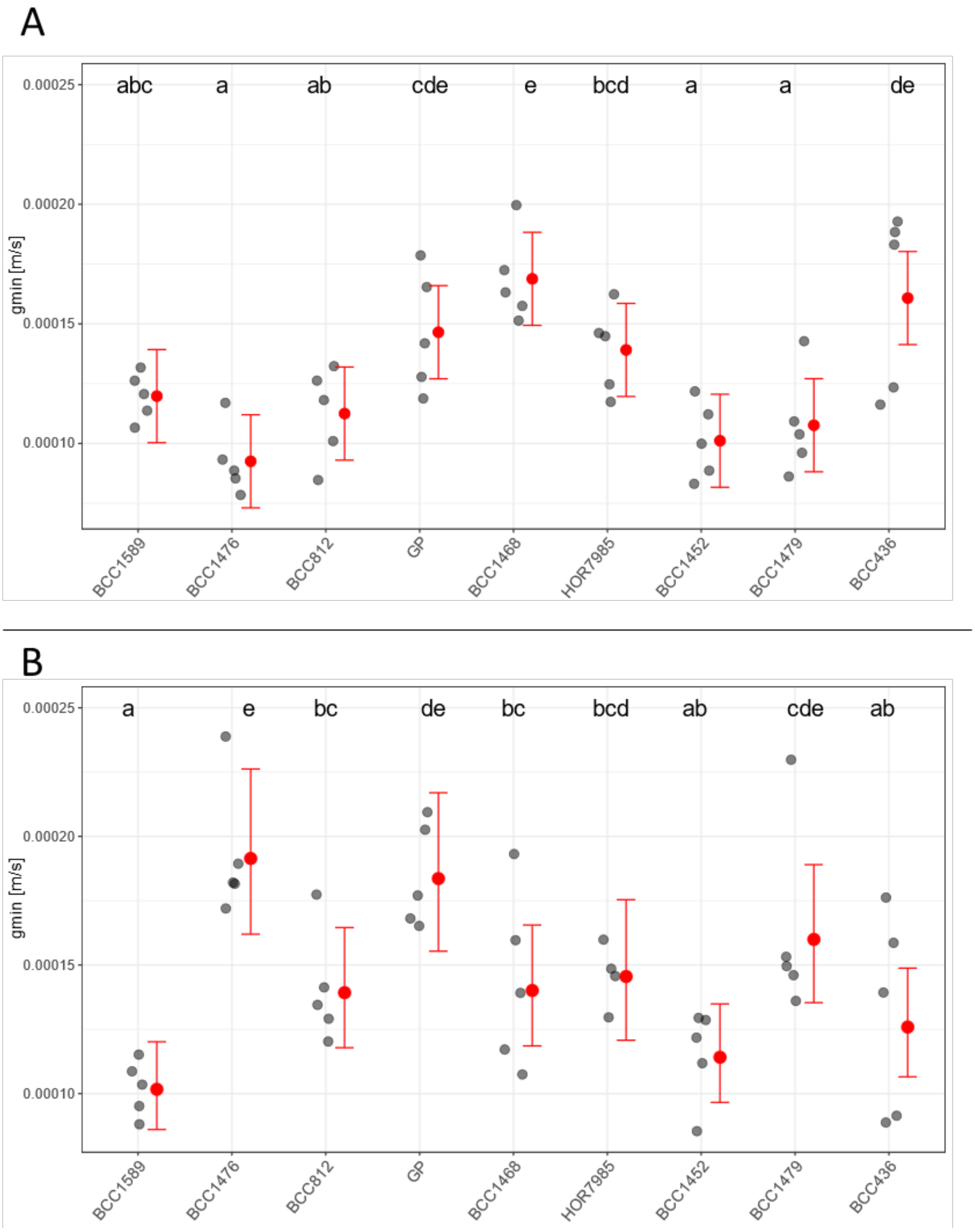


Figure 34. Minimum conductance of barley leaves to water of genotypes differing in their susceptibility towards *Blumeria graminis* f.sp. *hordei* race A6. GP: Golden Promise; HOR7985: JK_1_HOR7985; grey dots: raw data; red dots/error bars: adjusted means with 95% confidence interval, different letters indicate significant differences ($p<0.05$); n=5 (except for inoculated JK_1_HOR7985: n=4); A: non-inoculated leaves; B: inoculated with *Blumeria graminis* f.sp. *hordei* race A6.

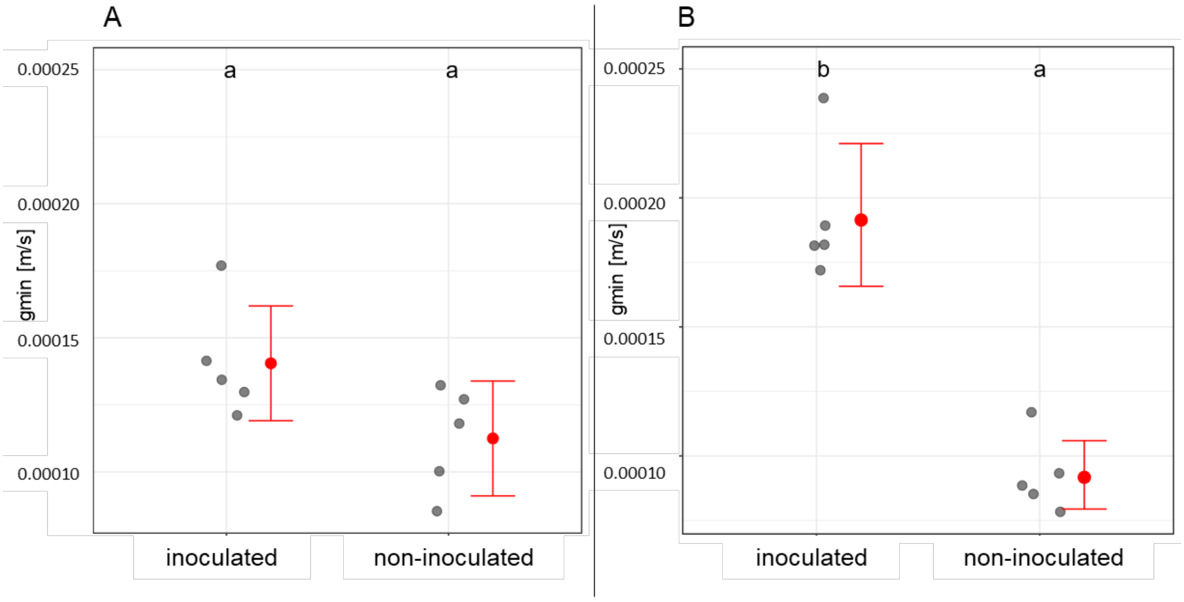


Figure 35. Influence of an inoculation with *Blumeria graminis* f.sp. *hordei* race A6 on the minimum conductance of leaves to water from two resistant barley genotypes. grey dots: raw data; red dots/error bars: adjusted means with 95% confidence interval, different letters indicate significant differences ($p<0.05$); n=5; A: BCC812; B: BCC1476;

CropCover or giant knotweed leaf extract treated plants were analysed for the g_{min} of their secondary leaf to find out whether these biologicals alter g_{min}. No significant differences ($p>0.05$) were found between biological treated and the respective water treated leaves of GP for both biologicals. Additionally, no significant differences ($p>0.05$) were found between the two application time points (Figure 36).

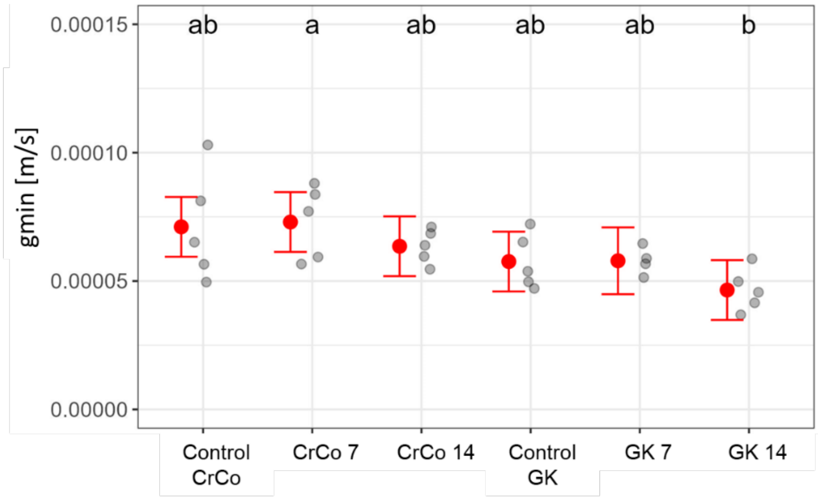


Figure 36. Influence of biological treatments on the minimum conductance of leaves to water of barley genotype Golden Promise. Control treated with water; CrCo: CropCover; GK: giant knotweed leaf extract; number after product indicates the application day as days after sowing; grey dots: raw data; red dots/error bars: adjusted means with 95% confidence interval; different letters indicate significant differences ($p<0.05$); n=5 (except for GK 7: n=4).

3.17 The cuticle composition of barley genotypes is variable

Cyclohexane and ethyl acetate soluble molecules of barley cuticle were studied to identify molecules on barley leaf surfaces that could interfere with Bgh A6 infection. Molecules whose identity could neither correctly be identified by the automated database comparison connected to the GC-MS nor by manual comparison with NIST were numbered.

The largest percentage of total extract was made up in both, cyclohexane and ethyl acetate extract, of molecules similar to hexacosanol, labelled here as hexacosanol derivatives 1 and 2 (Figure 37). Together they account for between 32.43 ± 8.33% (BCC436 in cyclohexane) and 47.61 ± 2.64% (BCC1589 in ethyl acetate) of the total extract, depending on extraction solvent and genotype (Table 10). Further identified molecules with a share of more than 3% were 1-tetracosanol and 1-octacosanol, found in both the cyclohexane and ethyl acetate extract of the four genotypes. Thereby, the proportion of 1-tetracosanol in the cyclohexane extract was lower than that in the ethyl acetate extract. Molecule 212 was only found in the cyclohexane extract, but in the four genotypes, whereas molecule 222 was only soluble in cyclohexane and was exclusively found in genotype BCC436 (Figure 37).

Table 10. Mean and standard deviation of the percentage of hexacosanol derivatives from the total extract of four barley genotypes. Given is the sum of the percentage of hexacosanol derivative 1 and 2 from the total extract and the standard deviation (SD); n=2.

	Cyclohexane		Ethyl acetate	
	Mean percentage	SD	Mean percentage	SD
BCC1479	33.92	1.86	46.43	0.82
BCC436	32.43	8.33	41.21	4.93
BCC1589	38.47	1.42	47.61	2.64
BCC192	38.06	0.12	41.95	4.97

On average, with cyclohexane as solvent, six molecules were exclusively found on either BCC1479 or BCC436 thus on one of the two Bgh A6 susceptible genotypes (Figure 38), examples are molecule 220, 221 and 222 that were only found in BCC436 extract. In the ethyl acetate extracts this was the case for 14 molecules (Figure 39). Examples are, molecule A20 and x91 that were only detected in BCC1479 extract, while x11 was only found in BCC436. In the cuticle extract of resistant genotypes, eleven molecules were exclusively found in the ethyl acetate extract of one resistant genotype (Alkane 1 or x59 on BCC1589, x14 on BCC192) and in the cyclohexane extract, nine molecules were exclusively found in resistant genotypes (A18 on BCC192), one of which (x107) was also extracted via ethyl acetate.

The extraction with cyclohexane additionally shows an unidentified molecule (x75) that was exclusively detected in both susceptible genotypes (chapter S 9, S Figure 9 A), while one molecule was detected exclusively in both resistant genotypes. Its mass spectrum resembled that of heneicosane, but it could not be identified, which is why it was named heneicosane like molecule (chapter S 9, S Figure 9 B). The heneicosane like molecule was also detected in the ethyl acetate extract, but only in one of the resistant genotypes (BCC1589). The x75 molecule, on the other hand, was not extracted with ethyl acetate.

A large group of molecules was detected in the extracts of all four tested genotypes (resistant and susceptible) (Figure 40). Eighteen of these molecules were only found in ethyl acetate including three saccharides, 14 could only be extracted with cyclohexane and a further 14 molecules were extractable with both solvents, including 1-octacosanol, 1-tetracosanol, hexacosanoic acid, lignoceric acid, octadecanoic acid, palmitic acid and docosanoic acid. The overall share of these molecules differed only very little between resistant and susceptible genotypes. The biggest difference in the overall share of a molecule was 0.99% for hexacosanoic acid with resistant genotypes having a higher percentage of this molecule (chapter S 9, S Table 13).

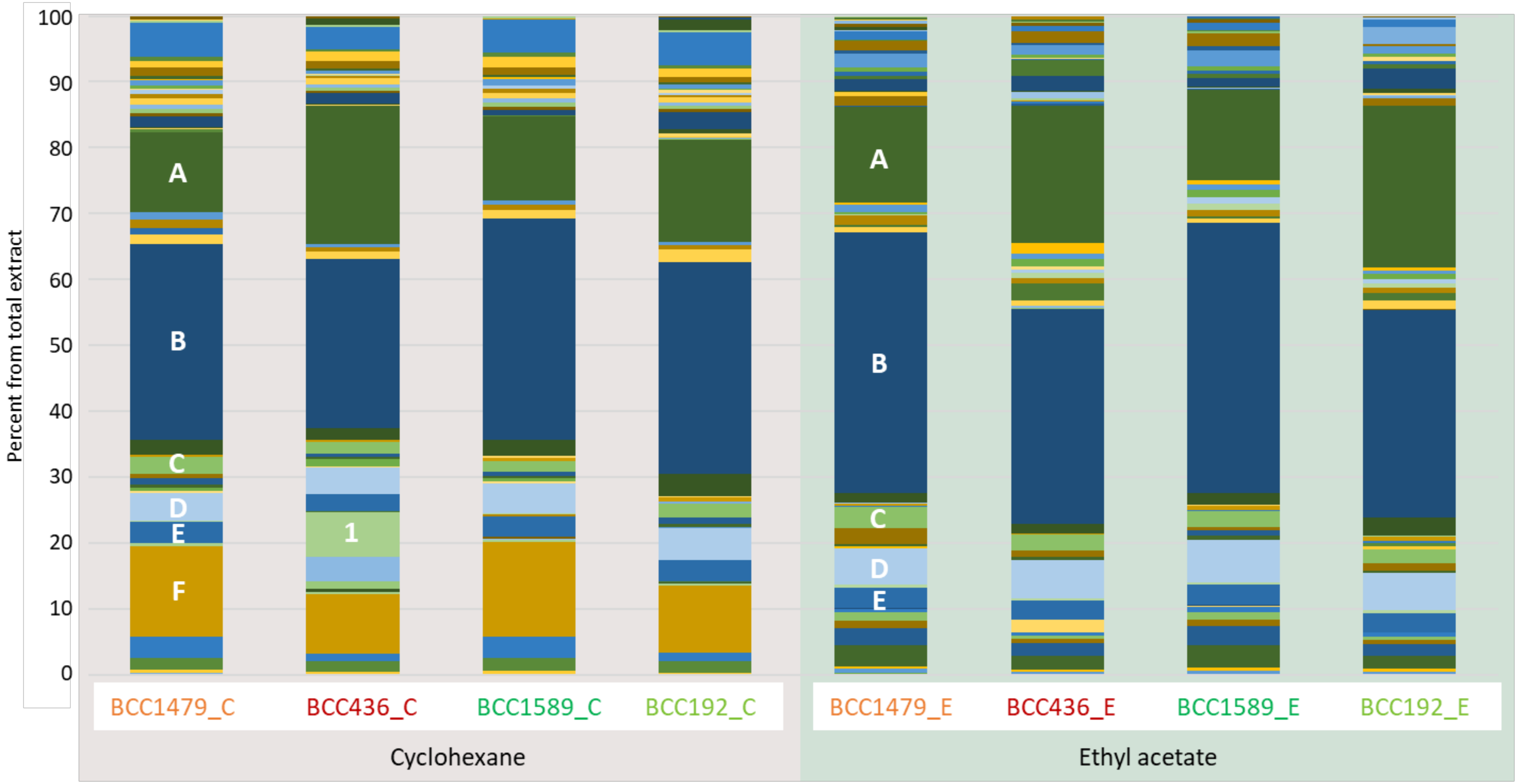


Figure 37. Proportion of cuticle molecules in the cyclohexane and ethyl acetate extract of four barley genotypes. Given is the mean share of the molecules from two repetitions. A: hexacosanol derivative 1; B: hexacosanol derivative 2; C: alkane_2; D: 1-tetracosanol; E: 1-octacosanol; F: molecule 212; 1: molecule 222; orange/red letters: susceptible genotypes towards *Blumeria graminis* f.sp. *hordei* race A6 (Bgh A6); green letters: resistant genotypes towards Bgh A6.

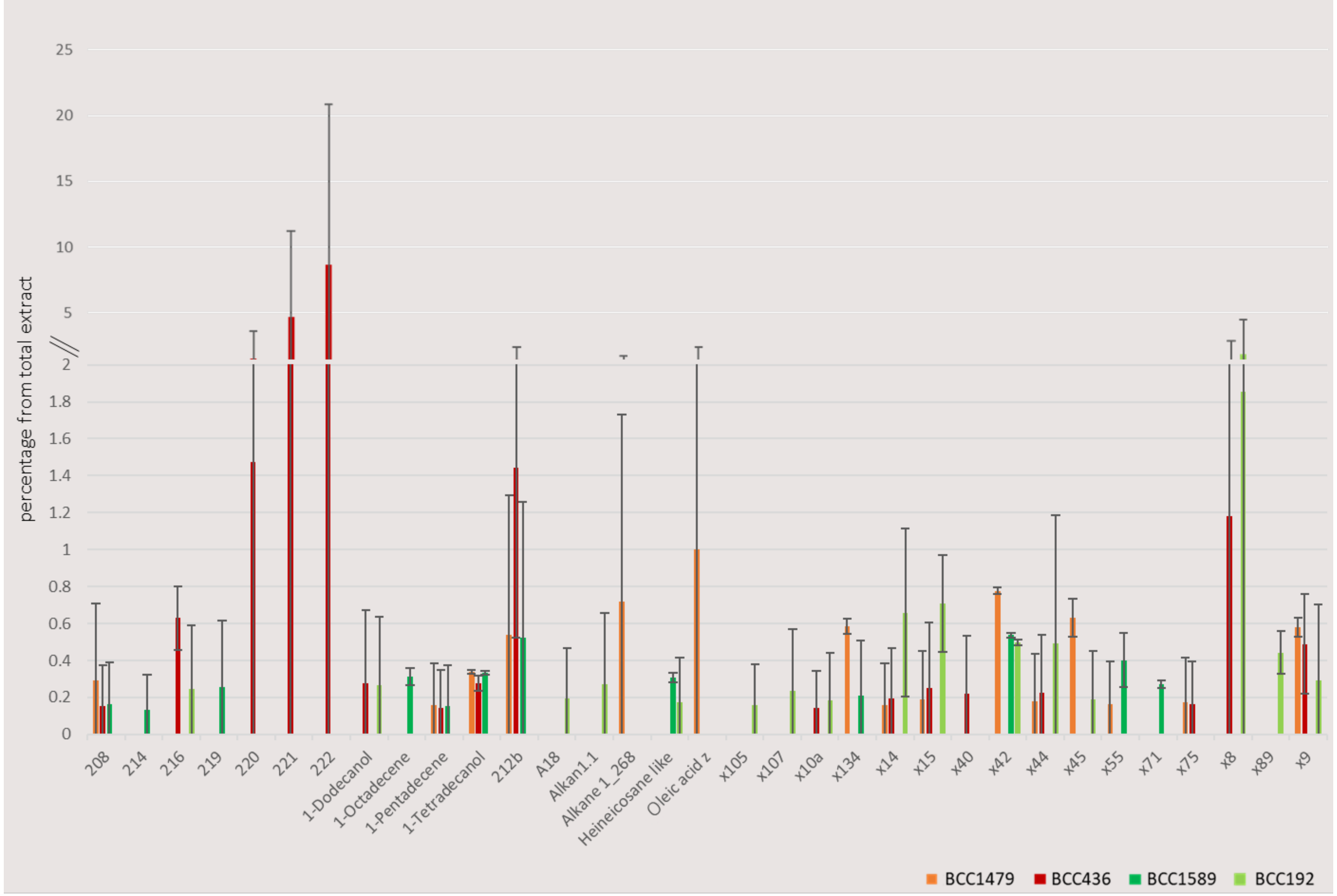


Figure 38. **Proportion of cuticle molecules in the cyclohexane extract with variable occurrence in barley genotypes resistant or susceptible towards *Blumeria graminis* f.sp. *hordei* race A6.** Shown is the mean per genotype and the standard deviation (n=2); numbered molecules could not be identified; orange and red bars: susceptible genotypes; green bars: resistant genotypes.

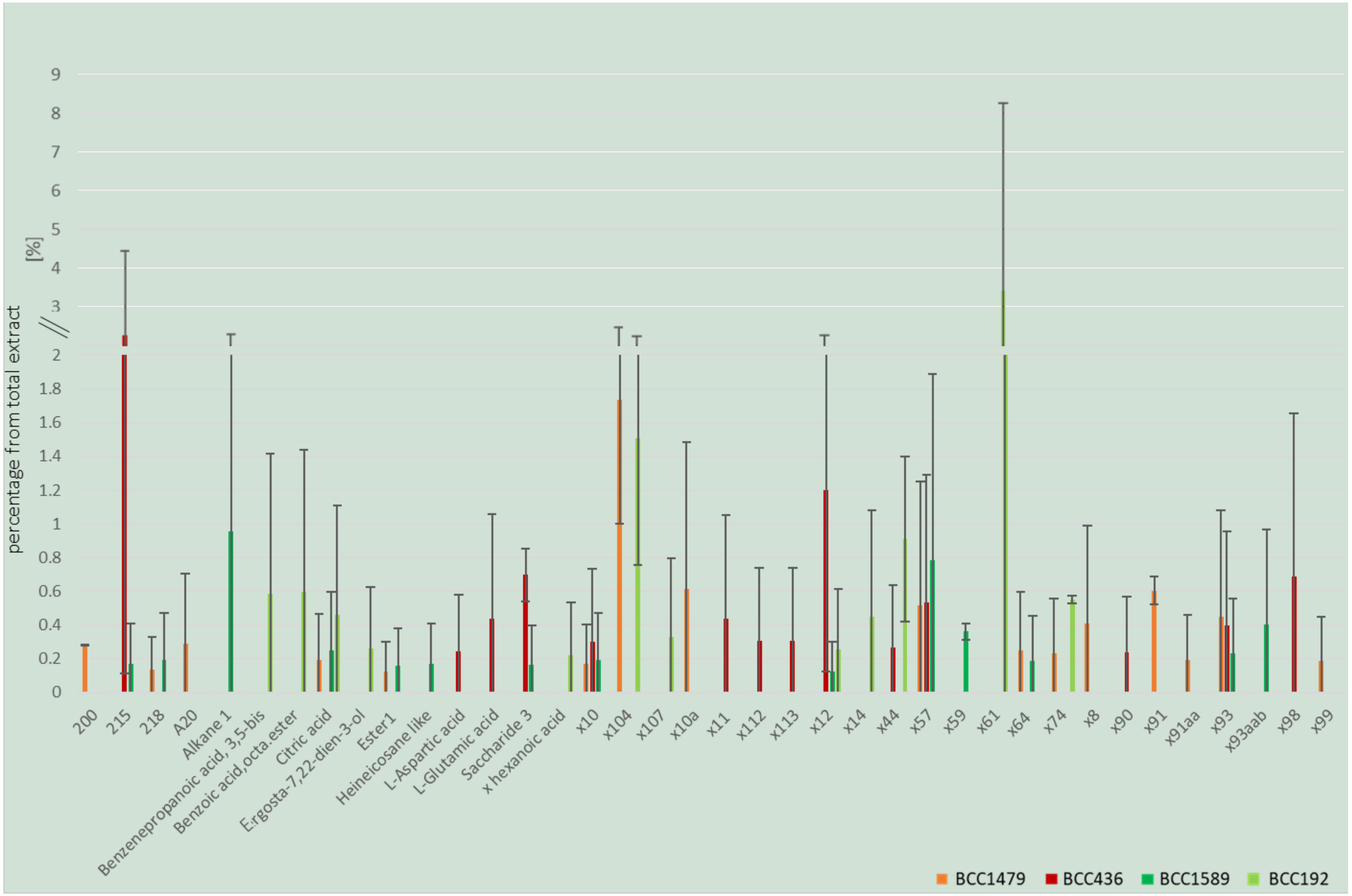


Figure 39. **Proportion of cuticle molecules in the ethyl acetate extract with variable occurrence between in barley genotypes resistant or susceptible towards *Blumeria graminis* f.sp. *hordei* race A6.** Shown is the mean per genotype and the standard deviation (n=2); numbered molecules could not be identified; orange and red bars: susceptible genotypes; green bars: resistant genotypes.

Figure 40. **Proportion of cuticle molecules whose frequency differs little between resistant and susceptible barley genotypes towards *Blumeria graminis* f.sp. *hordei* race A6.** Shown is the mean per genotype and the standard deviation (n=2); numbered molecules could not be identified; orange and red bars: susceptible genotypes; green bars: resistant genotypes; A: cyclohexane extract; B: ethyl acetate extract

4. Discussion

This study aimed to 1) characterise the susceptibility of 40 spring barley genotypes towards *Blumeria graminis* f.sp. *hordei* race A6 (Bgh A6), 2) to find cuticle molecules attributed to Bgh A6 susceptibility, 3) to describe the potential of biologicals to prevent Bgh A6 infection on different genotypes also in the presence of drought, 4) to search for off-target effects of biologicals (biomass, BBCH, chlorophyll content, nitrogen balance index (NBI)), 5) to reveal the mode of action of three biologicals and 6) to find out if the minimum conductance predict Bgh susceptibility.

To achieve these goals an inoculation protocol was established, and its features are discussed whereby its outstanding characteristic of being statistically validated is pointed out (chapter 4.1). Implementing this protocol a susceptibility study was conducted showing a susceptibility continuum of 40 spring barley genotypes towards Bgh A6. The results of the individual genotypes are integrated into the context of current knowledge to address the question whether resistant genotypes are race-specific or -unspecific resistant (chapter 4.2). Causes of susceptibility are discussed and the results of expression studies of pathogen-associated genes in response to Bgh infection are highlighted (chapter 4.3). Thereby, the expression pattern reflects the quantitative resistance of genotype GP.

The preventive efficacy of 21 biologicals against Bgh A6 on seedlings of four genotypes, differing in their susceptibility, was studied in greenhouse trials, revealing mostly genotype-dependent effects (chapter 4.4). The effects of biologicals were additionally studied under the simultaneous occurrence of drought stress and Bgh, picturing a mosaic-like pattern of biological efficiency with diverse effects, ranging from genotype- and water status-independent to genotype- and water status-dependent effects (chapter 4.4). The results are contextualised in the current state of research, compared with results from other cultures, mode of actions are discussed (chapter 4.5), and conclusions drawn about their mode of action. These are supported by the results of an expression study of pathogen-associated genes in response to a biological treatment and Bgh A6 inoculation in genotype GP. The two biologicals with the best efficacy are shown to have a multi-layered mode of action comprising the induction of defence genes, priming and presumably also direct fungicidal effects (chapter 4.6).

In addition to the effectiveness of biological treatments against Bgh A6, the effects of the preparations on above-ground dry biomass, plant height, developmental stage as well as chlorophyll content and NBI were studied and are discussed as off-target effects (chapter 4.7, chapter 4.8). Furthermore, the relationship between the minimum conductance of leaves to water and the Bgh A6 susceptibility is discussed (chapter 4.9).

Finally, summarising the results made it possible to identify predominantly supportive biologicals and genotypes that are particularly suitable for biological application (chapter 4.10).

4.1 The inoculation method should be anchored in the statistical model for analysing plant trials

The concept of airborne inoculation using an inoculation tower is a well-established concept in the field of Bgh infection studies (Reifenschneider and Boiteaux 1988; Bell *et al.* 1952; Reuber *et al.* 1998; Nielsen *et al.* 1999; Vogel and Somerville 2000). This study shows that the conidia are distributed evenly, without significant differences between the border, middle and central areas of the low cost and easy to build inoculation tower. Due to the proven uniform inoculation over the entire area, no border plants have to be excluded from the analysis. These findings are essential for statistical analysis as well as for planning, interpretation and understanding of experimental data. The inoculation event determines the disease severity of experimental plants (Figure 7). There are several reasons for this: 1) new conidia were harvested for each inoculation process, which can differ in their vitality, 2) the microclimate in the tower is variable and depends, for example, on the pot humidity and climatic conditions and thus influences the infection success and 3) different quantities of conidia are swirled in each inoculation process and the conidia mass correlates with the severity of Bgh symptoms (Figure 8). The fact that a decrease in Bgh symptoms occurs with conidia masses above 7.4 mg could be due to increased clustering of the conidia or to mutual inhibition of germination. It is therefore, and because of the lower Akaike Information Corrected Criterion (AICc), recommended to integrate the inoculation process into the model for the statistical evaluation of experimental data instead of the conidial mass, which further simplifies the experimental procedure by eliminating the need for weighing of conidia. Inconsistencies in the conidial mass used, in their vitality and in climatic conditions favouring or inhibiting infections are considered by integrating the inoculation process as a fixed effect. To avoid not only the weighing of the conidia but also their harvesting, whole mildew-infested plants, e.g. from a permanent culture, could be placed on the platform in the inoculation tower. Their conidia would then be swirled by the fan-suspended air currents. However, it remains to be seen whether there is no position effect with this approach; it can be assumed that the area under the pot is disadvantageous in terms of conidia distribution. Such an effect would have to be considered when positioning and randomising test plants. The most suitable inoculation method may vary depending on the issue in question, but in summary, the here presented inoculation tower enables high accuracy in inoculation while saving time and space for the researcher.

4.2 Quantitative and qualitative resistance may form the susceptibility continuum of 40 spring barley genotypes towards Bgh A6

As in Central Europe more than 200 Bgh pathotypes are found (Dreiseitl 2019), each study with a respective Bgh isolate contributes to complete the picture of Bgh susceptibility of genotypes. Through race-specific resistance, a genotype or accession can be resistant to one Bgh pathotype but susceptible to others. For example, barley accession P08B, has a known susceptibility towards Bgh D35/3 and Bgh RiIII, while being resistant towards Bgh CH4.8 (Pogoda *et al.* 2020). Therefore, this study on susceptibility of barley towards Bgh A6 is important to further characterise the resistance and susceptibility of the genotypes studied. The investigation of 40 spring barley genotypes shows a continuum instead of a clustering towards Bgh A6 susceptibility (Figure 9). Likewise, for two poly-virulent isolates D35/5 and RiIII a range of susceptibilities is described (Pogoda *et al.* 2020). This reflects the complexity of plant pathogen defence based on a variety of metabolic-physiological processes. For example, different genetic pathways of race-specific and race-non-specific resistance are described (Peterhansel *et al.* 1997), although, the underlying genetic regulation of basal, race-specific and non-host resistance might overlap (Jansen *et al.* 2005). While more than half of the advanced/improved cultivars analysed here are classified as resistant, very low or low susceptible, only one-fifth of the traditional cultivars are categorised as such (Figure 10). This underlines that an important breeding objective is pathogen resistance.

For the resistant genotypes, the expression of resistance genes can be assumed. For most of the genotypes that are resistant towards Bgh A6, a susceptibility towards other Bgh races or isolates like Ty4, D35/3, RiIII or AEV7532 is known (Pogoda *et al.* 2020; Balkema-Boomstra and Mastebroek 1995). This leads to the assumption of race-specific resistance for these genotypes. Based on this study, A6-specific resistance is present in genotypes BCC1368, BCC1373, BCC1379, BCC1370, BCC1377, BCC1385, BCC1395, BCC812 and BCC1381. For both, BCC1368 and BCC1381 resistance towards Bgh A6 is shown earlier, while BCC1378 is described as susceptible to race A6 in former studies (Jensen *et al.* 1992). To my knowledge, no susceptibility data towards powdery mildew apart from the results shown here are available for Bgh A6 resistant BCC1589, BCC899, BCC1476, BCC1413 and BCC1387. Therefore, the question of race-nonspecific resistance remains open for these genotypes. It also remains open for the very low susceptible genotypes BCC432 and BCC1433, whether their reaction is specific towards race A6. In contrast to this, the low susceptible genotypes BCC817 is already described as susceptible to D35/3 and RiIII (Pogoda *et al.* 2020). The Bgh A6 susceptibility found in my studies in the medium, high, and very high susceptible genotypes is in accordance with the susceptibility of the genotypes to other isolates. For example, the genotype BCC1412 is susceptible to D35/3 and RiIII, as are BCC1468, BCC436, BCC852 and BCC875 (Pogoda *et al.* 2020). For GP a broad susceptibility is already described comprising several races and isolates like CC220, D35/3, RiIII, DH14 and K1 including race A6 (Pogoda *et al.* 2020; Walters *et al.* 2002; Aguilar *et al.* 2016; Seeholzer *et al.* 2010). The classification of genotype BCC913 and BCC903 as susceptible to Bgh A6 is the first description of the reaction of these genotypes towards powdery according to literature research.

To my knowledge, this study describes the susceptibility of nine barley genotypes towards powdery mildew that were not investigated so far. Seven of them are resistant, very low or low susceptible and two of them show intermediate susceptibility towards Bgh A6. In addition, the data extend the knowledge about the response of 20 genotypes, which have previously been studied only for other races and isolates. Furthermore, my data confirm the earlier described susceptibility of GP and the resistance of BCC1381, and BCC1368 to Bgh A6 (Jensen *et al.* 1992; Seeholzer *et al.* 2010; Aguilar *et al.* 2016). In contrast to previous studies, Bgh A6 resistance is found here for genotype BCC1378 (Jensen *et al.* 1992). This is not the first study whose results for some genotypes show a contradiction to previous data while confirming the resistance or susceptibility of other genotypes (Dreiseitl and Zavřelová 2018). Overall, the results demonstrate the poly-virulence of Bgh A6 and the wide range of Bgh A6 susceptibility of studied genotypes.

The assignment of BCC1589 to the resistant, GP to the mid susceptibility and BCC436 to the very high susceptible group is partly confirmed during the experiment on the combined occurrence of drought stress and Bgh also for later infection periods (chapter S 10, S Figure 10). Well-watered control plants of BCC1589 also have no or hardly any signs of infection at 13 and 21 days after infection (dai), while the difference between GP and BCC436 almost completely disappear and both genotypes show very strong signs of infection 21 dai (infection category of four, five). One possible reason for this could be the increasing infection pressure as the trial progresses. GP could withstand the initially low infection pressure better than higher infection levels attributable to the fact that all experimental plants were cultivated in one greenhouse cabin and heavily infected plants, such as control plants of BCC436, keep the infection pressure high. Under dry conditions, however, the control plants of the three genotypes show their increasing infection intensity from BCC1589 via GP to BCC436 for each of the points in time. Furthermore, it is shown that the resistance of BCC1589 is not lost due to drought stress (Figure 17, chapter S 10, S Figure 10), which suggests the existence of a monogenic, i.e. qualitative resistance in contrast to the assumed polygenic i.e. quantitative resistance of genotypes from the low susceptible group. Resistance to Bgh can withstand not only drought stress but also heat stress, for example, in the case of barley genotype HvHV07-17 (Schwarczinger *et al.* 2021). However, there are resistances that can be influenced by environmental conditions, such as *mlo* resistance leading to nearly total resistance, but with a variable efficacy along environmental conditions or fugal genotype (Lyngkjær *et al.* 2000).

4.3 Susceptibility has reasons

The underlying reasons for mildew resistance or susceptibility can be diverse. One trait influencing the susceptibility is the cuticle. Gas chromatography-mass spectrometry analyses of ethyl acetate and cyclohexane extracts were performed to determine whether the cuticular composition of genotypes classified here as resistant or highly susceptible to Bgh A6 have different extractable cuticular molecules. This has not yet been investigated for barley genotypes BCC1589 and BCC192 as representatives of the resistant genotypes and BCC1479 and BCC436 as representatives of the susceptible genotypes. The main component of the waxes are hexacosanol derivatives (Table 10, Figure 37). This is in agreement with studies on other barley genotypes (Zabka *et al.* 2008).

A molecule (x75) was found in the cyclohexane extract of both susceptible genotypes, that only accounts for a very small percentage of the total extract of both genotypes but was not found in resitante genotypes. The influence of this molecule on conidia could be investigated in germination tests. The mass spectrum of x75 resembles neither an alkane nor an alcohol or a carboxylic acid. However, it shows the typical peak of a functional group silylated by *N*-Methyl-*N*-trimethylsilyl-trifluoroacetamide. The heneicosane like molecule is also not identified, but its mass spectrum clearly assigns it to the alkanes group. The molecule was found in the cyclohexane extract of resistant genotypes, which was not present in the susceptible genotypes (Figure 38). An indication of a link between this molecule and Bgh infection in barley could not be found in literature, but heneicosane in wheat is associated with induced formation of appressoria in ascospores (Zhu *et al.* 2017). Differences between conidia and ascospores and between f.sp. of *Blumeria graminis* are conceivable. In general however, very long chain aldehydes favour infection partly dose- and chain- length depended (Zhu et al*.* 2017; Hansjakob *et al.* 2010). The C_{26} molecule hexacosanal induces appressorium formation in barley (Tsuba *et al.* 2002; Pham *et al.* 2019b), but it remains unclear whether the aldehyde altered surface properties or acts as a chemical signal. (Tsuba *et al.* 2002). In maize, docosanoic acid reduces conidial rate of germination (Hansjakob *et al.* 2011) what is unlikely for barley as docosanoic acid was found in Bgh A6 susceptible and resistant genotypes – and with them more often. Thereby, the difference in the percentage of docosanoic acid between susceptible and resistant genotypes is small (0.34%) making it unlikely that this difference can contribute to explain the different susceptibilities of the genotypes. Additionally to cuticle wax composition, also hydrophobicity is decisive for the development of phytopathogenic fungi (Lewandowska *et al.* 2020), which was not studied here but could contribute to the understanding of conidia-cuticle interactions. Nevertheless, by extracting with two solvents of different polarity, the diversity of the analysed molecules is increased. This method also makes it possible to determine more realistic proportions of the molecules that could be extracted with both solvents, as variable solubility in the two solvents is equalised.

Besides morphological features like the cuticle, several gene groups influence the susceptibility of barley genotypes towards Bgh. Isolate or race-specific resistance, as found here for numerous genotypes, is mediated by specific plant host resistance genes. These include the *Mildew locus a* (*Mla*), which has undergone enormous diversification into over 30 different alleles and is intensively researched (Seeholzer *et al.* 2010; Moscou *et al.* 2011). In contrast to race-specific resistance, the *Mlo* locus is known to mediate race-non-specific resistance with the contribution of two 'required for mlo-specified resistance` genes (Freialdenhoven *et al.* 1996).

Additionally, the expression of genes encoding pathogenesis-related proteins (PR) with antimicrobial activity in presence of a pathogen is part of plant defence response. The expression of *PR1b*, *PR5* and *PR17b* is Bgh infection associated (Wang *et al.* 2018; Christensen *et al.* 2002). In accordance with this, the presented study also found an association to Bgh infection for *PR1b*, *PR5* and *PR17b*. The expression of these three *PR* genes peaked 24 hours post inoculation (hpi) in mid susceptible genotype GP (Figure 31). This timing corresponds to haustoria formation of Bgh (Both *et al.* 2005) and is also shown for other barley genes involved in functional pathways of plant defence (Li *et al.* 2019) and proved in other studies for *PR1b* (Mouradov *et al.* 1994) and *PR17b* (Christensen *et al.* 2002). It is described, that PR17b proteins accumulate mostly in the barley mesophyll apoplast, but also in the epidermis (Christensen *et al.* 2002). Therefore it is assumed that their function is related either to cell wall metabolism, to signal transduction or is directly addressed to the pathogen (Christensen *et al.* 2002).

For *lesion simulation disease 1* (*LSD1*), *respiratory burst oxidase homologue F2* (*RBOHF2), glutathione reductase 1* (*GluRed1*) and *ascorbate peroxidase 1* (*AsPer1*) a downregulation, albeit small, is observed 72 hpi. Contrary to the upregulation 24 hpi, Li *et al.* (2019) also describes a downregulation of barley genes with a time delay to that peak, but already at 48 hpi. The decrease in gene expression, be it 72 hpi or 48 hpi, could be due to a declining plant immune response, which is suppressed by the successful establishment of a haustorium (Chisholm *et al.* 2006). LSD1 restricts cell death in response to pathogen attack (Mateo *et al.* 2004) and is associated with Bgh resistance (Johrde 2009). It is a zinc-finger protein monitoring a superoxide-dependent signal and negatively regulating cell death (Dietrich *et al.* 1997). In this way, LSD1 protects from oxidative stress induced cell death (Kaminaka *et al.* 2006). Respiratory burst oxidase homologues (RBOH) are sources of reactive oxygen species (ROS), leading to higher susceptibility when knocked down, as they influence the accumulation of hydrogen peroxide (Proels *et al.* 2010). Conversely, this means that a normally regulated RBOH could increase during an infection and cause the accumulation of hydrogen peroxide, which in turn is part of HR, going together with the early (one hpi) increase in *RBOHF2* expression. At later stages of infection, this immune response might no longer be necessary, as indicated by the declined *RBOHF2* expression. The expression pattern of *AsPer1* resembled that of *RBOHF2* with a decline 72 hpi and a small increase at earlier stages of infection (five hpi). Ascorbate peroxidase takes part in the balance of plant homeostasis via ROS-scavenging mechanisms (Foyer and Noctor 2005; Davletova *et al.* 2005) and its activity increases in susceptible barley lines post infection with Bgh (El-Zahaby 1995). This enzyme seems to contribute to early Bgh response. Although, El-Zahaby (1995) found no changes in glutathione reductase expression after infection, the presented study shows an early but low increase in expression of *GluRed1* from one hpi on and a reduction 24 and 72 hpi respectively. Glutathione reductase provides glutathione for detoxification (Smith *et al.* 1989). This is necessary as H_2O_2 accumulates in response to Bgh penetration attempt in small papillae at early stage of infection (An *et al.* 2006). Other studies also found in a longer-term perspective, that the activity of antioxidants such as peroxidase and glutathione reductase are induced five to seven days after inoculation (Harrach *et al.* 2008). This could not be validated with the experimental setup of the presented study.

The discussed expression patterns of pathogenesis and even explicitly Bgh associated genes explain the categorisation of GP as mid susceptible in the susceptibility continuum. The pattern emphasises the quantitative immune response.

4.4 The effectiveness of most biologicals depends on the genotype

The preventive application of 21 biologicals as well as milk-associated products against Bgh A6 on spring barley genotypes GP, Morex, JK1_HOR7985 and BCC436 shows genotype-dependent and -independent effects already at seedling stage (Figure 11, Figure 12). It is shown that the efficacy of biologicals exhibits a mosaic-like pattern with differential effects of biologicals on the infection with Bgh. It is moreover concluded that the infection pressure may also be relevant for the effects of the biologicals, which is in accordance with Matzen *et al.* (2019). As expected, the positive control Kumulus WG exhibits its known fungicidal effect preventively on all tested genotypes with and without drought stress – the same applies to Blattgrün – one of three tested biologicals and milk-associated products with genotype-independent preventive efficacy. In case of Blattgrün, this preventive genotype-independent effect is maintained even under simultaneous drought stress six dai (Figure 15). Thereby, little is known about the potential of biologicals to control Bgh under the combined occurrence of drought stress and powdery mildew although the pathosystem has been subject of considerable research (Hoseinzadeh *et al.* 2019; Walters *et al.* 2002; Wright *et al.* 2002). After a second spray (p-i) and 20 dai, Blattgrün only lead to a significant reduction of infection in GP, but not in BCC436. It must therefore be assumed that the effect of Blattgrün on GP is more long-lasting whereas it is not maintained on the highly mildew-susceptible genotype BCC436, possibly due to the higher infection pressure. It is conceivable that a weekly application beyond two weeks could significantly reduce the infection on BCC436. This assumption is supported by the observation that there are biologicals whose application on GP and BCC436 resulted in constantly low infection signs from six to 13 dai, but showing more infection 20 dai (Figure 14) - at this point, two weeks went by without repeated application.

Blattgrün consists of field-horsetail extract, plant-based emulsifiers and essential oils. Horsetail is known to be rich on silicon (Labun *et al.* 2013), a molecule that inhibits hyphal development of powdery mildew (Bowen *et al.* 1992). Moreover, antifungal activity of horsetail is reported to rely on peptides (Rogozhin *et al.* 2020). Also field-horsetail-based Equisetum Plus is able to significantly reduce Bgh symptoms in three of the four genotypes in the 'biological trial', whereas Ackerschachtelhalm Extrakt Compositum, also field-horsetail-based, has no effect on infection (Figure 11). It can be assumed that ingredients like emulsifiers contribute to mildew reduction and according to van Oosten *et al.* (2017), the composition of commercial formulations varies, which can make the choice of manufacturer a key factor for efficacy - a statement reinforced by my results. In addition, the variability of the chemical composition of biologicals, especially botanicals, is challenging. When comparing experiments with plant extracts it must be remembered that the weather conditions and the location of the harvested plant influence the formation of secondary plant metabolites (Docimo *et al.* 2024) and therefore the effectiveness of extracts might vary. Likewise, the extraction method influences the extract ingredients - both critical factors for the effectiveness, which may have contributed to the different efficiencies of horsetail extracts in this study.

Differences in efficacy are also observed for raw milk, underlining natural fluctuations in composition, like field horsetail extract, influencing the effectiveness against phytopathogens (Figure 11, Figure 12). Raw milk has a reducing effect on the Bgh infection of JK1_HOR7985 in the 'biological trial', but not in the trial on the mildew-reducing effect of milk-associated products. With Morex it is the other way round. The trials differ in their infection pressure (higher infection in the 'biological trial'), and in the raw milk batch applied – both factors that might influence the effectiveness. However, further studies are needed to fully understand this effect. Raw milk (100%) is preventively effective against Bgh under well-watered and dry conditions as well as in later stages of infection on GP and BCC436 (Figure 15, Figure 16). Raw milk, a listed basic substance for use on different plants like grapevine, cucumbers or soybeans against powdery mildew (EU pesticide database), is also known to protect wheat from powdery mildew (Drury *et al.* 2003). This study expands the current knowledge, because it shows that raw milk (100%) was genotype-independently effective on barley genotype GP and BCC436, and at the same time drought stress-independently effective. Which components or mechanisms were responsible for this effectiveness? To obtain indications on this, milk ingredients as well as milk-associated products were analysed (Figure 12). Living cells in raw milk are not responsible for the effect. Thus, in addition to raw milk, UHT milk, pasteurized milk and milk powder are also mildew-reducing on at least three of the four genotypes. Due to the processing and heating of the milk during pasteurization and in the production of UHT milk, it must be assumed that living cells are eliminated in these products - therefore they cannot be solely responsible for the mildew-reducing effect. It is conceivable that many components contribute to the effectiveness of the milk products. One factor seems to be the formation of oxygen radicals, which cause hyphal collapse and conidia damage (Crisp *et al.* 2006b). Additionally, the action of fatty acids or proteins play a role (Crisp *et al.* 2006a), indicating a direct pathogen damaging mode of action. Indeed, a sole direct antipathogenic effect would have to be genotype-independent what is not shown in the trials. But raw milk was effective independently of the simultaneous occurrence of drought stress. I therefore agree with the hypothesis of a multi-layered mode of action postulated by Wagner (1999). The author claims that salts and amino acids contained in milk could indirectly induce systemic resistance in addition to the direct effect of oxygen radical formation. A direct influence of raw milk on immune response-associated genes is described here, as is the induction of such genes (chapter 4.6).

Besides, the described effects of raw milk, Blattgrün and other field-horsetail based products, six biologicals have no influence on Bgh infection in the ‘biological trial’ independent of the tested genotype and three worsen the infection on at least one genotype. Furthermore, genotype-dependent preventive efficacy is found among others for the microbial-based products T-Gro, Rhizovital 42 and Serenade ASO in the ‘biological trial’. This is not surprising as the interaction of microbial-based products and crops is reported to be individual and variable between bacterial strains and plant genotypes (Creus *et al.* 2010).

Likewise, genotype-dependent is the preventive efficacy of giant knotweed leaf extract in the ‘biological trial’. Contrary to my results, Bgh is reduced due to its application on GP in earlier studies (Boyle and Walters 2006). Here, the extract only lead to a significant Bgh reduction in Morex but not on GP or JK1_HOR7985 (all classified as medium susceptible) and it is also ineffective in the highly Bgh susceptible genotype BCC436. This could be due to the aforementioned variability of secondary plant metabolites. However, the extract is described as effective for other cultures and pathogens. Thus, it is reported that giant knotweed leaf extract reduces downy mildew in grapevine (Taylor *et al.* 2022) and powdery mildew on cucumber cultivars of different susceptibility (Petsikos-Panayotarou *et al.* 2002). In courgette, the knotweed extract augments H_2O_2 release and callose formation (Margaritopoulou *et al.* 2020), both traits belong to plant immune response also towards powdery mildew and indicate an indirect mode of action. My results extend the knowledge of giant knotweed leaf extract effects by the finding that powdery mildew can be significantly reduced on genotype Morex after preventive application and that a genotype-dependent effect exists in barley. Genotype-dependent effects are found for several other biologicals, which suggests an immune response-inducing effect rather than a direct fungicidal effect for these products.

In addition to the biological itself, the effectiveness is influenced by genotype, environmental conditions such as drought stress and genotypes' ability to cope with it. For example, a genotype-dependent preventive efficacy is found for baking powder, burdock root extract, CropCover and raw milk (10%) (Figure 15). In drought stress tolerant BCC436, drought stress-independent preventive effectiveness of these biologicals is found, whereas in drought stress sensitive GP the mildew reducing effect disappears under dry conditions. Moreover, high disease pressure may further influence the effectiveness, as is observed by experiment comparison for baking powder and CropCover on well-watered GP and BCC436: in the 'biological trial' a significant increase of Bgh infection is observed in the presence of high infection pressure (mean number of pustules on the first leaf of control GP in the 'biological trial': 18.5; BCC436: 51.9) but a significant disease reduction under lower pressure (mean number of pustules on the first leaf of control GP: 16.5; BCC436: 39.1) in the 'combined experiment' – underlining the influence of disease pressure on biological effectiveness. Reasons for this must be further investigated. A repeated application of baking powder or CropCover, as in the p-i application, apparently ensures that even at higher infection pressures, as at the end of the 'combined experiment', a significant reduction in infection can still be achieved and that after a second application Bgh can also water status-independently be reduced via baking powder and CropCover treatment - possibly via induced resistance. This contrasts with the partly infection-promoting effect of Biplantol mycos V forte, Elot-Vis Green, giant knotweed leaf extract and Serenade ASO 21 dai (Figure 11). These products have no initial effect after a preventive treatment in this trial.

4.5 Repeated application of biologicals and mode of actions

The additional treatment with biologicals after inoculation (p-i) was applied to increase the concentration of effective substances and to obtain evidence on whether repeated application at the first symptoms of infection can mitigate the course of the infection. For baking powder, raw milk (10%) and CropCover, re-spraying results in enhanced disease reduction (Figure 16). While initially ineffective on GP of the dry group, p-i lead to a significant reduction of infection symptoms for all of these biologicals. Furthermore, a change in effectiveness is found after preventive plus p-i treatment with giant knotweed extract on well-watered GP. Underlying mode of action might be induced resistance related, which may be associated with limited efficacy in case of high infestation as here, three weeks after sowing especially on highly Bgh A6 susceptible genotype BCC436.

For giant knotweed based product Milsana, induced resistance with the accumulation of chalcone synthase, chalcone isomerase and flavonoid compounds in response to curative application on powdery mildew infected cucumber plants is reported (Fofana *et al.* 2002). In addition, Milsana induced a rapid phytoalexin production after two applications, collapsing conidial chains of infected cucumber leaves underlining the curative potential of giant knotweed extract (McNally *et al.* 2003). Whether the additional p-i treatment with giant knotweed extract on well-watered GP is curative cannot be found out with the experimental setup carried out here, which also applies to possible eradicant effects. Besides for giant knotweed extract, a curative effect on barley powdery mildew through the application of Serenade ASO is described (Matzen *et al.* 2019). An infection reducing effect of Serenade ASO is not found in the 'combined experiment', probably due to the high infection pressure, thus Matzen *et al.* (2019) reports the curative potential only under early infection stages.

The found efficiency of burdock root extract might be ROS mediated. Because it is reported that fructooligosaccharides from burdock root extract induce a stomatal closure in *Pisum sativum* L., which goes together with ROS production (Guo *et al.* 2013). A similar reaction of barley to the burdock extract is possible. As ROS molecules are part of plant immune responses (Lee *et al.* 2020) they could contribute to the resistance inducing effect of burdock root extract. However, this induced effect is probably not sufficient to cause significant effects under higher disease pressure as on BCC436 of the well-watered group 20 dai. Overall, induced resistance could explain the variable, meaning genotype-dependent, response of biologicals in the different trials, as well as the reactions to the different water regimes.

In some cases of biological genotype water management, plants that initially showed no effect of their treatment on infection severity developed significantly increased infection symptoms during the course of the experiment and after p-i spraying. This is observed for Biplantol mycos V forte, Elot-Vis Green, giant knotweed extract and Serenade ASO in different genotype and water regimes. It is assumed that this is due to the increasing infection pressure as the trial progressed.

4.6 Biologicals activate and prime barleys´ immune response

Plant defence related gene expression can be modulated, among others, via biologicals. This is demonstrated by analysing the gene expression of selected genes as a function of biological treatment in Bgh A6 inoculated and non-inoculated GP. The data collected enables a distinction between directly activated gene expression and priming through the application of Blattgrün, raw milk and giant knotweed extract. Directly activated is the expression of *peroxidase 40* (*Perox*) treated with Blattgrün or raw milk. These plants show a clear increase in the expression of *Perox* (fold-change of up to 33.4). Other then most peroxidases, Perox is secreted into the apoplast of epidermal cells and is associated with basal resistance of barley, but not with ROS production whereby its defence-related function remains currently unclear (Johrde and Schweizer 2008). However, it is shown that its transient overexpression leads to increased penetration resistance against Bgh (Johrde and Schweizer 2008).

Increased penetration resistance could therefore be one of the reasons for the mildew-reducing effect of preventively applied raw milk or Blattgrün. In this way, the preventive application of these products could block the infection before the pathogen has even entered the host cell. As *Perox* is naturally expressed in epidermal cells (Johrde and Schweizer 2008), a genotype-independent overexpression of the enzyme gene is conceivable, explaining the genotype-independent mode of action of Blattgrün and raw milk. But how long does the induced overexpression and thus the penetration protection by *Perox* last? The downregulation of *Perox* 96 hours post application (hpa) of Blattgrün could indicate that the resistance promoting effect is not maintained for too long - it seems to be similar to the effects of raw milk. A treatment with giant knotweed leaf extract also improves *Perox* expression, but not throughout the whole timeline and remarkably only for one repetition 24 hpa (fold-change of up to 22.3). This regulation is therefore referred to as 'variable', which could explain the lower efficiency of giant knotweed treatment against Bgh infection compared to the other preparations.

Besides *Perox*, the *PR* genes are affected by leaf treatments particularly 48 hpa in case of Blattgrün or 29 and 48 hpa for raw milk, but also 24 and 29 hpa in case of giant knotweed extract. In giant knotweed treated non-inoculated GP plants, an increase in *PR1b*, *PR5* and *PR17b* expression occurs firstly 24 hpa, while other genes such as *Perox*, *ethylene response factor 1* (*ERF1*) or *non-expressor of pathogenesis-related genes 1* (*NPR1*) are higher expressed 1 hpa, albeit at a low level. This phenomenon is found less pronounced in the other treatments, where the *Perox* expression dominates, as already discussed. In courgette, giant knotweed leaf treatment induces salicylic acid (SA) production which in turn translocate the cytoplasmatic SA receptor NPR1 to the nucleus which then activates *PR1* transcription (Margaritopoulou *et al.* 2020; Kinkema *et al.* 2000). This could explain the delayed *PR1b* expression relative to *NPR1* in knotweed treated non-inoculated barley. The SA receptor is considered a main regulator of systemic acquired resistance in salicylic acid pathway and also *PR5* is *NPR1* associated (Wang *et al.* 2018), which goes along with the here presented results. In addition, also *PR17b* could be *NPR1* associated. Furthermore, NPR1 seems to be involved in the establishment of mutualistic symbiosis of beneficial bacteria and crops (Kumar *et al.* 2021), underlying the important role of this protein for plant health. Giant knotweed induces also *PR* expression 29 hpa and slightly 48 hpa, a finding that was reported earlier for *PR1* at a similar time, in courgette (Margaritopoulou *et al.* 2020).

In addition to the direct effects of the applied products on gene expression, evidence of priming effects is found. In water treated and inoculated plants, a *PR* peak (*PR1b*, *PR5*, *PR17b*) occurs 24 hpi. In contrast, after Blattgrün or raw milk treatment and a following inoculation, a *PR* peak is observed as early as five hpi and for *PR5* even one hpi, while in Blattgrün or raw milk treated non-inoculated plants, a *PR* peak is recorded 48 hpa, which corresponds to the same time as 24 hpi. The treatments accelerate the immune response after Bgh A6 infection. Therefore, in addition to the direct gene expression inducing effects, a priming effect of Blattgrün and raw milk can also be assumed. In giant knotweed treated plants this priming effect is lower, and the results mostly show inconsistencies between replicates. This fits with the variable Bgh reduction by foliar treatment with giant knotweed. In addition to these biologicals, biotic and abiotic agents can have a resistance-inducing effect (Walters *et al.* 2013). Thereby, not only leaf treatments are able to modulate PR gene expression, but also the transplantation of rhizosphere microbiome or a root treatment can enhance the expression of *PR1b*, *PR17b* or *PR5* and additionally reduce Bgh susceptibility (Bziuk *et al.* 2022). Comparable results are achieved via GP treatment with *Piriformospora indica* (Sebacinales) prior to Bgh A6 infection resulting in earlier and faster PR gene expression in early infection phase, which shows that also 'mycorrhiza-induced' resistance is possible (Molitor *et al.* 2011). Besides, also insect herbivores or pathogens can challenge plants, resulting in an induced resistance (Bostock 2005). Priming effects in general provide fitness benefits in an environment with pathogens as they enable rapid reactions in response to pathogen attack (van Hulten *et al.* 2006). In this way, resources are deployed in a demand-orientated way.

The question remains why the two replicates show divergent results. Inconsistencies in environmental conditions (inavoidable) could influence the expression as well as differences in conidia vitality, thus in infection pressure. However, also other studies report huge differences in elicitor effectiveness against pathogens (also Bgh) between different years (Walters *et al.* 2014). Differing results between repetitions are even described for the expression of *PR1b* and *PR17b* (Bziuk *et al.* 2021). It is therefore highly advisable to repeat the experiment a third time despite the high costs of molecular analyses.

4.7 Biologicals influence biomass production and BBCH under Bgh infection

The above-ground dry biomass of test plants was determined both at the end of the 'biological trial' and at the end of the 'combined experiment' with Bgh and drought stress. Remarkably, differences in dry biomass between genotypes and biologicals are already observed 14 das, meaning seven days after the application of the products in the 'biological trial' (Figure 18) and 28 das in the 'combined trial' (Figure 19).

It was found that GP biomass production is generally lower 14 das than that of Morex or JK1_HOR7985 and that, 28 das, GP control plants are not as tall as plants of BCC436 and BCC1589, which might be due to the semi-dwarf mutant characteristics of GP (Schreiber *et al.* 2020). In addition to genetic determinations, also environmental conditions, health, and biologicals influence the above-ground plant biomass. My results suggest that a reduction in the above-ground biomass is primarily due to the application of the biologicals and not due to Bgh, as this was the case with both, 'reliably', thus mostly effective mildew-reducing products such as raw milk, as well as with 'unreliably', so products with variable effectiveness such as burdock root extract, CropCover or ineffective products such as AMN

BioVit. Similarly, Elot-Vis Green, for example, leads to an increase in biomass 14 das and 28 das in well-watered GP plants, although it is ineffective against powdery mildew (preventive and p-i). This attests the strong growth-modelling properties of biologicals and makes them interesting for agricultural issues as the above-ground biomass is a predictor of yield and grain quality (Křen *et al.* 2014). Additionally, the biomass production under harsh conditions corresponds to the ability to withstand the stressor. Golden Promise is classified as drought stress sensitive and BCC436 as drought stress tolerant (Töpfer unpublished) and the average above-ground biomass of their control plants differs slightly (+/-17 mg) in the well-watered treatment, with plants of BCC436 being heavier. Contrary to expectations, in the dry treatment the control plants of drought stress sensitive genotype GP are on average eight mg heavier than those of the drought stress tolerant genotype BCC436. This emphasizes that drought stress tolerance combines many traits, and that biomass alone is not sufficient for predicting drought tolerance, other factors like water use efficiency or yield are important. Over and above that it proves that defence mechanisms against pathogens rely on plant resources, as BCC436 is much more susceptible to Bgh A6 than GP.

None of the biologicals tested is able to promote biomass production significantly under dry conditions 28 das, although non-significant increases of 10 to 30% are found for Kumulus WG and Elot-Vis Green in GP and BCC436, for giant knotweed extract in GP and for Serenade ASO in BCC436. It should be emphasised that the significant increase in biomass after preventive plus p-i treatment of Kumulus WG and giant knotweed extract on well-watered GP is accompanied by a mildew-reducing effect. It can be assumed that in some cases plants treated with a biological preventively and p-i, which significantly reduces powdery mildew infection, have sufficient resources left for growth. In contrast, reactions based on induced responses in the plant or the occurrence of spray films may stress a sensitive plant to the detriment of its biomass. This can be assumed for raw milk, baking powder, CropCover, and burdock root extract on GP and BCC436. However, this is not always the case, as shown for Biplantol mycos V forte and Elot-Vis Green, which do not significantly reduce infection in GP or BCC436 in any of the water regimes, but nevertheless lead to a significant increase in above-ground biomass under well-watered conditions. In this case, comparable to discussed results above, it can be assumed that the increase in growth is due to the direct effect of the biological and not indirectly due to a reduction in infection.

In addition to infection intensity, biomass and plant height, biologicals can also influence the stage of development. The application of Serenade ASO on well-watered GP plants, for example, gives indications of an induced accelerated entry into the tillering phase. It remains to be seen whether the acceleration of development could also affect the time of harvest. In principle, shorter cultivation times allow the use of climatically favourable periods and reduce the risk of an undesirable change in the weather during the cultivation period.

4.8 The interpretation of chlorophyll content and NBI is multi-layered

The chlorophyll content of plant tissues is an indicator for plant health and decreases in stressed plants (Liang *et al.* 2017), also under water restriction (Velicevici *et al.* 2013). Considering this, it is initially surprising that the chlorophyll content of water treated control plants of drought stress sensitive genotype GP is higher under dry conditions than under well-watered conditions. In addition to abiotic stress, the chlorophyll content is also influenced by biotic stress, such as a Bgh infection, which lowers photosynthetic performance (Brugger *et al.* 2017). Therefore, it is assumed that in GP the chlorophyll-reducing effect of drought stress in the control plants is lower than that of severe powdery mildew infection, as found in well-watered control plants. Following this logic, it is hardly surprising that chlorophyll data of drought stress tolerant and Bgh resistant genotype BCC1589 vary less than with the other genotypes (Figure 27). Additionally, it is known that the chlorophyll content of drought tolerant barley genotypes is less effected by drought than that of sensitive ones (Li *et al.* 2006). In susceptible genotypes, whether for Bgh or drought stress, however, the biologicals can cause larger variations in barley chlorophyll content - as they do in other crops. In biological treated tomato plants infected with powdery mildew, a higher chlorophyll content is associated with the application of milk (Abdel-Maksoud Abada *et al.* 2018). My results do not allow this statement to be transferred unrestrictedly to barley. Although, the chlorophyll content of raw milk (10%, 100%) treated GP plants increases by more than 10% under well-watered conditions, it is reduced by more than 10% under dry conditions. In addition, chlorophyll-reducing effects of raw milk are observed on the BCC436 genotype. Spray residues could be responsible for the chlorophyll-reducing effects of raw milk. Since, these residues can reduce photosynthesis even if they are not obviously phytotoxic (Crisp *et al.* 2006a).

The NBI is used for crop surveys and monitoring of nitrogen nutrition (Cerovic *et al.* 2012), as it is an indicator of N content, corresponds to chlorophyll concentration and is told to be less sensitive to phenology than chlorophyll alone (Fan *et al.* 2022). The index therefore provides information about the fitness of a plant. In the case of drought stress tolerant genotypes BCC436 and BCC1583, the index is not significantly influenced by drought in the water treated control plants (Figure 27). The increased NBI values of all dry cultivated BCC1589 plants compared to their well-watered counterparts suggest that high soil moisture negatively affects their metabolism. That would fit with the environmental conditions of BCC1589’s southern European origin. The observed tendency for NBI to decrease between the 7th and 28th das (Figure 26) could be due to decreasing N availability in the soil - a nutrient analysis of the soil at the end of the trial would clarify this.

4.9 All under one roof?

It is hypothesised that the minimum conductance of a leaf to water (g_{min}) can provide information on the Bgh susceptibility of a genotype. The g_{min} indicates how much water escapes from a leaf per unit of time when the stomata are maximally closed. The value thus summarises the water loss via incompletely closed stomata with the diffusion via the epidermis and the cuticle (Duursma *et al.* 2019). The underlying considerations for the hypothesis of a relation between g_{min} and Bgh susceptibility are: a) the conductance via the cuticle might be related to the cuticle composition and b) the cuticle composition influences the Bgh susceptibility of barley genotypes. Additionally, g_{min} could provide information on the drought stress tolerance of genotypes, as the ability to avoid water-loss under dry conditions is one of the drought stress tolerance traits. Besides a Bgh infection could influence g_{min} as the conidia penetrate the cuticle to enter host cells (Nielsen *et al.* 2000), thereby the transpiration barrier of the cuticle might become leaky. Furthermore, the modification of g_{min} via biological application is feasible and could provide information on altered surface structure and/or composition. It is known that silicates from biologicals containing horsetail, for example, are deposited on the leaf surface and strengthen it (Guerriero *et al.* 2018; Taylor *et al.* 2022), thus influencing the aforementioned mildew and drought stress tolerance, possibly measurable via g_{min}. In this way, g_{min} could reflect the findings of the experiments conducted - and could be understood as an overarching variable. A requirement for this is a natural variability of g_{min} that is large enough within species to reflect differences. The minimum conductance is at least variable among species (Duursma *et al.* 2019). Thus, experiments were carried out on differences in g_{min} of barley leaves from resistant and susceptible genotypes, healthy and infected leaves and plants treated with biologicals.

In both non-inoculated and inoculated barley leaves, the genotype has a significant influence on g_{min} and the values of inoculated and non-inoculated leaves both show great variability (Figure 34). However, g_{min} did not correlate with Bgh susceptibility. The hypothesis must therefore be rejected. Besides, neither significant differences are found between water treated and CropCover or giant knotweed leaf extract treated leaves of GP, nor between different application times (Figure 35). It can therefore neither be assumed that the two products cause a direct change in the leaf structure that has a significant influence on g_{min}, nor that this happens indirectly. Nevertheless, the g_{min} can provide an indication of the Bgh resistance type of an genotype.

The genotype BCC812 is described as Bgh A6-specific resistant (chapter 4.2). Inoculated leaves of BCC812 have a higher g_{min}, i.e. lose more water per unit time than non-inoculated plants (Figure 34), although not significantly ($p=0.065$). This could be because germinated conidia penetrated the cuticle and the epidermis, damaging the water loss barrier - in this case Bgh resistance should be intracellular and e.g. prevent the establishment of a haustorium. It is thereby also possible that BCC812 resistance, as with other race-specific resistant genotypes, is based on an HR (Schulze-Lefert and Vogel 2000; Prats *et al.* 2006). The increased g_{min} could then be due to the fact that genotypes of this resistance type lose the ability to close their stomata locally in consequence of HR (Prats *et al.* 2006). In the case of the genotype BCC1476 whose resistance type could not be described, as to my knowledge no further data on its Bgh susceptibility are available, it can be assumed that it is an HR mediated race-specific resistance as inoculated leaves have a significantly ($p<0.001$) higher g_{min} than the non-inoculated leaves. Thus, the minimum conductance cannot be used as an overarching variable, but allows conclusions to be drawn about HR.

4.10 The look beyond

In consideration of the diverse and partly variable plant reactions to biological applications, this study illustrates the need for further research in this field. At the same time, it contributes to the understanding of interrelationships and provides the basis for further research.

The analyses of the cuticular composition contribute to the development of a comprehensive knowledge of resistance-mediating cuticular molecules which can open the door to genetic modifications of genotypes regarding their cuticular characteristics or provide new screening methods for extracellular resistance. In addition, there may be indications for certain molecules in plant extracts, e.g. those used as plant strengtheners, which make the cuticle unattractive to conidia, which could guide the search for new extracts. In addition to the plant cuticle, the cuticle of pathogens such as Bgh plays a role in host-pathogen interaction. While chitin and glucan usually trigger a defence reaction, chitosan and glucans do so to a lesser extent (Hadwiger 2013; Pham *et al.* 2019a). Oligosaccharides, originating for example from burdock, have the potential for an application in sustainable agriculture for crop protection (Wang *et al.* 2009). Beyond that, biodegradable glycans and glycan mimics may be safe alternatives to conventional chemicals (Chaliha *et al.* 2018) thereby, studies like mine provide a basis for identifying promising molecules.

The complexity of plant reactions to the application of biologicals, is well known (Du Jardin 2015). The complexity is also shown by this study, as the effects of biologicals in combination with Bgh with and without drought stress are diverse. This makes differentiated interpretations necessary and sometimes difficult. On the one hand, some of the contradictory results of individual experiments could likely be attributed to the different levels of infection pressure between the experiments but on the other hand the results on the preventive effectiveness of biologicals on four genotypes, varying in Bgh resistance, pictured a mosaic like pattern with genotype-dependent efficacy of numerous products. As induced resistance is influenced by genotype and environment (Walters *et al.* 2005), it can be postulated that genotype-dependent efficacy against Bgh was at least among other possible mechanisms based on resistance inducing effects of most of the tested biologicals. In contrast, the mostly genotype- and drought stress-independent efficiency of Blattgrün and raw milk has to be interpreted as a combination of direct fungicidal effects and an additional indirect effect. This additional indirect effect might be seen in the durability differences of p-i application efficiency against Bgh between GP and BCC436. On genotype GP Blattgrün and raw milk application directly influences the expression of *peroxidase 40*. The enzyme is associated with basal and penetration resistance. Additionally, priming effects of both treatments were found as *PR* genes (*PR1*, *PR5*, *PR17b*) respond faster to an infection after pretreatment with Blattgrün or raw milk. The results are in line with studies showing that it is possible to boost plant immune response through biological application and that primed plants show a faster immune response in the event of a subsequent infection, e.g. with Bgh (Molitor *et al.* 2011; Margaritopoulou *et al.* 2020; Bziuk *et al.* 2022). Biologicals have the potential to promote plant resilience to biotic and abiotic stress and to promote plant growth (Nada Parađiković *et al.* 2019; Sharma *et al.* 2014; van Oosten *et al.* 2017; Daayf *et al.* 2000; Pylak *et al.* 2019), as supported by this study. Thereby, repeated application of biologicals in cases of genotype-dependent efficiency can increase the effectiveness, also under simultaneous drought stress. Dry cultivation conditions thereby also significantly reduce the severity of mildew infection in the water treated control group in some cases, which is consistent with the observation that abiotic stress can reduce susceptibility to biotrophic pathogens (Saijo and Loo 2020).

As **milk was mostly effective against powdery mildew** regardless of genotype and drought stress and as it is hypothesised that its efficiency is multisided, preventing pathogen resistance, raw milk could be a promising candidate for broad use. But, the above-ground biomass is significant negatively influenced, i.e. reduced, depending on the genotype. And since the biomass correlates with the yield and raw milk also had a reducing effect on the chlorophyll content, the use of raw milk cannot be recommended. The user may have to choose between yield losses due to powdery mildew infection and yield losses due to a biomass-reducing and cost-intensive but effective biological application. However, it remains to be seen how raw milk and the other biologicals will affect yield and whether they are also effective against other pathogens occurring under field conditions. The use of milk is however questionable from an ethical point of view. Outside of private gardens and small-scale application and even there it is not sustainable without proof of a waste avoidance strategy or a circular economy to spray milk on fields.

The responsiveness of a genotype to biologicals can help to make sowing decisions. Thereby, more important than the general responsiveness, however, is the ratio of supportive to negative effects. With a ratio of 8:1 and 2.43:1, 6-row barley BCC436 and 6-row barley Morex respectively show the best combination of effects in the ‘biological trial’: a highly positive responsiveness and a low negative influenceability of physiological and morphological parameters - desirable for agriculture. In the ‘combined experiment’ the ratio of supportive to negative effects was slightly higher for 2-row barley GP (1.68:1) than for 6-row barley BCC436 (1.22:1) and much higher than for 2-row barley BCC1589 (0.09:1). According to Mitterbauer *et al.* (2015), 6-row barley genotypes are more responsive to higher CO_2 concentrations than 2-row barley, whether this also applies to the responsiveness to biologicals must be shown in further studies with larger sample size, however a first hint in this direction is given. Such ‘response ratios’ of genotypes to biologicals could be interesting for seed selection - even if numerous studies would still be necessary to create a comprehensive catalogue of relevant cultures and genotypes with their ratios.

This work provides **new insights, raises future research questions** and is the basis for follow-up research hypotheses. In this sense, the found high number of genotype-dependent effects of biologicals raises the question of whether the effect of biologicals depends not only on the genotype but also on the Bgh race or isolate. This should be the subject of research in subsequent studies. Moreover, follow-up projects could in microscopic tests focus on the influence of biologicals on conidial germination and their vitality. This could clarify whether biologicals such as Blattgrün cause an increased conidial mortality, i.e. are antifungal, prevent the penetration of conidia into the plant cell, e.g. through a field-horsetail based silicate accumulation in the cell wall or cuticle, or even conceal the host cell-typical structures and thus prevent their recognition by conidia, which would be expected with the same mortality but lower germination rate than in the control. One hypothesis to be tested in this context is related to the following observation: In conidia of Bgh, chitin deacetylase expression was detected, indicating that chitin is deacetylated to chitosan in the conidial cell wall provoking a lower plant immune response - a way of escaping the host defence system (Pham *et al.* 2019a). In this context, it could subsequently be tested whether a genotype-independent efficacy against Bgh is associated with a reduced chitin deacetylase activity in the cuticle of Bgh and whether this is related to an altered pH on the leaf surface as a consequence of biological application shifting the pH outside the enzyme optimum. If so, this could lead to increased chitin in the conidial cell wall and thus to an increased immune response of the host plant. Such an interaction could result in genotype-independent efficacy without being antifungal.

There is **currently no broad application of biologicals** in use on the field. Despite the discussed potential of biologicals, their widespread implementation may not only be inhibited by the diverse plant reactions to biological application, but also by high costs and the often still inadequately understood effects. In addition, the variability of botanicals and the influence of additional ingredients make it difficult to draw recommendations for botanicals’ practical implementation. The small sample of microbial and homeopathic products analysed here (three and two products respectively) makes it difficult to draw conclusions about their potential and limitations. For both, however, the data collected show that their effects were only supportive, if at all, under certain conditions (genotype, water regime), but never reliably reduced powdery mildew, as was the case with the botanical Blattgrün and the animal-based basic substance raw milk. Blattgrün may be the best candidate in this study for widespread use in practice due to its plant origin, which, unlike the use of raw milk, does not raise ethical or climate issues.

4.11 Conclusion

Climate change and associated weather extremes confront the world population with major challenges, as does the development of resistance of pest organisms to pesticides. Knowledge of biological and sustainable solutions to mitigate resistance breakdowns in plants are therefore essential as well as knowledge of tolerances of crops to pathogens. Thereby, the large number of Bgh pathotypes makes it difficult to create a comprehensive tolerance catalog of agronomically important genotypes. As part of this work, a statistically validated inoculation protocol was developed for studies with airborne inoculation, which can be used for the answering of different questions - including work on a comprehensive tolerance catalogue. The experiments conducted expand the knowledge of 40 genotypes in relation to Bgh A6, which includes the description of seven resistant, very low or low susceptible genotypes and two with intermediate susceptibility towards Bgh A6, about whose interaction with Bgh, according to my literature research, nothing was known so far. Additionally, a susceptibility continuum of spring barley towards Bgh A6 was found, reflecting i.e. the quantitative resistance of the genotypes. Over and above hexacosanol derivates were confirmed as the most common molecules in both susceptible and resistant genotypes. Furthermore, a hint on a molecule not previously mentioned in this context, with an heneicosane like mass spectra, which was only found in resistant genotypes, was identified, as well as an unknown molecule that was exclusively found on susceptible genotypes.

The application of biologicals influenced the interaction of all tested genotypes with Bgh A6 in various forms. The investigation of several genotypes showed the complexity of plant reactions. The examination of multiple traits made it possible to determine the responsiveness of the genotypes to the biologicals and to calculate a ratio of growth and health-promoting to detrimental effects. In addition, the cross-trait supportive potential of the biologicals could be assessed, Blattgrün and raw milk having significant positive effects on several traits while having negative effects on the fewest. The effect of both biologicals against Bgh A6 was shown to involve direct activation of pathogen defence-associated genes and priming effects.

The study illustrates that the isolated consideration of one parameter is insufficient to describe the effects of a biological and that multi-parametric tests are necessary to validate plant responses to biological applications. To assess the potential of biologicals, experiments should include combinations of simultaneous stressors, investigations of physiological and growth parameters as well as comparisons of different genotypes. The integration of physiological parameters such as the NBI and studies on stage-dependent effects would also be advisable. Furthermore, future plant protection strategies, including biologicals, should rely on individualised plant protection that involves not only the crop and the pathogen but also the genotype/variety, the location, the climate and the local availability and infrastructure of resources. Because then, biologicals can unify further services like climate-neutrality in production, or the integration into a circular economy which can be space- and resource-saving.

Supplementary data

S1 Additional information on plant height of 50 barley genotypes

Supplementary Table 1 a-d. Results of statistical analysis of plant height from 50 barley genotypes at four time points. Given are the estimate, the confidence interval (CI) and the p-value; genotypes named with JK_ are of unknown identity, genotypes named with x_ were found not to be genetically pure.

days after sowing	4			5		
Predictors	Estimates	CI	p	Estimates	CI	p
(Intercept)	3.36 ***	2.70 – 4.01	<0.001	6.76 ***	5.92 – 7.60	<0.001
gen [BCC1370]	0.09	-0.85 – 1.02	0.854	-0.38	-1.58 – 0.82	0.534
gen [BCC1373]	0.03	-0.90 – 0.96	0.953	-0.22	-1.42 – 0.97	0.713
gen [BCC1377]	-0.98 *	-1.91 – -0.05	0.039	-1.75 **	-2.95 – -0.56	0.004
gen [BCC1378]	-0.75	-1.69 – 0.18	0.114	-0.98	-2.19 – 0.22	0.109
gen [BCC1379]	0.23	-0.70 – 1.16	0.623	0.55	-0.65 – 1.75	0.369
gen [BCC1381]	-0.61	-1.55 – 0.33	0.201	-0.35	-1.56 – 0.86	0.57
gen [BCC1385]	0.24	-0.69 – 1.16	0.613	0.38	-0.81 – 1.57	0.534
gen [BCC1387]	-0.96 *	-1.90 – -0.02	0.045	-1.01	-2.21 – 0.19	0.099
gen [BCC1395]	0.38	-0.55 – 1.31	0.425	0.41	-0.80 – 1.61	0.507
gen [BCC1412]	-1.09 *	-2.02 – -0.16	0.022	-1.21 *	-2.40 – -0.02	0.046
gen [BCC1413]	-0.01	-0.94 – 0.93	0.991	0.31	-0.88 – 1.51	0.609
gen [BCC1430]	-0.42	-1.35 – 0.51	0.371	-0.45	-1.64 – 0.75	0.466
gen [BCC1433]	0.38	-0.55 – 1.31	0.42	0.76	-0.44 – 1.95	0.216
gen [BCC1452]	0.06	-0.94 – 1.06	0.905	-1.19	-2.42 – 0.04	0.058
gen [BCC1455]	0.11	-0.82 – 1.03	0.823	0.82	-0.38 – 2.02	0.179
gen [BCC1468]	-0.13	-1.05 – 0.80	0.787	0.08	-1.11 – 1.27	0.891
gen [BCC1476]	-1.85 ***	-2.78 – -0.92	<0.001	-2.28 ***	-3.47 – -1.09	<0.001
gen [BCC1479]	0.58	-0.35 – 1.52	0.222	0.42	-0.78 – 1.62	0.494
gen [BCC1589]	0	-0.93 – 0.93	0.995	-0.53	-1.72 – 0.66	0.38
gen [BCC432]	-0.7	-1.63 – 0.23	0.141	-0.83	-2.03 – 0.36	0.172
gen [BCC436]	-1.06 *	-1.98 – -0.13	0.025	-0.98	-2.17 – 0.21	0.105
gen [BCC526]	-0.95 *	-1.89 – -0.02	0.045	-0.87	-2.07 – 0.34	0.158
gen [BCC768]	-0.47	-1.40 – 0.47	0.326	-0.69	-1.89 – 0.51	0.258
gen [BCC812]	0.07	-0.86 – 1.00	0.88	-0.1	-1.29 – 1.10	0.874
gen [BCC817]	-0.93 *	-1.86 – -0.00	0.05	-1.32 *	-2.51 – -0.12	0.031
gen [BCC847]	0.36	-0.58 – 1.29	0.457	-0.34	-1.54 – 0.87	0.584
gen [BCC852]	-0.55	-1.48 – 0.38	0.247	-1.04	-2.23 – 0.15	0.087
gen [BCC875]	0.93	-0.00 – 1.87	0.05	1.28 *	0.07 – 2.49	0.039
gen [BCC899]	-0.91	-1.85 – 0.03	0.057	-1.13	-2.34 – 0.08	0.067
gen [BCC903]	-0.23	-1.16 – 0.70	0.628	-0.44	-1.65 – 0.76	0.471
gen [BCC913]	-0.29	-1.22 – 0.63	0.533	0.04	-1.15 – 1.23	0.949
gen [Golden Promise]	-1.40 ***	-2.21 – -0.60	0.001	-2.13 ***	-3.17 – -1.10	<0.001
gen [HOR2800]	-1.33 **	-2.27 – -0.40	0.005	-1.34 *	-2.54 – -0.13	0.029

Supplementary Table 1 b.

days after sowing	4			5		
Predictors	Estimates	CI	p	Estimates	CI	p
gen [JK_1_HOR7985]	0.03	-0.90 – 0.95	0.953	-0.02	-1.21 – 1.17	0.978
gen [JK_2_BCC192]	-2.04 ***	-2.97 – -1.10	<0.001	-2.29 ***	-3.50 – -1.09	<0.001
gen [JK_5_BCC1367]	-0.69	-1.63 – 0.24	0.146	-1.53 *	-2.72 – -0.34	0.012
gen [JK_6_BCC1402]	-0.54	-1.47 – 0.39	0.256	-0.82	-2.01 – 0.37	0.176
gen [JK3_HOR11374]	0.13	-0.80 – 1.06	0.782	0.72	-0.47 – 1.92	0.236
gen [JK4_BCC801]	-2.18 ***	-3.23 – -1.14	<0.001	-3.55 ***	-4.81 – -2.28	<0.001
gen [Morex]	-1.05 *	-1.98 – -0.12	0.026	-1.28 *	-2.47 – -0.09	0.035
gen [x_BCC1371]	-0.29	-1.22 – 0.64	0.54	-0.82	-2.01 – 0.37	0.176
gen [x_BCC1374]	-1.81 ***	-2.74 – -0.88	<0.001	-2.07 ***	-3.26 – -0.88	0.001
gen [x_BCC1415]	-0.09	-1.03 – 0.85	0.852	0.17	-1.04 – 1.38	0.785
gen [x_BCC1417]	0.43	-0.49 – 1.36	0.359	0.32	-0.87 – 1.51	0.596
gen [x_BCC1470]	-0.97 *	-1.90 – -0.04	0.042	-0.74	-1.95 – 0.46	0.226
gen [x_BCC846]	0.43	-0.50 – 1.37	0.364	1.19	-0.01 – 2.39	0.053
gen [x_BCC857]	-0.23	-1.16 – 0.70	0.63	-0.7	-1.91 – 0.50	0.254
gen [x_BCC868]	-0.18	-1.11 – 0.74	0.698	-0.55	-1.74 – 0.64	0.365
gen [x_BCC921]	0.59	-0.34 – 1.53	0.213	0.35	-0.87 – 1.56	0.576
	Random Effects					
σ2	0.52			1.11		
τ00 pot	0.17			0.25		
ICC	0.24			0.18		
N pot	102			102		
Observations	838			853		
Marginal R2 / Conditional R2	0.418 / 0.558			0.402 / 0.511		
	* p<0.05 ** p<0.01 *** p<0.001					

Supplementary Table 1 c.

days after sowing	6			7		
Predictors	Estimates	CI	p	Estimates	CI	p
(Intercept)	11.12 ***	10.21 – 12.02	<0.001	14.23 ***	13.36 – 15.09	<0.001
gen [BCC1370]	0.31	-0.98 – 1.60	0.638	0.77	-0.46 – 2.00	0.222
gen [BCC1373]	-0.5	-1.79 – 0.80	0.451	-0.36	-1.61 – 0.88	0.568
gen [BCC1377]	-1.35 *	-2.64 – -0.06	0.04	-0.63	-1.86 – 0.60	0.317
gen [BCC1378]	-0.67	-1.97 – 0.63	0.311	-0.52	-1.76 – 0.72	0.411
gen [BCC1379]	0.67	-0.62 – 1.96	0.307	0.77	-0.46 – 2.00	0.218
gen [BCC1381]	0.19	-1.12 – 1.49	0.777	0.8	-0.45 – 2.04	0.209
gen [BCC1385]	0.83	-0.45 – 2.12	0.204	1.27 *	0.04 – 2.49	0.042
gen [BCC1387]	0.18	-1.12 – 1.49	0.782	1.07	-0.18 – 2.31	0.093
gen [BCC1395]	0.87	-0.41 – 2.15	0.182	1.18	-0.05 – 2.41	0.061
gen [BCC1412]	-1.39 *	-2.68 – -0.11	0.033	-1.09	-2.31 – 0.13	0.081
gen [BCC1413]	0.89	-0.40 – 2.18	0.174	1.41 *	0.18 – 2.64	0.024
gen [BCC1430]	-0.28	-1.57 – 1.00	0.666	0.45	-0.78 – 1.69	0.472
gen [BCC1433]	1.79 **	0.50 – 3.08	0.006	2.17 ***	0.93 – 3.41	0.001
gen [BCC1452]	-0.53	-1.86 – 0.80	0.434	-0.81	-2.05 – 0.43	0.2
gen [BCC1455]	1.27	-0.02 – 2.57	0.054	1.76 **	0.53 – 2.99	0.005
gen [BCC1468]	0.94	-0.34 – 2.23	0.148	1.57 *	0.34 – 2.79	0.013
gen [BCC1476]	-1.90 **	-3.18 – -0.62	0.004	-1.30 *	-2.52 – -0.08	0.037
gen [BCC1479]	-0.38	-1.66 – 0.91	0.567	0.5	-0.73 – 1.74	0.425
gen [BCC1589]	-0.88	-2.16 – 0.40	0.176	-0.94	-2.17 – 0.29	0.135
gen [BCC432]	-0.47	-1.76 – 0.82	0.474	-0.1	-1.33 – 1.13	0.871
gen [BCC436]	-0.18	-1.46 – 1.10	0.786	1.14	-0.10 – 2.38	0.071
gen [BCC526]	0.49	-0.81 – 1.79	0.46	1.09	-0.16 – 2.35	0.088
gen [BCC768]	-0.23	-1.54 – 1.08	0.735	-0.2	-1.50 – 1.10	0.765
gen [BCC812]	0.56	-0.74 – 1.85	0.401	0.5	-0.73 – 1.73	0.424
gen [BCC817]	-0.73	-2.02 – 0.56	0.267	0.07	-1.16 – 1.30	0.911
gen [BCC847]	-0.17	-1.48 – 1.13	0.796	-0.38	-1.62 – 0.87	0.554
gen [BCC852]	0.26	-1.02 – 1.55	0.687	0.58	-0.65 – 1.81	0.352
gen [BCC875]	1.95 **	0.65 – 3.26	0.003	2.27 ***	1.02 – 3.51	<0.001
gen [BCC899]	-1.08	-2.39 – 0.22	0.104	-0.95	-2.20 – 0.31	0.138
gen [BCC903]	0.33	-0.97 – 1.62	0.621	0.65	-0.59 – 1.88	0.305
gen [BCC913]	1.11	-0.17 – 2.39	0.089	2.05 **	0.82 – 3.27	0.001
gen [Golden Promise]	-3.10 ***	-4.21 – -1.98	<0.001	-3.49 ***	-4.56 – -2.43	<0.001
gen [HOR2800]	-0.72	-2.02 – 0.57	0.275	0.04	-1.19 – 1.28	0.945

Supplementary Table 1 d.

days after sowing	6			7		
Predictors	Estimates	CI	p	Estimates	CI	p
gen [JK_1_HOR7985]	1.39 *	0.11 – 2.67	0.034	1.58 *	0.36 – 2.80	0.011
gen [JK_2_BCC192]	-1.43 *	-2.72 – -0.13	0.031	-0.36	-1.59 – 0.87	0.57
gen [JK_5_BCC1367]	-1.21	-2.50 – 0.07	0.065	-0.92	-2.15 – 0.31	0.141
gen [JK_6_BCC1402]	-0.65	-1.94 – 0.64	0.324	-1.1	-2.33 – 0.13	0.08
gen [JK3_HOR11374]	1.05	-0.24 – 2.34	0.111	1.26 *	0.03 – 2.49	0.044
gen [JK4_BCC801]	-3.05 ***	-4.37 – -1.73	<0.001	-1.94 **	-3.22 – -0.66	0.003
gen [Morex]	-0.26	-1.54 – 1.03	0.696	0.36	-0.87 – 1.58	0.569
gen [x_BCC1371]	-0.59	-1.88 – 0.70	0.369	-0.58	-1.81 – 0.65	0.352
gen [x_BCC1374]	-1.15	-2.44 – 0.15	0.083	0.41	-0.83 – 1.65	0.516
gen [x_BCC1415]	1	-0.32 – 2.32	0.137	1.36 *	0.11 – 2.61	0.032
gen [x_BCC1417]	0.83	-0.45 – 2.11	0.205	0.94	-0.28 – 2.17	0.13
gen [x_BCC1470]	0.43	-0.87 – 1.72	0.518	0.82	-0.47 – 2.11	0.211
gen [x_BCC846]	2.10 **	0.79 – 3.40	0.002	2.49 ***	1.10 – 3.89	<0.001
gen [x_BCC857]	-0.04	-1.34 – 1.27	0.957	0.18	-1.07 – 1.43	0.78
gen [x_BCC868]	-0.35	-1.64 – 0.94	0.596	-0.02	-1.24 – 1.21	0.979
gen [x_BCC921]	0.98	-0.33 – 2.29	0.142	1.50 *	0.15 – 2.84	0.029
	Random Effects					
σ2	1.46			1.34		
τ00 pot	0.26			0.24		
ICC	0.15			0.15		
N pot	102			102		
Observations	841			812		
Marginal R2 / Conditional R2	0.441 / 0.526			0.493 / 0.571		
	* p<0.05 ** p<0.01 *** p<0.001					

S2 Additional information on gene expression

Supplementary Table 2 a-b. Cycle threshold values of *Ubiquitin* over time and under use of biologicals and *Blumeria graminis* f.sp. *hordei* (Bgh) race A6 inoculation. Given are sample name, time (A = hours after application; B = hours after inoculation with Bgh); status (h = healthy and uninfected; i = inoculated), treatment and mean cycle threshold (CT) values of three technical replicates.

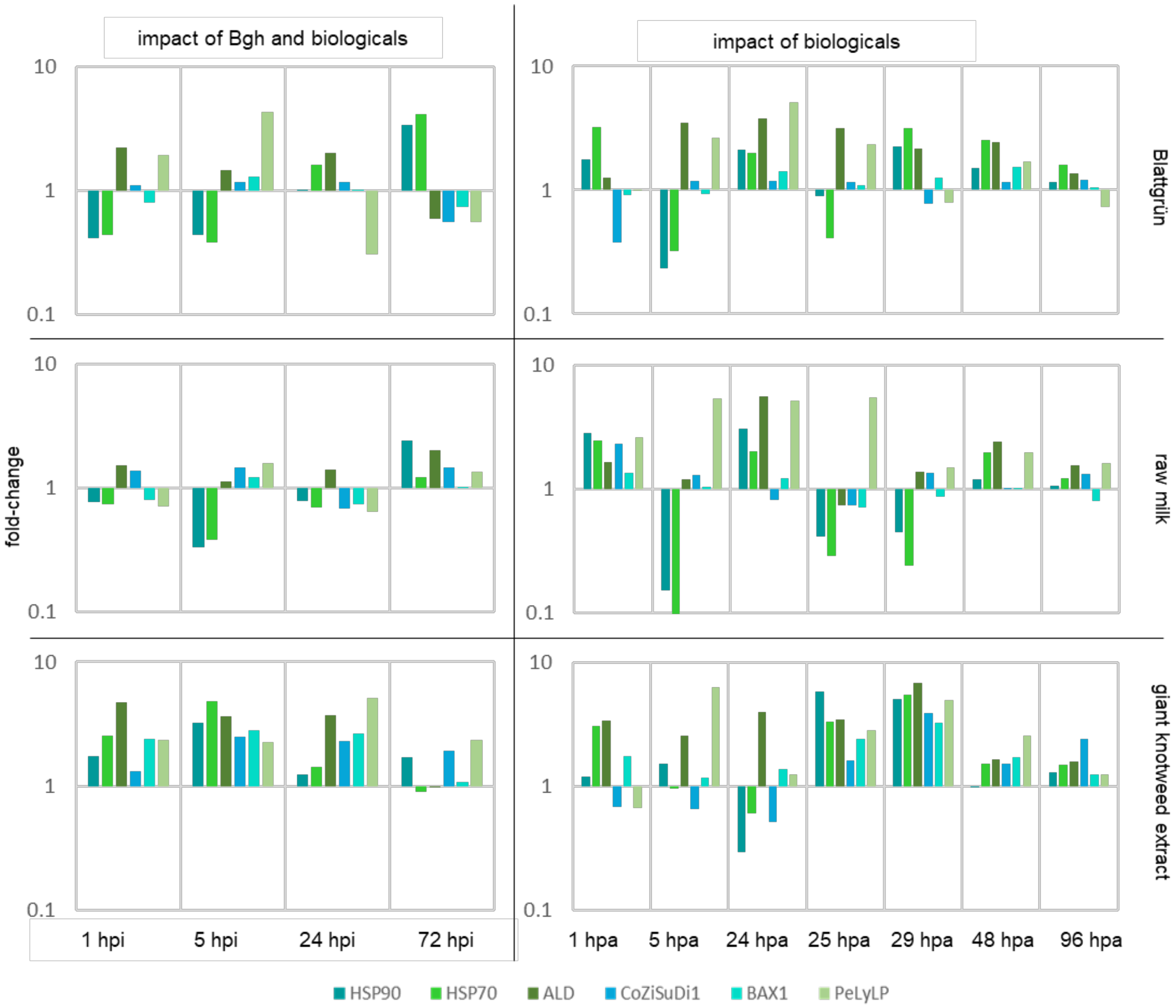
sample name	time	status	treatment	mean CT
Bl1A.1	1A	h	Blattgrün	24.129
Bl5A.3	5A	h	Blattgrün	23.572
Bl0B.1	24A	h	Blattgrün	23.285
Bl-1B.2	25A	h	Blattgrün	23.138
Bl-5B.1	29A	h	Blattgrün	23.502
Bl-24B.2	48A	h	Blattgrün	22.642
Bl-72B.1	96A	h	Blattgrün	23.192
SKE1A.1	1A	h	Giant knotweed leaf extract	24.120
SKE5A.1	5A	h	Giant knotweed leaf extract	23.287
SEK0B.1	24A	h	Giant knotweed leaf extract	23.891
SKE-1B.1	25A	h	Giant knotweed leaf extract	23.379
SKE-5B.1	29A	h	Giant knotweed leaf extract	23.723
SKE-24B.1	48A	h	Giant knotweed leaf extract	22.719
SKE-72B.1	96A	h	Giant knotweed leaf extract	23.692
Y1A.1	1A	h	Raw milk	24.435
Y5A.1	5A	h	Raw milk	23.428
Y0B.2	24A	h	Raw milk	23.292
Y-1B.2	25A	h	Raw milk	23.467
Y-5B.1	29A	h	Raw milk	22.413
Y-24B.2	48A	h	Raw milk	22.050
Y-72B.1	96A	h	Raw milk	22.348
C0A.1.1	0A	h	Control	23.747
C1A.1.1	1A	h	Control	23.572
C5A.1.1	5A	h	Control	23.409
C0B.1.1	24A	h	Control	22.221
C-1B.1.1	25A	h	Control	22.442
C-5B.1.1	29A	h	Control	22.197
C-24B.1.1	48A	h	Control	21.987
C-72B.1.1	96A	h	Control	23.240
Bl.Bgh-1B.1	1B	i	Blattgrün	23.128
Bl.Bgh-5B.3	5B	i	Blattgrün	21.952
Bl.Bgh-24B.2	24B	i	Blattgrün	22.594
Bl.Bgh-72B.1	72B	i	Blattgrün	24.981
SKE.Bgh-1B.1	1B	i	Giant knotweed leaf extract	23.779
SKE.Bgh-5B.1	5B	i	Giant knotweed leaf extract	24.207
SKE.Bgh-24B.1	24B	i	Giant knotweed leaf extract	24.228
SKE.Bgh-72B.1	72B	i	Giant knotweed leaf extract	23.924

Supplementary Table 2 b.

sample name	time	status	treatment	mean CT
Y.Bgh-1B.1	1B	i	Raw milk	23.743
Y.Bgh-5B.1	5B	i	Raw milk	22.698
Y.Bgh-24B.1	24B	i	Raw milk	22.368
Y.Bgh-72B.1	72B	i	Raw milk	23.266
C.Bgh-1B.1.1	1B	i	Control	22.373
C.Bgh-5B.1.1	5B	i	Control	21.604
C.Bgh-24B.1.1	24B	i	Control	22.788
C.Bgh-72B.1.1	72B	i	Control	22.801

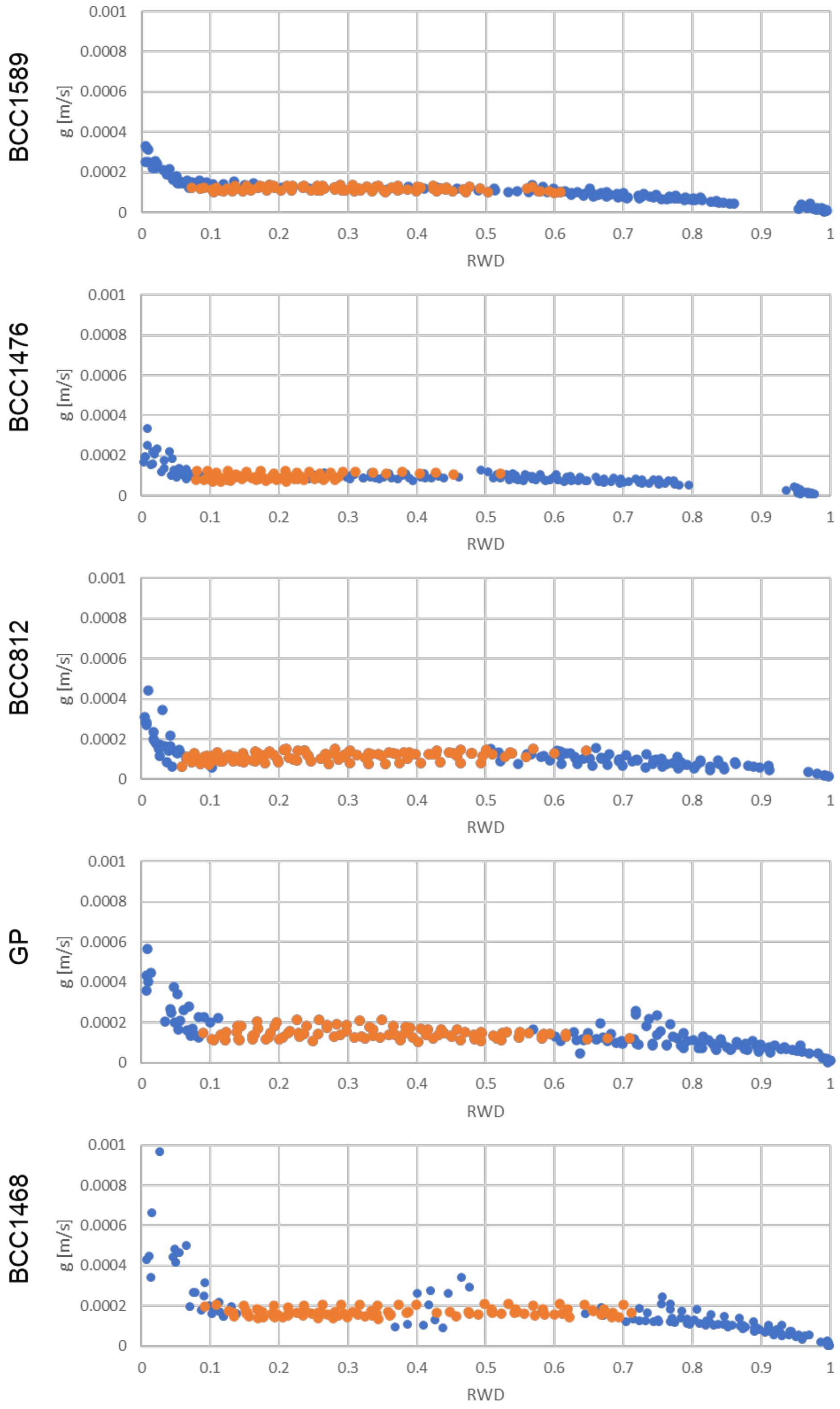
Supplementary Table 3. Pretest results on primer efficiency of 17 primer pairs. Written in bold type: primer pairs that were further studied and the corresponding primer efficiency.

Gene	Primer efficiency
Alcohol dehydrogenase	2.033
Ascorbate peroxidase 1	**1.985**
BAX inhibitor 1	2.003
Copper-zinc superoxide dismutase 1	1.977
Glutathione reductase 1	**2.024**
Heat shock protein 90-1	2.066
Heat shock protein 70	2.932
Pathogenesis-related protein 17b	**2.023**
Ubiquitin	**2.057**
Respiratory burst oxidase homologue F2	**1.982**
Pathogenesis-related protein 1b	**1.933**
Pectin-lyase like Protein	2.137
Peroxidase 40	**1.963**
Lesion simulation disease 1	**2.037**
Non-expressor of pathogenesis-related genes 1	**2.101**
Pathogenesis-related protein 5	**2.029**
Ethylene response factor 1	**1.911**

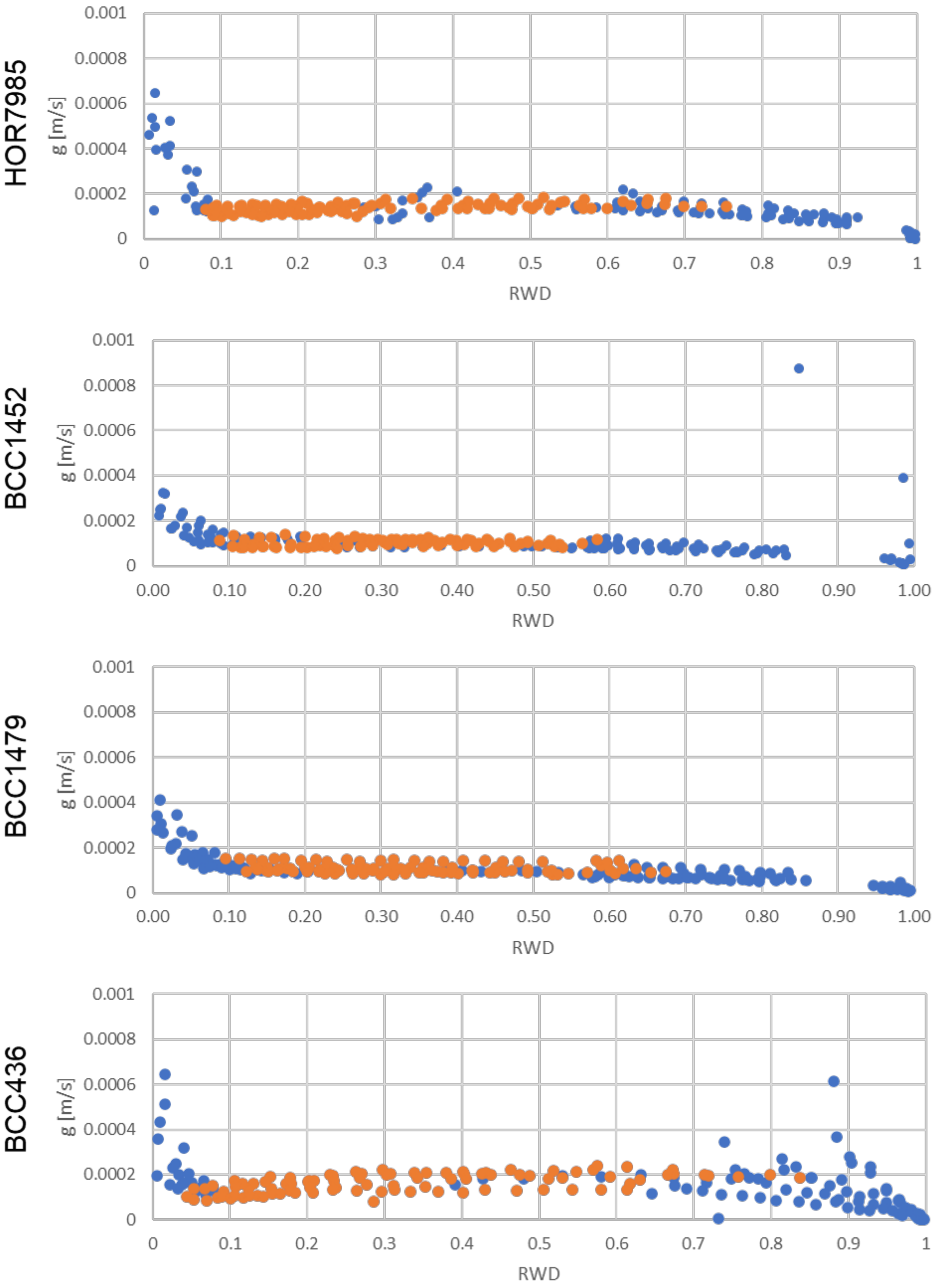


Supplementary Figure 1. Fold-change of gene expression in response to a biological application and in combination with an *Blumeria graminis* f.sp. *hordei* inoculation over time. Shown is the fold-change of genes whose expression was not studied with repetition; hpi: hours post inoculation; hpa: hours post application; HSP90, HSP70: *heat shock protein 90* or *70* respectively; ALD: *alcohol dehydrogenase*; CoZiSuDi1: *copper-zinc superoxide dismutase 1*; BAX1: *BAX inhibitor 1*; PeLyLP: *pectin-lyase like protein*.

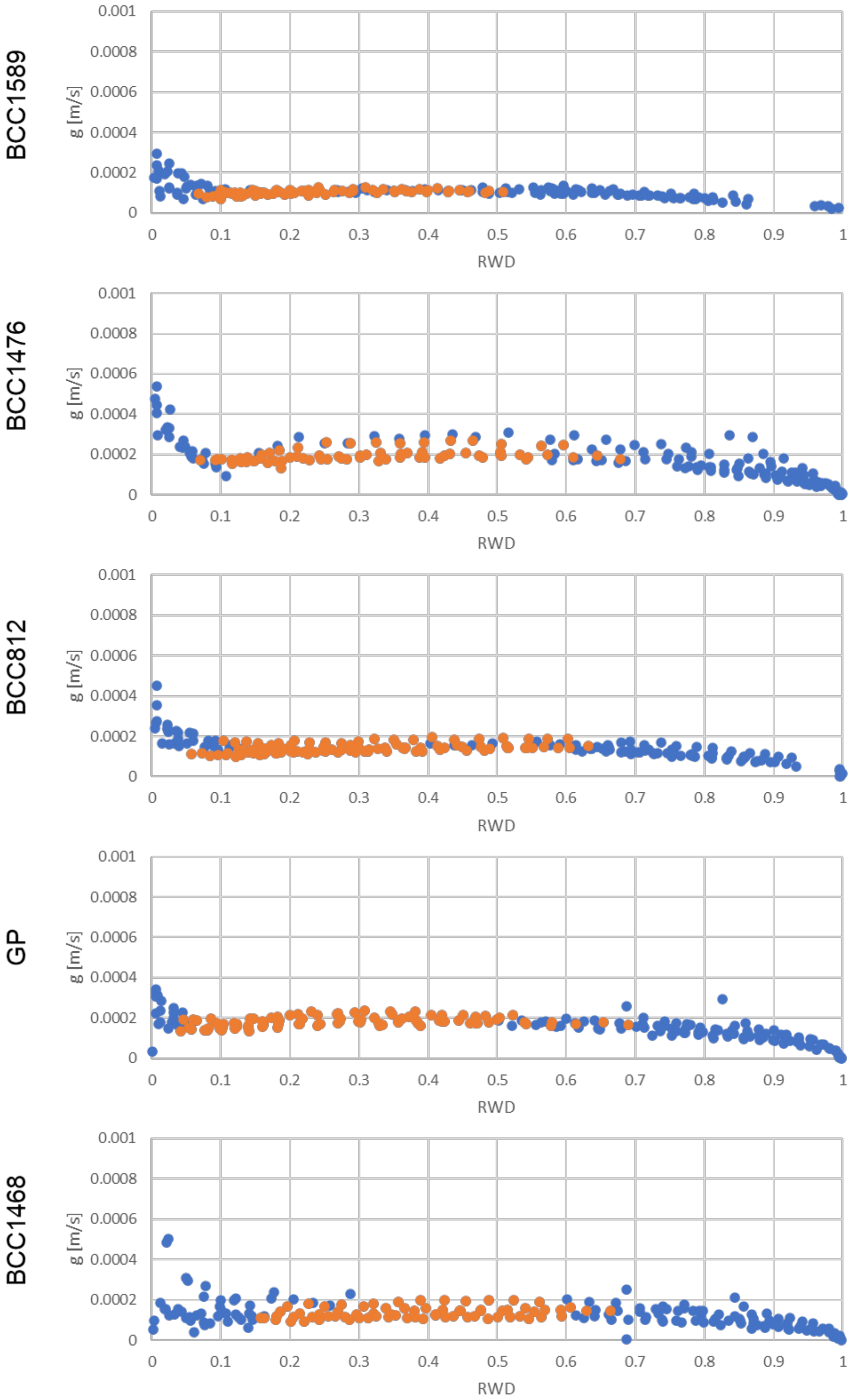
S3 Additional information about minimum conductance of leaves to water



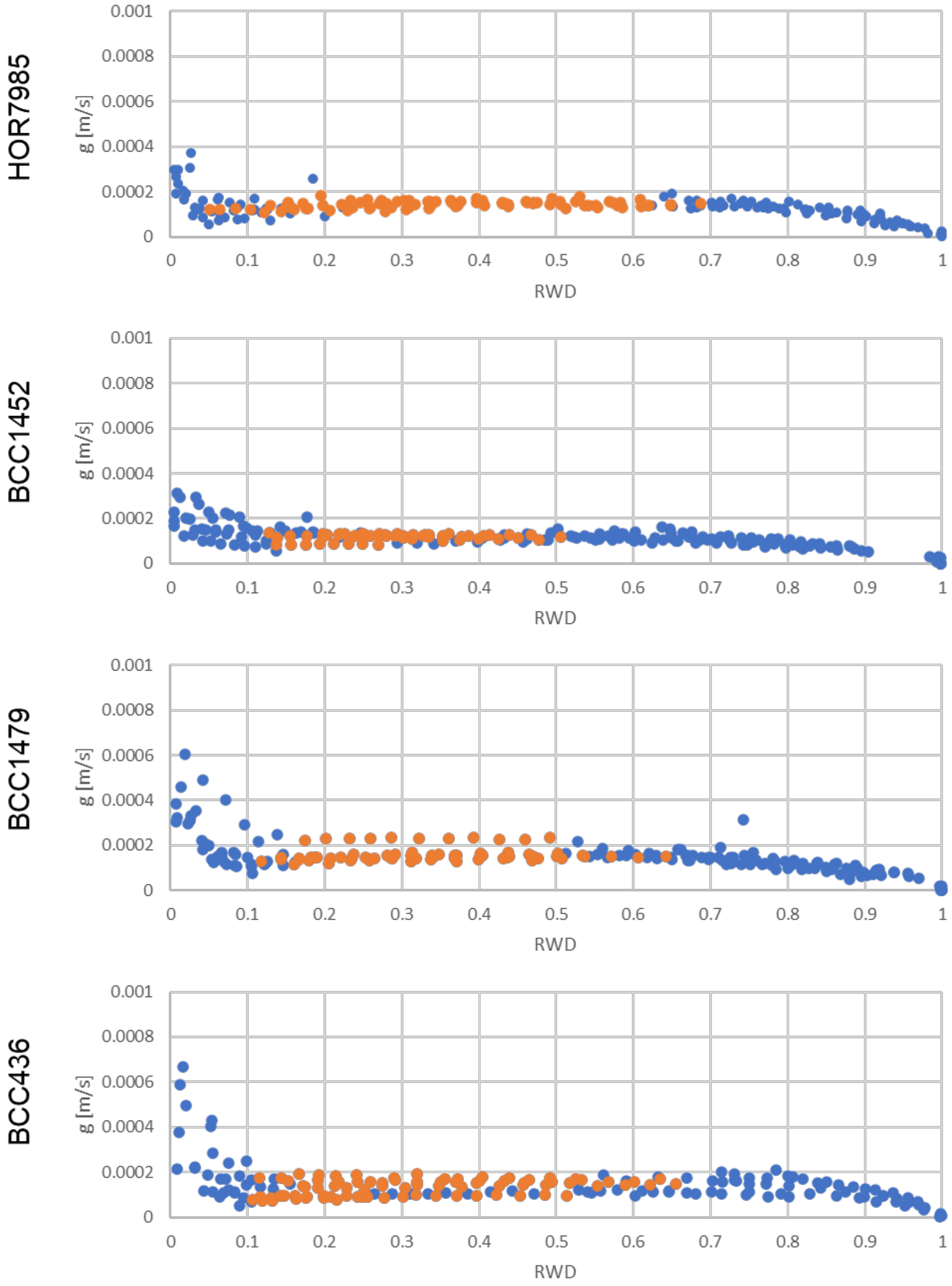
Supplementary Figure 2. Dehydration curves of *Blumeria graminis* f.sp. *hordei* resistant and mid susceptible genotypes. Plotted are the values of five leaves per genotype; RWD: relative water deficit; GP: Golden Promise; blue dots: not included in the minimum conductance; orange dots: included in the minimum conductance; for the conductance only positive values and for RWD only those not higher than one are shown.



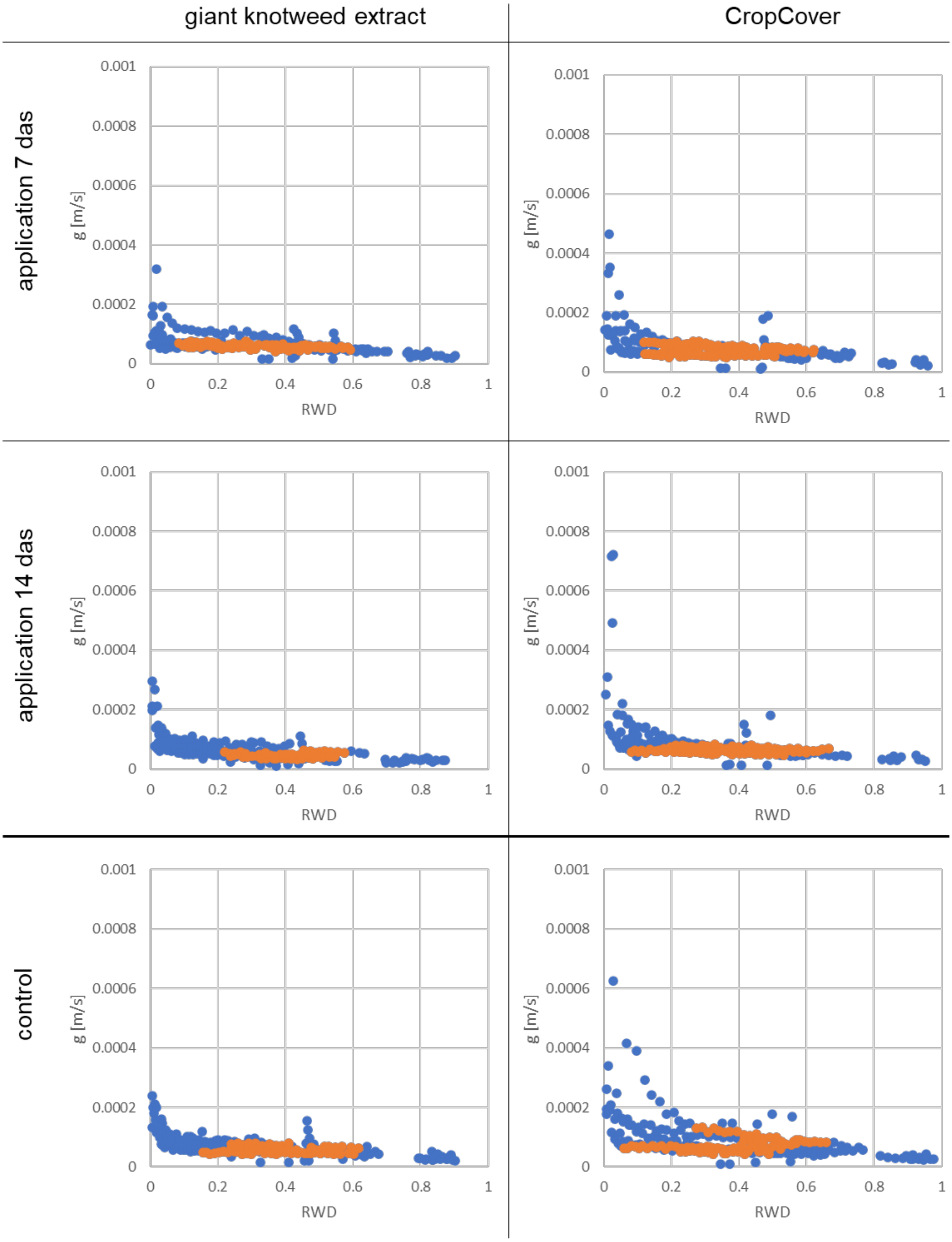
Supplementary Figure 3. Dehydration curves of *Blumeria graminis* f.sp. *hordei* mid, high and very high susceptible genotypes. Plotted are the values of five leaves per genotype; RWD: relative water deficit; HOR7985: JK_1_HOR7985; blue dots: not included in the minimum conductance; orange dots: included in the minimum conductance; for the conductance only positive values and for RWD only those not higher than one are shown.



Supplementary Figure 4. Dehydration curves of *Blumeria graminis* f.sp. *hordei* (Bgh) inoculated plants resistant and mid susceptible towards Bgh race A6. Plotted are the values of five leaves per genotype; RWD: relative water deficit; GP: Golden Promise; blue dots: not included in the minimum conductance; orange dots: included in the minimum conductance; for the conductance only positive values and for RWD only those not higher than one are shown.



Supplementary Figure 5. Dehydration curves of *Blumeria graminis* f.sp. *hordei* (Bgh) inoculated plants mid, high and very high susceptible towards Bgh race A6. Plotted are the values of five leaves per genotype, in case of JK_1_HOR7985 four leaves; RWD: relative water deficit; HOR7985: JK_1_HOR7985; blue dots: not included in the minimum conductance; orange dots: included in the minimum conductance; for the conductance only positive values and for RWD only those not higher than one are shown.



Supplementary Figure 6. Dehydration curves of barley genotype Golden Promise treated with CropCover or giant knotweed leaf extract at two time points. Plotted are the values of five leaves per plot, in case of CropCover 7 das four leaves; control: water treated; RWD: relative water deficit; das: days after sowing; blue dots: not included in the minimum conductance; orange dots: included in the minimum conductance; for the conductance only positive values and for RWD only those not higher than one are shown.

S4 Additional information (sample size, descriptive statistics) from the ‘biological trial’ and the ‘milk experiment’

Supplementary Table 4. Sample size (n) and mean of the number of pustules on the first leaf six days after inoculation in the biological trial.

	BCC436		Golden Promise		JK_1_HOR7985		Morex	
treatment	mean	n	mean	n	mean	n	mean	n
control	51.858	120	18.494	395	32.715	379	39.394	398
Kumulus WG	10.583	36	1.691	327	5.268	332	6.937	331
Ackerschachtelhalm Extrakt Compositum	57.063	32	13.854	41	35.366	41	45.925	40
Alginure Agro Support F	40.556	36	22.051	39	40.000	41	46.725	40
AMN BioVit	37.167	36	13.545	33	34.861	36	40.129	31
ASL Kombi Power	53.031	32	19.098	41	47.744	39	58.694	36
Baking powder	80.125	40	33.278	36	22.030	33	42.809	47
Biplantol agrar	14.300	20	14.811	37	28.225	40	36.475	40
Biplantol mycos V forte	16.667	30	12.184	38	35.111	36	30.150	40
Blattgrün	12.667	36	1.976	41	2.325	40	6.263	38
Brennessel Extrakt Compositum	54.906	32	16.585	41	45.784	37	48.525	40
CropCover	58.538	39	22.639	36	29.531	32	53.000	46
Elot-Vis Green	32.833	36	17.000	39	37.976	42	51.854	41
Equisetum Plus	56.875	32	15.125	40	20.268	41	26.513	39
FytoSave	63.094	32	14.949	39	20.405	38	32.514	37
Burdock root extract	60.813	32	16.500	32	34.324	34	25.273	33
Giant knotweed leaf extract	33.028	36	12.080	50	28.588	34	28.323	31
Potta Sol	50.969	32	15.750	48	28.981	52	40.426	47
RhizoVital 42	39.344	32	12.025	40	32.381	42	34.195	41
Raw milk	17.250	40	4.944	36	9.486	35	15.361	61
Serenade ASO	37.278	36	13.525	40	19.927	41	26.450	40
T-Gro	41.548	31	13.205	39	21.927	41	26.784	37
Tillecur	24.531	32	10.805	41	27.756	41	44.350	40

Supplementary Table 5. Sample size of the experiment on the preventive efficacy of milk and milk associated products against *Blumeria graminis* f.sp. *hordei* race A6 on four spring barley genotypes.

treatment	Morex	JK_1_HOR7985	BCC436	Golden Promise
Lactoperoxidase	23	24	28	32
Milk powder	23	24	24	32
Lactoferrin	24	24	28	32
Lactose	24	24	24	32
Whey	24	24	28	28
UHT milk	24	24	24	32
Pasteurized milk	24	24	24	32
Control	35	36	48	59
Raw milk	61	35	40	36

S5 Additional information (sample size, descriptive statistics) from the ‘combined experiment’

Supplementary Table 6. Means and standard errors of infection signs on three barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei* race A6 and cultivated under dry conditions. BRE=burdock root extract, GKLE: giant knotweed leaf extract, dai: days after infection; Biplantol mycos: Biplantol mycos V forte. The units of the mean values are: 6 dai in pustules on the first leaf, 13 dai in percentage of infected leaf area and 20 dai in infection categories. For information on sample sizes see S. Table 8; empty field = not studied.

	dry																	
	mean									standard error								
	Golden Promise			BCC436			BCC1589			Golden Promise			BCC436			BCC1589		
dai Treatment	6	13	20	6	13	20	6	13	20	6	13	20	6	13	20	6	13	20
Control	2.855	5.007	2.891	13.417	8.863	3.983	0.030	0.000	1.207	0.406	0.846	0.132	2.399	0.931	0.151	0.030	0.000	0.091
Kumulus WG	0.207	0.124	1.596	0.906	0.469	2.400	0.000	0.000	1.000	0.064	0.055	0.079	0.292	0.099	0.102	0.000	0.000	0.000
Baking powder	3.905	4.708	2.208	1.842	0.781	2.250				1.359	1.868	0.134	0.663	0.259	0.162			
Biplantol mycos	4.129	8.164	3.371	13.419	10.035	4.781	0.000	0.000	1.114	0.520	1.496	0.159	2.207	1.150	0.133	0.000	0.000	0.068
Blattgrün	0.417	0.715	2.389	2.606	2.465	3.824	0.000	0.000	1.000	0.175	0.250	0.121	1.100	0.850	0.107	0.000	0.000	0.000
CropCover	3.071	2.000	2.174	4.313	1.615	2.273				1.247	0.694	0.162	1.762	0.488	0.248			
Elot-Vis Green	1.125	2.757	3.371	9.394	8.438	4.853	0.000	0.000	1.088	0.194	0.525	0.143	1.359	1.021	0.075	0.000	0.000	0.049
BRE	6.833	7.063	2.292	6.235	1.840	2.870				2.107	1.845	0.221	2.493	0.484	0.254			
GKLE	1.676	3.243	2.941	10.857	8.688	4.722	0.000	0.000	1.000	0.315	0.551	0.179	1.931	1.065	0.110	0.000	0.000	0.000
Raw milk (100%)	0.842	0.167	1.455	0.700	0.313	1.739				0.414	0.078	0.109	0.282	0.217	0.094			
Raw milk (10%)	1.063	0.667	1.609	1.000	0.646	1.913				0.359	0.262	0.137	0.332	0.173	0.107			
Serenade ASO	3.848	5.309	3.656	13.333	9.396	4.727	0.031	0.000	1.000	0.777	0.995	0.159	2.290	1.220	0.090	0.031	0.000	0.000

Supplementary Table 7. Means and standard errors of infection signs on three barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei* race A6 and cultivated under well-watered conditions. BRE=burdock root extract, GKLE: giant knotweed leaf extract, dai: days after infection; Biplantol mycos: Biplantol mycos V forte. The units of the mean values are: 6 dai in pustules, 13 dai in percentage of infected leaf area and 20 dai in infection categories. For information on sample sizes see S. Table 8.

	well-watered																	
	mean									standard error								
	Golden Promise			BCC436			BCC1589			Golden Promise			BCC436			BCC1589		
dai Treatment	6	13	20	6	13	20	6	13	20	6	13	20	6	13	20	6	13	20
Control	16.529	20.785	4.239	39.103	20.250	4.441	0.030	0.000	1.114	2.335	2.245	0.084	4.802	1.836	0.103	0.030	0.000	0.068
Kumulus WG	0.670	0.806	2.298	3.913	1.470	2.839	0.000	0.000	1.043	0.209	0.178	0.062	0.855	0.259	0.091	0.000	0.000	0.024
Baking powder	9.917	8.896	3.167	7.400	6.323	3.565				2.412	2.478	0.167	2.710	1.302	0.164			
Biplantol mycos	14.333	24.500	4.222	46.735	23.458	4.647	0.143	0.146	1.194	1.748	1.969	0.106	8.078	2.585	0.093	0.117	0.115	0.067
Blattgrün	1.600	0.875	3.029	14.056	6.722	4.250	0.059	0.029	1.059	0.394	0.316	0.126	3.459	1.276	0.115	0.059	0.029	0.041
CropCover	13.348	9.896	3.087	10.913	7.597	3.500				3.644	2.972	0.165	2.105	1.331	0.120			
Elot-Vis Green	8.057	12.646	4.314	46.176	22.236	4.824	0.000	0.000	1.028	1.379	1.823	0.090	7.541	2.330	0.066	0.000	0.000	0.028
BRE	15.870	18.125	3.783	22.250	11.083	3.944				3.738	3.705	0.088	5.375	1.967	0.235			
GKLE	9.882	13.042	4.000	37.594	18.563	4.875	0.030	0.000	1.114	1.304	1.301	0.120	5.951	1.889	0.059	0.030	0.000	0.055
Raw milk (100%)	1.750	0.771	2.167	2.833	1.083	2.500				0.609	0.269	0.115	0.884	0.510	0.159			
Raw milk (10%)	3.211	3.063	2.316	7.455	3.104	3.130				1.260	1.565	0.134	1.652	0.582	0.170			
Serenade ASO	11.257	12.556	4.200	48.257	22.840	4.771	0.031	0.000	1.057	1.357	1.480	0.114	9.160	2.665	0.083	0.031	0.000	0.040

Supplementary Table 8. Sample size of infection measurements in the combined experiment. Given are the sample sizes (n) per water status (wc), time point (dai = days after inoculation), genotype and treatment; wet: well-watered cultivation conditions, dry: dry cultivation conditions.

		Golden Promise			BCC436			BCC1589		
Treatment	wc	6 dai	13 dai	20 dai	6 dai	13 dai	20 dai	6 dai	13 dai	20 dai
Control	dry	62	69	64	60	60	60	33	34	29
Kumulus WG	dry	82	93	89	85	96	90	71	72	71
Baking Powder	dry	21	24	24	19	24	24			
Biplantol mycos V forte	dry	31	35	35	31	36	32	35	35	35
Blattgrün	dry	36	36	36	33	36	34	35	36	36
CropCover	dry	14	24	23	16	24	22			
Elot-Vis Green	dry	32	36	35	33	36	34	34	34	34
Burdock Root Extract	dry	18	24	24	17	24	23	36	36	36
Giant Knotweed Extract	dry	34	35	34	35	36	36			
Raw milk (100%)	dry	19	24	22	20	24	23			
Raw milk (10%)	dry	16	24	23	20	24	23			
Serenade ASO	dry	33	34	32	33	36	33	32	36	36
Control	wet	70	72	71	58	60	59	33	36	35
Kumulus WG	wet	94	96	94	92	96	93	70	72	70
Baking Powder	wet	24	24	24	20	24	23			
Biplantol mycos V forte	wet	36	36	36	34	36	34	35	36	36
Blattgrün	wet	35	36	35	36	36	36	34	34	34
CropCover	wet	23	24	23	23	24	24			
Elot-Vis Green	wet	35	36	35	34	36	34	35	36	36
Burdock Root Extract	wet	23	24	23	20	24	18			
Giant Knotweed Extract	wet	34	36	36	32	36	32	33	36	35
Raw milk (100%)	wet	24	24	24	24	24	24			
Raw milk (10%)	wet	19	24	19	22	24	23			
Serenade ASO	wet	35	36	35	35	36	35	32	36	35

S6 Additional information on above-ground dry biomass of barley plants

Supplementary Table 9 a-b. Dry weight of above-ground plant biomass of 14-day old barley seedling of four genotypes treated with biologicals or Kumulus WG and inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Given is the number of samples (n), the mean, the lowest (min) and highest (max) dry weight of the biomass; mean, min and max are given in mg; bold type: highest and lowest value.

Genotype	**Golden Promise**				**Morex**				**JK1_HOR7985**				**BCC436**			
Treatment	n	mean	min	max	n	mean	min	max	n	mean	min	max	n	mean	min	max
Ackerschachtelhalm Extrakt Compositum	40	57.1	10	111	36	74.2	12	137	38	81.1	4	151	32	38.9	11	76
Alginure Agro Support F	39	62.2	17	104	40	74.6	36	124	41	84.8	29	158	36	42.9	18	93
AMN BioVit	31	34.8	12	52	31	47.9	16	83	31	43.8	20	84	36	45.9	20	93
ASL Kombi Power	40	53.4	18	87	36	79.1	32	134	39	68.2	8	108	31	36.7	**10**	69
Baking powder	35	28.8	15	40	25	**32.3**	11	**41**	30	31.6	19	51	24	39.3	21	81
Biplantol agrar	37	59.9	20	88	40	79.6	30	123	40	84.5	28	145	20	38.5	19	64
Biplantol mycos V forte	38	60.9	24	82	40	78.4	23	123	36	85.9	31	129	30	33.9	19	68
Brennessel Extrakt Compositum	41	65.4	28	92	40	79.1	36	130	37	76.1	16	131	32	38.4	17	72
Burdock root extract	28	36.1	7	53	32	46.6	11	71	33	56.8	19	214	32	44.8	19	86
Control	382	53.0	**4**	**198**	377	69.8	9	**550**	364	68.3	**4**	150	92	39.8	13	83
CropCover	33	**27.5**	13	**39**	25	35.0	17	48	30	32.0	14	47	23	41.7	21	**106**
Elot-Vis Green	39	62.2	31	101	41	80.0	12	136	42	88.8	16	134	36	43.6	13	74
Equisetum Plus	40	**70.7**	**34**	116	39	**85.1**	27	143	41	**89.0**	13	143	32	39.4	19	88
FytoSave	39	56.4	11	88	37	79.4	**5**	131	38	80.2	19	119	32	38.6	13	75

Supplementary Table 9 b.

Genotype	**Golden Promise**				**Morex**				**JK1_HOR7985**				**BCC436**			
Treatment	n	mean	min	max	n	mean	min	max	n	mean	min	max	n	mean	min	max
Giant knotweed extract	45	34.4	14	58	30	52.7	25	80	34	66.1	19	**540**	36	41.6	17	87
Kumulus WG	322	56.5	5	123	324	71.8	8	147	331	73.6	7	169	36	**56.8**	**28**	100
Potta Sol	48	62.9	16	100	47	76.7	**38**	127	52	76.2	11	128	32	40.0	20	94
Raw milk	34	29.4	13	42	40	33.2	9	47	33	**30.9**	7	**46**	24	**28.4**	13	**39**
RhizoVital®42	40	59.7	21	119	41	66.6	20	104	42	68.5	**34**	122	32	38.0	15	69
Blattgrün	41	46.9	16	76	38	73.3	15	117	40	65.2	18	114	36	35.9	16	68
Serenade ASO	40	55.6	19	87	40	76.4	6	118	41	77.1	11	158	36	48.1	16	95
T-Gro	39	59.4	20	86	37	78.5	25	117	41	78.0	28	132	30	41.8	20	68
Tillecur	41	51.9	27	85	40	74.9	26	121	41	69.5	33	122	32	37.0	11	77

Supplementary Table 10 a-b. Descriptive data of above-ground dry biomass of three barley genotypes 28 days after sowing treated with biologicals or Kumulus WG and inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Given are the number of investigated plants (n; sample size corresponds to the sample size of the dualex data), the mean of above-ground dry biomass in mg, the standard deviation (sd), the minimum (min) and the maximum (max) weight of the plants, for each genotype and product under well-watered and dry cultivation conditions.

well-watered															
	Golden Promise					**BCC436**					**BCC1589**				
Treatment	n	mean	sd	min	max	n	mean	sd	min	max	n	mean	sd	min	max
Control	71	237.552	105.527	28.000	441.200	58	255.028	120.382	46.000	636.400	22	356.691	123.564	13.800	543.200
Kumulus WG	94	260.745	90.068	58.000	427.500	93	319.431	127.758	43.100	630.900	69	365.770	100.325	13.000	578.700
Baking Powder	24	133.875	59.018	24.000	236.000	23	165.652	45.059	31.000	237.000					
Biplantol mycos V forte	36	272.481	79.107	136.700	464.200	33	316.476	113.210	59.800	535.000	35	396.271	127.885	17.600	661.200
Blattgrün	34	253.438	71.257	96.700	377.600	36	313.672	120.810	67.100	556.100	34	378.712	117.870	93.500	561.200
CropCover	23	142.391	49.028	17.000	237.000	24	157.417	43.447	70.000	250.000					
Elot-Vis Green	35	292.300	73.694	185.400	511.400	34	339.603	84.653	133.900	502.400	36	389.508	107.492	47.300	623.900
Burdock Root Extract	23	129.657	46.191	28.000	206.100	18	172.111	57.199	96.000	305.000					
Giant Knotweed Extract	36	268.719	72.891	85.000	395.000	32	333.247	121.658	48.500	562.700	32	389.434	112.652	193.200	714.000
Raw milk (100%)	24	155.750	53.697	60.000	257.000	24	167.875	49.352	39.000	254.000					
Raw milk (10%)	19	153.211	50.220	82.000	280.000	23	179.870	55.790	43.000	271.000					
Serenade ASO	35	283.226	111.575	140.200	784.300	35	324.277	102.026	25.700	504.700	32	425.472	138.503	192.700	749.300

Supplementary Table 10 b.

dry															
	Golden Promise					BCC436					BCC1589				
Treamtnet	n	mean	sd	min	max	n	mean	sd	min	max	n	mean	sd	min	max
Control	64	71.619	37.757	15.000	143.400	60	63.408	38.223	5.400	198.800	21	125.810	44.579	58.800	231.800
Kumulus WG	89	80.146	45.801	6.000	264.800	90	77.299	41.525	2.000	190.000	71	101.118	39.210	13.000	195.200
Backing Powder	24	45.333	15.491	15.000	72.000	24	46.958	26.709	6.000	99.000					
Biplantol mycos	35	63.900	30.327	16.300	152.000	32	67.241	27.317	20.700	145.700	35	102.126	42.912	17.900	210.600
SBG	36	66.256	30.170	10.000	129.300	34	62.165	55.565	2.800	215.600	36	67.156	35.456	15.200	170.700
CropCover	23	45.043	26.965	5.000	92.000	21	37.810	16.467	1.000	72.000					
Elot-Vis Green	35	86.737	30.607	39.600	176.300	34	73.394	50.592	10.600	237.100	34	119.224	83.233	51.000	539.100
Burdock Root Extract	24	43.088	22.996	12.000	78.000	23	47.652	23.163	11.000	88.000					
Giant Knotweed Extract	35	84.243	36.327	24.200	179.000	36	69.425	38.956	5.900	198.600	36	91.100	32.138	44.000	178.500
Raw milk (100%)	22	45.882	23.820	3.000	88.000	23	46.565	20.324	10.000	87.000					
Raw milk (10%)	23	37.739	17.410	7.000	83.000	23	38.565	22.006	9.000	103.000					
Serenade ASO	33	65.267	29.644	9.200	133.800	33	73.200	43.864	13.600	165.500	35	80.251	33.295	19.100	172.700

S7 Additional information on plant height in the ‘combined experiment’

Supplementary Table 11 a-d. Plant height of three barley genotypes over time that were treated with biologicals or Kumulus WG and inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Given is the number of plants, mean and standard error per genotype, treatment and timpoint; n= sample size, se= standard error, wet= well-watered cultivation conditions, dry: dry cultivation conditions.

7 days after sowing		wet								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	58	10.059	0.159	57	14.088	0.267	21	12.176	0.255
	Kumulus WG	83	9.804	0.154	91	13.722	0.204	56	11.695	0.193
	Backing Powder	24	10.054	0.272	23	12.948	0.385			
	Biplantol mycos V forte	24	10.233	0.259	34	13.479	0.376	23	12.374	0.232
	Blattgrün	23	9.526	0.191	34	13.065	0.317	24	11.925	0.156
	CropCover	22	10.014	0.398	24	13.171	0.405			
	Elot-Vis Green	23	10.170	0.163	34	13.874	0.353	11	11.036	0.209
	Burdock root extract	23	10.183	0.278	19	13.642	0.562			
	Giant knotweed leaf extract	24	10.138	0.205	32	13.525	0.420	10	11.160	0.385
	Raw milk (100%)	24	9.979	0.257	23	12.922	0.473			
	Raw milk (10%)	19	10.211	0.289	21	13.705	0.304			
	Serenade ASO	23	10.330	0.232	34	14.126	0.239	9	12.187	0.273
		dry								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	54	8.057	0.210	54	11.256	0.290	23	10.513	0.280
	Kumulus WG	64	7.978	0.225	84	10.174	0.282	59	9.798	0.183
	Backing Powder	16	7.388	0.343	16	8.400	0.586			
	Biplantol mycos V forte	22	8.323	0.380	30	11.197	0.424	24	10.404	0.276
	Blattgrün	24	8.592	0.223	29	10.876	0.458	18	10.196	0.204
	CropCover	15	7.560	0.411	17	10.247	0.797			
	Elot-Vis Green	19	9.247	0.271	35	11.157	0.474	12	9.692	0.342
	Burdock root extract	12	5.758	0.644	17	10.006	0.508			
	Giant knotweed leaf extract	22	8.764	0.236	35	11.880	0.423	12	9.433	0.357
	Raw milk (100%)	15	7.060	0.245	15	10.207	0.690			
	Raw milk (10%)	15	6.873	0.246	19	9.126	0.368			
	Serenade ASO	23	8.752	0.193	33	11.552	0.386	11	9.700	0.181

Supplementary Table 11 b.

14 days after sowing		wet								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	57	21.828	0.374	57	23.707	0.678	12	27.667	0.301
	Kumulus WG	82	21.128	0.350	92	24.025	0.478	46	27.326	0.489
	Backing Powder	24	20.871	0.701	24	20.275	0.884			
	Biplantol mycos V forte	24	23.046	0.448	33	23.715	0.858	11	25.773	0.712
	Blattgrün	22	19.805	0.735	34	22.703	0.773	12	26.817	0.320
	CropCover	22	20.618	0.649	24	21.788	0.956			
	Elot-Vis Green	23	23.104	0.315	34	25.021	0.818	11	24.655	0.625
	Burdock root extract	23	20.596	0.757	18	23.100	1.313			
	Giant knotweed leaf extract	24	22.508	0.468	31	23.187	0.850	10	26.220	0.947
	Raw milk (100%)	24	20.521	0.750	24	21.317	1.049			
	Raw milk (10%)	19	20.768	0.691	23	20.600	1.128			
	Serenade ASO	23	22.600	0.420	33	24.494	0.720	9	26.789	0.526
		dry								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	49	11.069	0.274	55	16.011	0.344	12	13.483	0.592
	Kumulus WG	75	11.404	0.309	85	13.800	0.344	46	12.822	0.404
	Backing Powder	24	10.250	0.418	18	13.978	0.983			
	Biplantol mycos V forte	23	11.100	0.494	31	15.277	0.463	12	11.217	0.491
	Blattgrün	23	10.226	0.297	30	14.957	0.649	12	11.792	0.201
	CropCover	21	11.176	0.640	18	13.917	0.666			
	Elot-Vis Green	23	11.296	0.336	32	16.122	0.605	12	13.867	0.884
	Burdock root extract	23	11.096	0.785	22	14.427	0.685			
	Giant knotweed leaf extract	20	11.125	0.411	33	16.509	0.539	12	12.692	0.605
	Raw milk (100%)	21	12.057	0.856	21	14.543	0.358			
	Raw milk (10%)	20	10.830	0.319	23	15.261	1.003			
	Serenade ASO	21	10.848	0.297	33	16.094	0.564	11	11.482	0.265

Supplementary Table 11 c.

21 days after sowing		wet								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	59	28.605	0.455	46	29.683	0.747	21	38.262	0.764
	Kumulus WG	82	28.368	0.332	80	32.073	0.471	68	36.622	0.373
	Backing Powder	24	26.592	0.644	23	27.891	1.015			
	Biplantol mycos V forte	24	30.654	0.672	22	31.073	0.865	35	37.057	0.893
	Blattgrün	23	28.970	0.757	24	30.975	1.017	34	36.862	0.421
	CropCover	23	26.465	0.994	24	29.342	1.065			
	Elot-Vis Green	22	31.068	0.458	23	32.278	0.773	34	38.512	0.535
	Burdock root extract	23	27.061	0.798	16	30.075	0.950			
	Giant knotweed leaf extract	24	30.154	0.991	20	30.245	1.359	33	37.048	0.468
	Raw milk (100%)	24	26.296	0.738	24	28.604	0.932			
	Raw milk (10%)	18	28.367	0.523	23	28.717	1.163			
	Serenade ASO	24	31.425	0.658	22	32.186	0.840	32	38.094	0.526
		dry								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	46	17.759	0.711	46	18.880	0.757	20	24.415	0.923
	Kumulus WG	77	17.958	0.487	76	17.324	0.472	70	20.453	0.551
	Backing Powder	24	15.988	0.881	23	17.913	1.119			
	Biplantol mycos V forte	23	16.935	0.941	18	17.072	1.115	34	21.324	1.001
	Blattgrün	23	18.739	0.746	21	18.276	1.464	32	17.719	0.775
	CropCover	22	17.077	1.077	21	15.410	0.887			
	Elot-Vis Green	23	20.635	0.861	23	18.783	1.305	34	22.482	0.672
	Burdock root extract	24	15.854	0.958	22	17.795	1.320			
	Giant knotweed leaf extract	23	20.039	0.917	24	17.163	1.235	36	21.253	0.803
	Raw milk (100%)	21	17.229	1.143	17	17.324	0.821			
	Raw milk (10%)	21	16.481	0.994	22	16.405	0.833			
	Serenade ASO	22	18.082	0.902	20	19.085	1.289	32	19.672	0.948

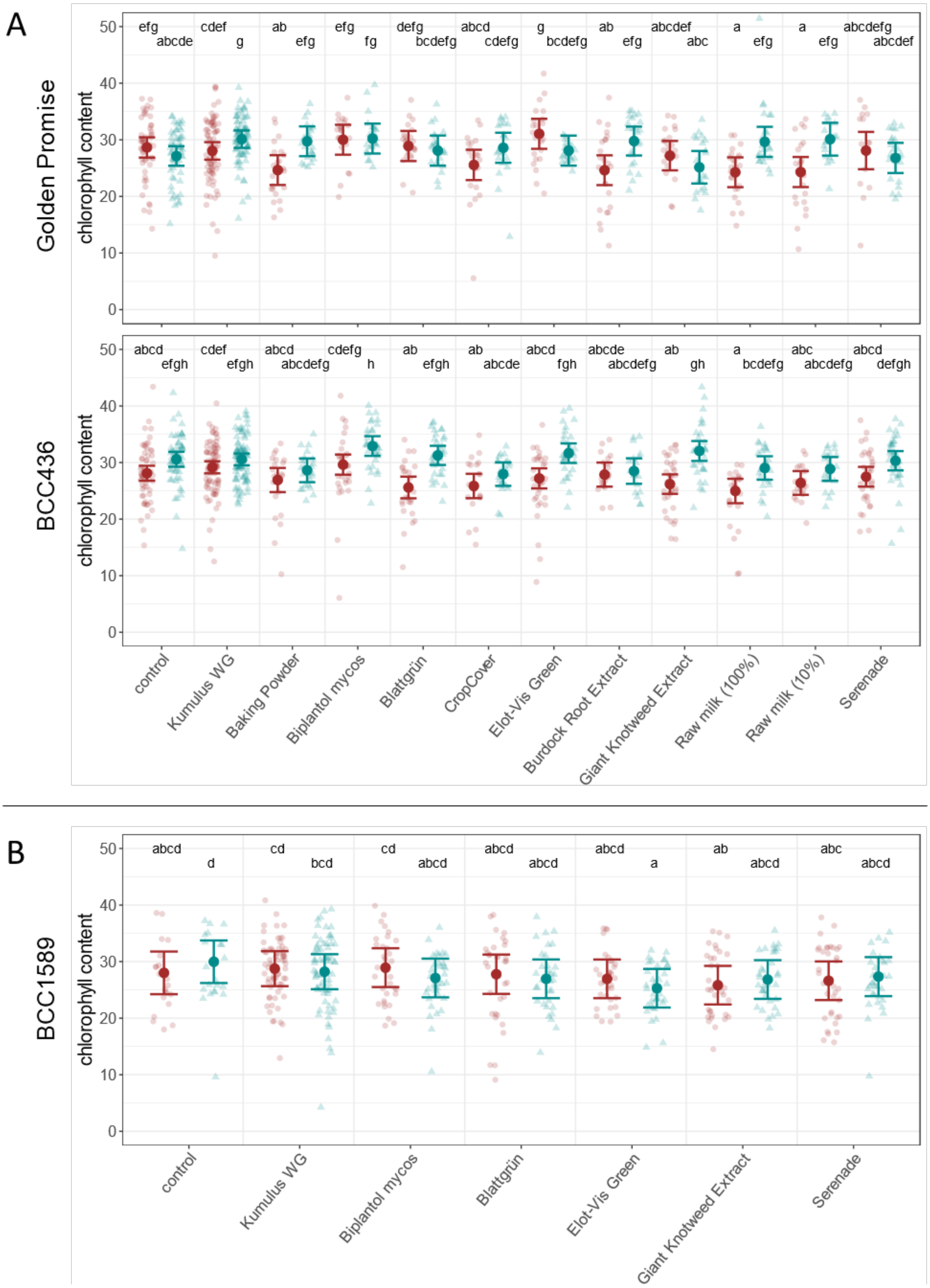
Supplementary Table 11 d.

28 days after sowing		wet								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	59	31.063	0.375	58	35.288	0.785	22	40.155	1.461
	Kumulus WG	82	31.587	0.294	92	37.192	0.513	70	40.630	0.590
	Backing Powder	24	30.729	0.593	21	35.119	1.200			
	Biplantol mycos V forte	24	31.438	0.727	33	36.109	0.934	35	40.269	0.957
	Blattgrün	23	31.043	0.612	36	35.708	0.893	34	40.724	0.584
	CropCover	23	30.152	0.819	24	35.679	0.898			
	Elot-Vis Green	23	34.030	0.559	34	37.406	0.803	33	41.764	0.633
	Burdock root extract	22	30.082	0.632	18	36.189	1.245			
	Giant knotweed leaf extract	20	31.615	0.764	31	35.687	1.133	32	40.572	0.721
	Raw milk (100%)	24	30.221	0.712	21	35.019	1.011			
	Raw milk (10%)	18	31.317	0.510	22	37.005	1.008			
	Serenade ASO	23	32.470	0.649	35	36.154	0.871	32	42.288	0.571
		dry								
		Golden Promise			BCC436			BCC1589		
		n	mean	se	n	mean	se	n	mean	se
	Control	54	20.661	0.662	59	22.641	0.821	20	32.600	0.722
	Kumulus WG	75	21.599	0.494	87	24.046	0.668	69	29.116	0.701
	Backing Powder	24	20.529	0.759	22	22.945	1.246			
	Biplantol mycos V forte	24	20.300	0.988	28	22.163	0.990	33	29.121	1.078
	Blattgrün	23	19.248	0.738	26	22.423	1.336	29	23.959	1.092
	CropCover	22	20.314	1.081	21	19.810	1.027			
	Elot-Vis Green	23	20.900	0.682	33	23.133	1.491	34	28.491	0.909
	Burdock root extract	24	20.317	1.146	22	21.795	1.464			
	Giant knotweed leaf extract	23	20.270	0.913	34	23.029	1.204	35	28.171	0.755
	Raw milk (100%)	22	19.600	1.286	20	20.780	1.016			
	Raw milk (10%)	22	17.882	1.034	21	20.271	1.242			
	Serenade ASO	14	17.793	0.948	31	23.597	1.366	35	26.031	1.152

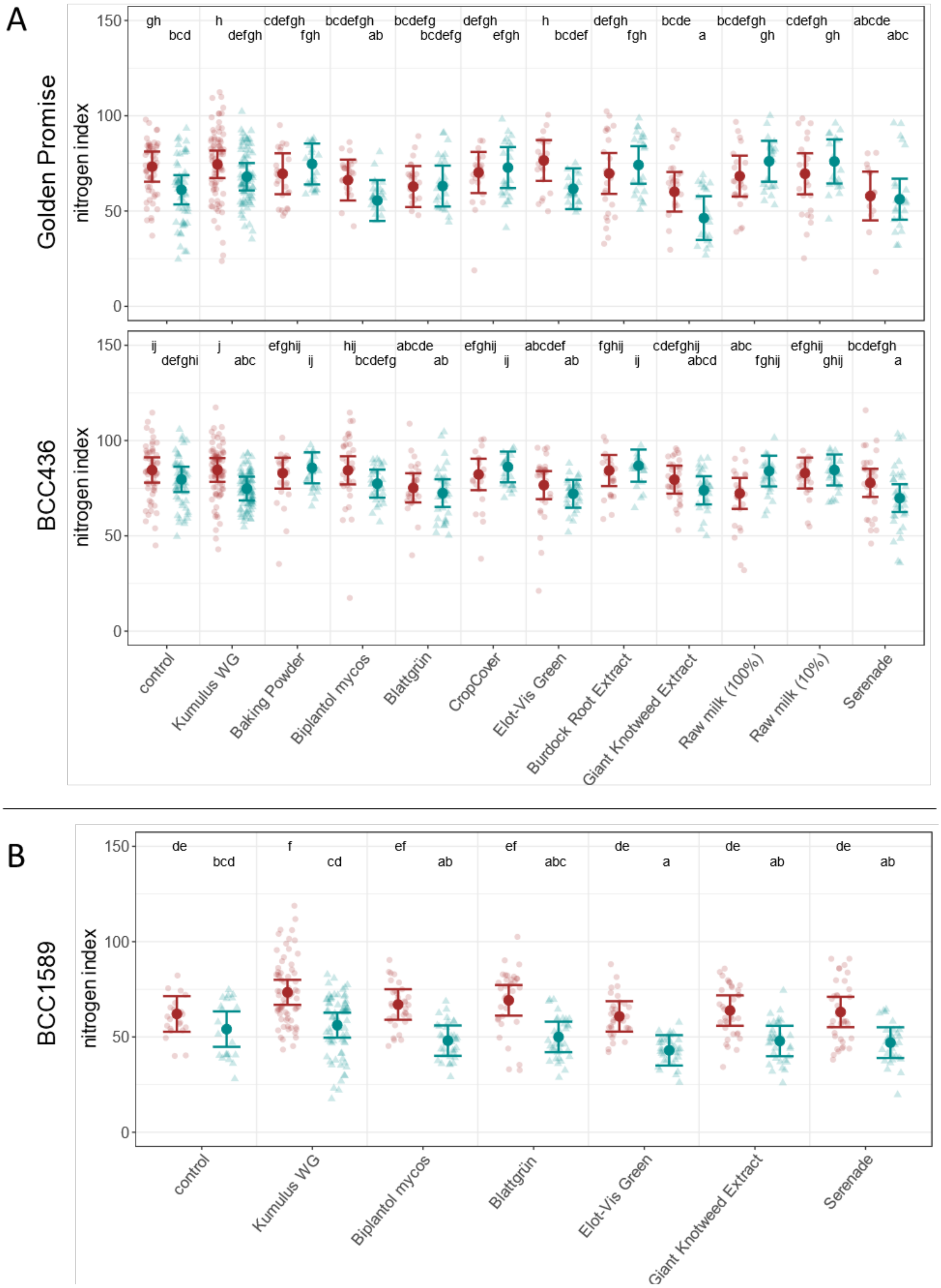
S8 Additional information on Dualex data

Supplementary Table 12. Sample size of Dualex measurements of barley plants from three genotypes at four time points that were treated with biologicals or Kumulus WG and inoculated with *Blumeria graminis* f.sp. *hordei* race A6. Das = days after sowing; Biplantol mycos: Biplantol mycos V forte; Serenade: Serenade ASO, wet/dry: well-watered or dry cultivation conditions. On genotype Golden Promise the Dualex measurement was not done for chlorophyll content of burdock root extract, raw milk or CropCover treated plants 14 das. In these cases the given sample size only represents the NBI measurements, in all other cases the sample size of chlorophyll content and NBI is equal to each other.

	Golden Promise							
	dry				wet			
das treatment	7	14	21	28	7	14	21	28
control	54	146	170	164	58	169	189	176
Kumulus WG	64	225	244	231	83	245	258	245
Baking Powder	16	72	72	72	24	72	72	72
Biplantol mycos	22	68	83	72	24	72	84	72
Blattgrün	24	69	79	69	23	69	80	69
CropCover	15	63	66	66	22	66	69	69
Elot-Vis Green	19	69	80	69	23	69	81	69
Burdock Root Extract	12	69	72	72	23	67	69	68
Giant Knotweed Extract	22	60	76	69	24	72	84	60
Raw milk (100%)	15	63	63	72	24	72	72	70
Raw milk (10%)	15	59	65	68	19	56	55	55
Serenade	23	65	76	42	23	69	81	69
	BCC436							
	dry				wet			
control	54	165	138	177	57	171	137	174
Kumulus WG	84	255	228	261	91	275	242	277
Baking Powder	16	56	68	68	23	72	69	66
Biplantol mycos	30	95	54	96	34	99	66	99
Blattgrün	29	90	63	80	34	104	72	108
CropCover	17	57	61	63	24	72	72	72
Elot-Vis Green	35	96	69	99	34	102	68	102
Burdock Root Extract	18	66	68	66	19	54	51	52
Giant Knotweed Extract	35	99	72	102	32	93	61	95
Raw milk (100%)	15	63	50	64	23	72	71	65
Raw milk (10%)	19	69	66	65	21	69	69	68
Serenade	33	99	60	93	34	101	66	103
	BCC1589							
	dry				wet			
control	23	69	60	62	21	63	66	65
Kumulus WG	59	174	210	207	56	168	206	207
Biplantol mycos	24	72	102	99	23	69	105	104
Blattgrün	23	62	96	95	24	72	100	100
Elot-Vis Green	24	72	102	102	23	69	104	102
Giant Knotweed Extract	24	69	108	105	21	66	98	99
Serenade	22	69	96	104	20	60	96	96



Supplementary Figure 7. Chlorophyll content of biological or Kumulus WG treated barley genotypes 28 days after sowing. Red: dry water regime, petrol: well-watered water regime; big dots and error bars: adjusted means with 95% confidence interval, different letters indicate significant differences ($p<0.05$); Sample size see S. Table 10. A: genotype Golden Promise and BCC436; B: genotype BCC1589.

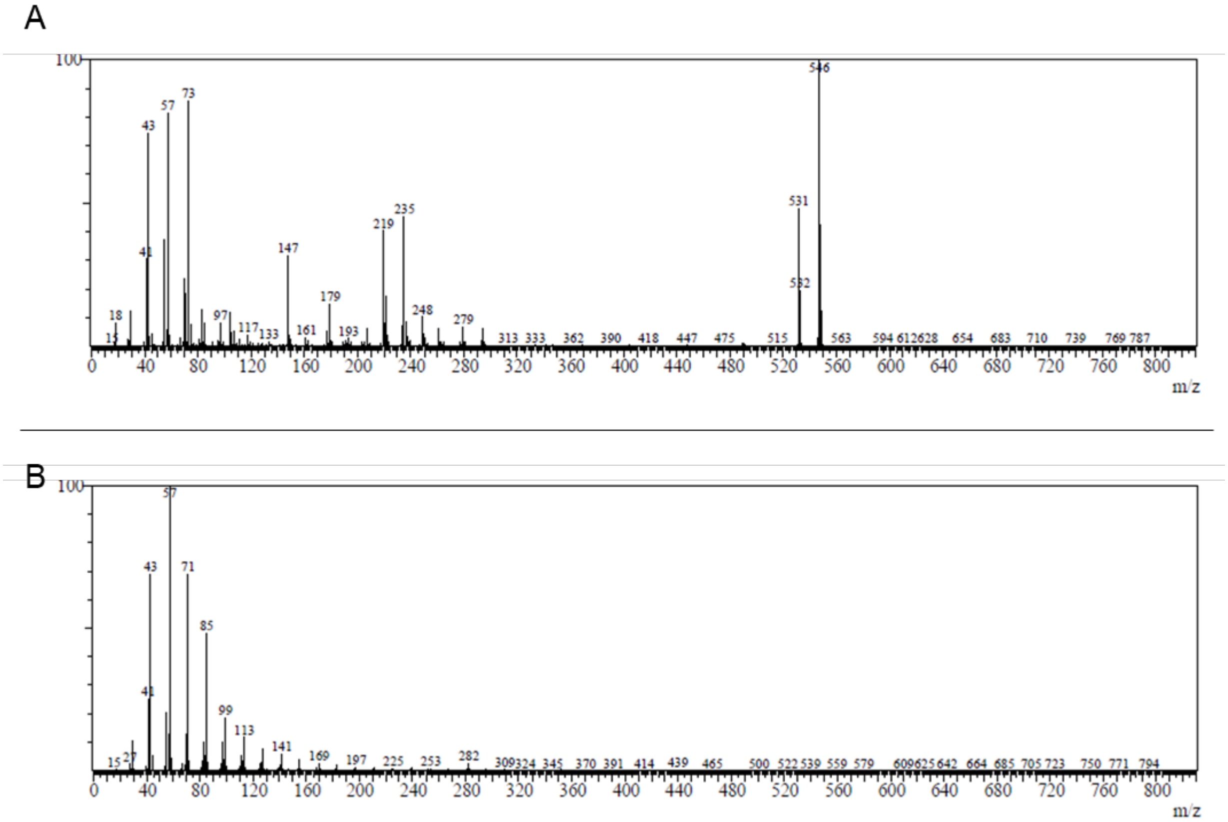


Supplementary Figure 8 Nitrogen balance index of biological or Kumulus WG treated barley genotypes 28 days after sowing. Red: dry regime, petrol: well-watered water regime. Sample size see S. Table 10.; big dots and error bars: adjusted means with 95% confidence interval, different letters indicate significant differences (p<0.05); A: genotype Golden Promise and BCC436; B: genotype BCC1589.

S9 Additional information on cuticle molecules

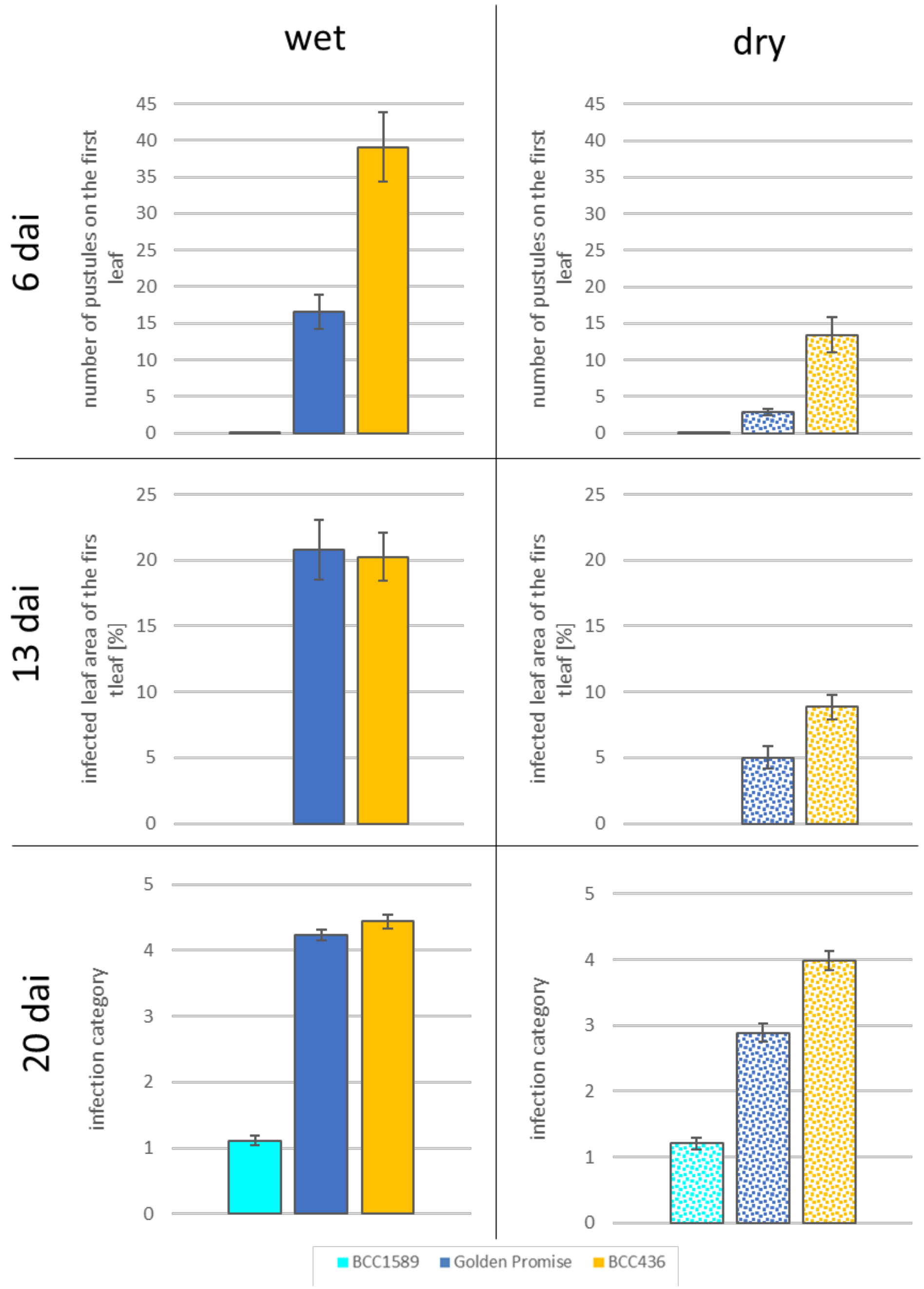
Supplementary Table 13. Proportion of cuticular molecules soluble in cyclohexane and ethyl acetate that were found in barley genotypes susceptible or resistant towards *Blumeria graminis* f.sp. *hordei*. The total proportion of molecules was determined by adding the mean proportions of molecules in the two extracts (cyclohexane, ethyl acetate) and dividing by two - this value is given here, followed by the mean proportion of each molecule in susceptible (mean sus (BCC1479, BCC436)) and resistant (mean res (BCC1589, BCC192)) genotypes; also the difference between mean sus and mean res is indicated.

	BCC1479	BCC436	BCC1589	BCC192	mean sus	mean res	difference (mean sus - mean res)
Hexacosanoic acid	2.08	2.06	2.40	3.72	2.07	3.06	-0.99
1-Tetracosanol	5.63	6.17	6.34	6.60	5.90	6.47	-0.57
x7	3.75	2.63	3.58	3.66	3.19	3.62	-0.43
Lignoceric acid	1.23	1.19	1.21	2.00	1.21	1.60	-0.39
Docosanoic acid	0.43	0.36	0.60	0.85	0.40	0.73	-0.33
x1Docosanol	1.98	2.35	1.20	3.61	2.17	2.41	-0.24
1-Octacosanol	3.74	3.38	3.64	3.86	3.56	3.75	-0.18
211	2.13	1.07	2.36	1.20	1.60	1.78	-0.18
A3	0.47	0.48	0.54	0.48	0.47	0.51	-0.04
x53	1.69	1.30	1.99	1.03	1.49	1.51	-0.02
Palmitic acid	1.61	0.94	1.00	0.88	1.27	0.94	0.33
Octadecanoic acid	1.35	0.82	0.81	0.68	1.09	0.74	0.34
Alkane 2	3.32	2.55	2.32	2.76	2.94	2.54	0.40
x58	1.63	1.84	1.68	0.76	1.73	1.22	0.51



Supplementary Figure 9. Mass-spectra of two cuticle molecules extracted with cyclohexane from barley genotypes. A: unidentified molecule x75; mass-spectra from genotype BCC1479; B: heneicosane like molecule; mass-spectra from genotype BCC192.

S10 Additional information on genotype susceptibility towards powdery mildew



Supplementary Figure 10. Infection development of control plants from three genotypes over time in the presence and absence of drought. Given are mean and standard error per genotype and time point for well-watered (wet) and dry cultivated plants; dai: days after inoculation.

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Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutz habe, dass alle Stellen der Arbeit, die wörtlich oder sinngemäß aus anderen Quellen übernommen wurden, als solche gekennzeichnet sind, und dass die Arbeit in gleicher oder ähnlicher Form noch keiner Prüfungsbehörde vorgelegt wurde.

Mainz, den 31.01.2025

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