'On-Farm' Seed Priming: A Strategy for Ecological & Sustainable Agriculture

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Declaration

I hereby declare that this dissertation is an original piece of work that embodies the results of my own research. All work contained herein has not been submitted for any other degree. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

Signature of candidate

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List of abbreviations

%TG	Percentage of total germinated seeds
%TE	Percentage of healthy emerged seedlings
ΣCO_2	Cumulative CO_2
AHDB	Agriculture and Horticulture Development Board
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUDPC	Area under disease progress curve
CI	Confidence interval
Cl^-	Chloride
CO_2	Carbon dioxide
Cv	Cultivar
d	day(s)
df	Degrees of freedom
DAS	Days after sowing
DNA	Deoxyribonucleic acid
DSR	Disease severity of rhynchosporium
DSY	Disease severity of yellow rust
DW	dry weight
E	Plants emerged per m^2
E_{50}	Time to 50 % emergence
G_{50}	Time to 50 % germination
GN	Grain N concentration
G no.	Grain number per m^2
GS	Growth stages according to Zadock scale
GY	Grain yield
H	Crop height
HAD	Healthy area duration
HGCA	Home-Grown Cereals Authority
IPM	Integrated pest management
LC	Leaf chlorophyll content
LMM	Linear mixed-effects model
LSD	Fisher’s least significant differences for the interaction

Mc	Moisture content
MFA	Multiple factor analysis
N	Nitrogen
N_2	Atmospheric di-nitrogen
Na^+	Sodium
NO	Nitric oxide
PCA	Principal component analysis
PST	Percentage of senescent tissue
mRNA	Messenger ribonucleic acid
R^2	Coefficient of determination
Retention %	Percentage of grain retention
RSE	Residual standard error
SE	Standard error
SI	Sustainable intensification
SRR	Seed respiration rate
TCA	Tricarboxylic acid
TGW	Thousand grain weight
TPT	Total plant tissue
U	Uniformity of germination
$ZnSO_4$	Zinc sulphate

Abstract

There is an urgent need to intensify food production globally and reduce our reliance on synthetic agronomical inputs. Crop establishment is the most vulnerable stage in the crop cycle, therefore, sustainable strategies with the potential to alleviate unfavourable seedbed conditions are crucial to ensure yield potential is not restricted early in the season. Industrial seed priming is an effective strategy, but it is both energy-intensive and expensive. 'On-farm' seed priming offers a low-cost alternative; however, it is commonly underutilised in the developing world, and it has never before been evaluated in a European agricultural context. Therefore, this thesis sought to determine the potential contribution of 'on-farm' seed priming to increase food production in the developing world. In addition, the aim was to assess the effectiveness of 'on-farm' seed priming together with chitosan (an organic plant elicitor) to sustainably intensify barley (*Hordeum Vulgare* L.) production in conventional European agrosystems. Quantitative analysis showed that 'on-farm' seed priming had a significant positive effect on crop yields (on average, a 21 % increase over farmers' practice) in the developing world, and was most effective in dry climates, and nutrient deficient or salinity-stressed environments. In European conventional barley systems, 'on-farm' seed priming and chitosan-based seed treatments enhanced spring barley yields through improving emergence and seedling vigour, and led to a greater number and size of tillers being retained for grain filling. By contrast, winter barley did not benefit from seed priming treatments. Although seed treatments can promote emergence, it is likely that they alter the adaptative mechanism for overwinter acclimation and result in a fitness cost. It was found that a greater canopy size can provide a certain degree of tolerance to pre stem elongation powdery mildew (*Blumeria graminis*) infection and that rapid stem elongation limited secondary spreads of powdery mildew and, hence, provide a certain level of disease escape. Overall, this thesis provides the evidence for governmental institutions and policymakers in developing countries to promote 'on farm' seed priming as a recommended practice. In a European agricultural context, seed treatments can be included as one more management practice in spring-sown crops to ensure that yield potential is not restricted early in the season. Seed treatments may deliver disease tolerance and escape traits, but these benefits will be conditional upon conferring successful establishment and vigour first.

Chapter 1: General introduction

1.1 Food security and the need for sustainable agriculture

The continuous increase in the human population has resulted in greater food demands (Godfray et al. 2010). According to the Food and Agriculture Organisation of the United Nations (FAO), food production needs to be increased by 60 to 110 % by 2050 in order to meet the global demand for food (Rockström et al. 2017). The achievement of this goal has become one of the greatest challenges of this century, which is both a fundamental need for humanity and a major threat to the environment. Most current food demands have been addressed through the intensification of agricultural systems, i.e. producing more food per unit of cropland via investment and increased inputs (e.g. fertilisers, pesticides and high-yielding crop varieties), or through extensification, i.e. the conversion of natural land to agriculture; both of which can cause severe ecological harm (Rockström et al. 2017). The abuse and misuse of agronomical inputs such as fertilisers and pesticides compromise long-term soil fertility and water resources (Spiertz 2009; Popp et al. 2013); whilst extensification has been carried out at the expense of forests and other natural ecosystems, which can accelerate soil degradation and contribute to climate change (Schmidhuber and Tubiello 2007; Pimentel and Burgess 2013). Therefore, the improved use of both agronomical inputs and ecological resources is central for attaining greater food production and ecological sustainability, and, consequently, effectively contributing to present and future food security (Godfray and Garnett 2014; Pretty et al. 2018). As part of this process of agricultural transformation, the identification of strategies and technologies, which are synergistically productive and compatible with the environment, will be crucial for tackling worldwide hunger.

Food security is met when "*all people, at all times, have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life*" (FAO et al. 2019). It has been recently estimated that approximately 820 million people do not meet this criteria (FAO et al. 2019). Most of the undernourished people live in developing countries, with about 280 million in South Asia and 240 million in sub-Saharan Africa, which represents 15 and 23 % of their total respective population (FAO et al. 2019). Addressing this need for food will require global actions, although no single strategy will be

sufficiently effective (Godfray and Garnett 2014). Strategies for delivering food security include, a change of diet, freer trade of food or the expansion of aquaculture; however, the sustainable intensification of agricultural production will be key for increasing staple food production (Godfray et al. 2010; Godfray and Garnett 2014; Rockström et al. 2017). During any transformation of agricultural production systems, it is imperative that intensification does not dominate over sustainability in order to preserve future production (Rockström et al. 2017).

Mineral fertilisers have been crucial for increasing agriculture production during the past half century but, at the same time, have brought a large dependency for attaining high yields together with multiple environmental problems such as the exploitation of non-renewable sources (e.g. phosphate rock) and accentuation of land degradation (Cordell et al. 2009). Whilst nitrogen (N) fertilisers can ensure relatively high crop yields, a high proportion of these fertilisers are either volatilised or leached as ammonia, nitrogen oxides or nitrates, which pollute air and groundwaters (Spiertz 2009; Basosi et al. 2014). There are extreme regional disparities in fertiliser use across the globe, for example, in sub-Saharan agriculture, fertiliser use is low, because the degraded soils do not often respond well to fertiliser application or they are simply not accessible to farmers (Chianu et al. 2012; Vanlauwe et al. 2014). In contrast, fertilisers are excessively applied in high-input Chinese agricultural systems (using 30 % of global fertiliser consumption) to maximise production per unit of cropland, which is already compromising long-term fertility and, subsequently, arable land availability (Gu et al. 2017; Ouyang et al. 2018).

Similar to fertilisers, the use of synthetic pesticides since the early 1960s has significantly contributed to decreased crop losses due to pests and diseases (estimated to prevent up to 70 % of yield lost to pests) which, by extension, has also contributed to making food more affordable (Oerke and Dehne 2004; Popp et al. 2013). However, the side effects from the use and misuse of these chemicals are significant and diverse for both environment and human health. Spray drifts and off-target pesticide losses accumulate in soils and in aquatic environments poisoning water and affecting biodiversity (from microbial communities to small mammals) (Carvalho 2017). Prolonged exposure to pesticides and unsafe application techniques can cause chronic health issues such as cancer, asthma, diabetes, and

other diseases in humans (Kim et al. 2017; Carvalho 2017). However, the withdrawal of fertilisers and/or pesticides would increase the pressure on converting more natural land into agricultural soil. Land is a finite resource and further expansion of agricultural land would be at the expense of forests and other natural ecosystems entailing loss of carbon sequestration capacity and more soil erosion from the new croplands (Schmidhuber and Tubiello 2007; Pimentel and Burgess 2013). The majority of pasture and croplands worldwide suffer from moderate to severe soil erosion, and their productivity will decline if no conservative practices are carried out to mitigate the effects of erosion (Pimentel and Burgess 2013). The greatest soil losses occur in arid and semi-arid regions, which is exacerbated by climate change effects so that erosion will especially compromise the capacity of developing countries to grow their own food supplies (Schmidhuber and Tubiello 2007; Gomiero 2016). However, climate change may also increase the availability of croplands at higher latitudes accentuating differences between developing and industrialised countries (Schmidhuber and Tubiello 2007).

Increasing food production, while not leaving a footprint in the environment, represents a challenging task (Rockström et al. 2017), and there is clearly an urgent need for solutions that are both productively effective and environmentally sustainable. These efforts are encompassed under the umbrella term Sustainable Intensification (SI) of agriculture, which can be defined as a set of measures by which agricultural productivity is enhanced without negatively impacting the environment, and preferably also creating social and environmental benefits (Dicks et al. 2019). To achieve this goal, the integration of a wide array of efforts at the farm, landscape and regional level are needed to improve the options available for SI (Weltin et al. 2018; Dicks et al. 2019). Numerous SI practices can reduce the environmental impact of increased productivity whilst improving social wellbeing, and can also be profitable which makes them potentially attractive to farmers (Pretty et al. 2018). However, challenges and opportunities for SI are agrosystem and location specific, and trade-offs between sustainability and intensification are inevitable (Scherer et al. 2018).

The high-input agriculture practised in many industrialised countries already has a high degree of intensification in crop production systems. Yields above 70 % of the attainable yield are often obtained, e.g., wheat production in Northwest Europe

and rice production in Japan (Oerke and Dehne 2004). In these contexts, major SI benefits are to be gained by improving the environmental sustainability component on a long-term basis, while maintaining intensification as many of these countries are food self-sufficient (Pradhan et al. 2015; Pretty et al. 2018). Practices towards increasing resource use efficiency, i.e. reducing external inputs and expenses within the existing system configuration (e.g. integrated pest management schemes and fertilizer rationalisation), but also the adoption of systems that integrate biodiversity and longer-term benefits (e.g. multiple cropping or organic agriculture) are currently being implemented as well as being active fields of research (Reganold and Wachter 2016; Lechenet et al. 2017; Pretty et al. 2018). Thus, much of the contribution to the SI goal carried out by many industrialised countries tends to be towards “de-intensification” (Struik and Kuyper 2017). European Union regulations are directed towards progressively reducing the list of pesticides available and promoting low pesticide-input pest management, which will require the development of more SI practices with the potential to replace chemicals with non-synthetic based approaches that also ensure food production stability (Hillocks 2012).

In developing countries, the intensification component is a prime necessity due to the food production deficit per capita and, hence, strategies may differ from those for high-input agriculture (Tittonell and Giller 2013; Pradhan et al. 2015). In small-holder farming systems, like those of rural Africa and some parts of Southern Asia, agriculture still remains relatively low-input and attainable yields are largely improvable (Tittonell and Giller 2013; Pretty et al. 2018). In these contexts, external inputs can be justified by their large responses, i.e. economical and agronomical profit (Chianu et al. 2012; Struik and Kuyper 2017). However, farmers face major challenges that restrict intensification such as limited access to agricultural inputs (good quality seeds, fertilisers and pesticides) and a high demand for labour (due to low mechanisation), which compromise their capacity to raise capital to invest in improvements (e.g. soil amendments to build more productive soils) (Chianu et al. 2012; Tittonell and Giller 2013). Environmental sustainability is, in some ways, imposed by the reduced availability of resources rather than the main goal and returns can gradually decrease due to this lack of investments constituting a ‘poverty trap’ (Tittonell and Giller 2013). Therefore, pathways towards SI of small-holder farmers must have minimal or no financial cost, resilience to the adverse and

changing conditions and short-term productivity benefits in order to be adopted (Vanlauwe et al. 2014). Thus, these farming systems urge practices with potential to replace the lack of inputs and make better use efficiency of practices already available. Inexpensive practices with reduced risks of failure that can be easily accessed by farmers, such as water harvesting and the incorporation of organic residues in to soil, combined with appropriate management (e.g. timely sowing, weeding and harvesting) may provide entry points towards food production intensification (Aune and Bationo 2008; Branca et al. 2013). Further intensification can then be escalated when some capital is accumulated to invest. For example, adding organic amendments to soil (compost or animal, and green manures) and increasing legume densities in to crop rotations can re-establish soil health in the medium to long term (Aune and Bationo 2008; Branca et al. 2013).

Agricultural intensification of small-holder farming systems is important for addressing the related challenges of increasing food security and self-sufficiency in developing countries. In industrialised countries, the priority is to increase ecological sustainability, so that there are no environmental repercussions (either locally or globally) that further constrain future food production. Hence, food security is a global challenge that requires appropriate technologies and practices that can synergistically bring both intensification and environmental sustainability to current agrosystems.

1.2 Model crops: Barley (*Hordeum vulgare* L.)

1.2.1 Barley as a worldwide crop

Barley is the fourth most produced cereal in the world after maize, rice and wheat (FAOSTAT, 2018). Its production takes up 48 million hectares distributed across more than 100 countries and yields over 141 million tonnes (FAOSTAT, 2018). Barley is considered to be a highly resilient crop compared to most other small grains, and can adapt to extreme temperatures, poor soils, salinity and drought (Newton et al. 2011; Gürel et al. 2016), hence, it is one of the most widely distributed crops in the world. It is cultivated in the temperate maritime climate of the UK & Ireland, semi-arid regions of Ethiopia and the arid conditions of the Middle East, as well as at the high altitude of Tibet or high latitude of Iceland (Newton et al. 2011; Kishore et al. 2016).

About 70 % of barley production is used for animal feed and 21 % for the malting, brewing and distilling industries (FAOSTAT, 2018). Although the use of barley for human consumption only represents about 6 % of the total production, this is important for providing food security for some of the most marginalised people (FAOSTAT, 2018). Apart from some marginal use that remains in northern Europe, barley grain for human consumption is confined to people in the least developed areas of the world where it is used as staple food, whilst the remaining straw after harvest is used to feed livestock (Forster et al. 2004; Kishore et al. 2016). The adaptability of barley to environmentally stressed conditions makes it the preferred choice among farmers of developing countries as it ensures more yield stability than other grain cereals (Forster et al. 2004; Newton et al. 2011).

1.2.2 Barley physiology: growth stages and leaf number terminology

The developmental stages of barley plants, from germination to maturity, are commonly described using Decimal Code system keys known as the Zadoks decimal scale (Figure 1.1) (Tottman et al. 1979). Following germination, seedling emergence concludes when the coleoptile emerges from the soil surface (GS10). The first four leaves emerge relatively quickly, each one unfurling from the sheath of the previous one, in the order that they were pre-formed in the seed embryo (leaf primordia) (Slafer et al. 2009; Kennedy 2015). The formation of the top four leaves will start with stem elongation (GS31) and end when the flag leaf blade is visible (GS39) (Figure 1.2); crop protection strategies are mainly focused on preserving these four leaves (Blake et al. 2016).

After the development of the first three leaves, a number of tillers (side-shoots/branches) begin to emerge from the axils of the basal leaves of the main stem (GS21), and secondary tillers may emerge from the axils of the basal leaves of the primary tiller stems (Kennedy 2015). At some point just after floral initiation, the number of tillers typically reaches its maximum, and then decreases rapidly before ear emergence.

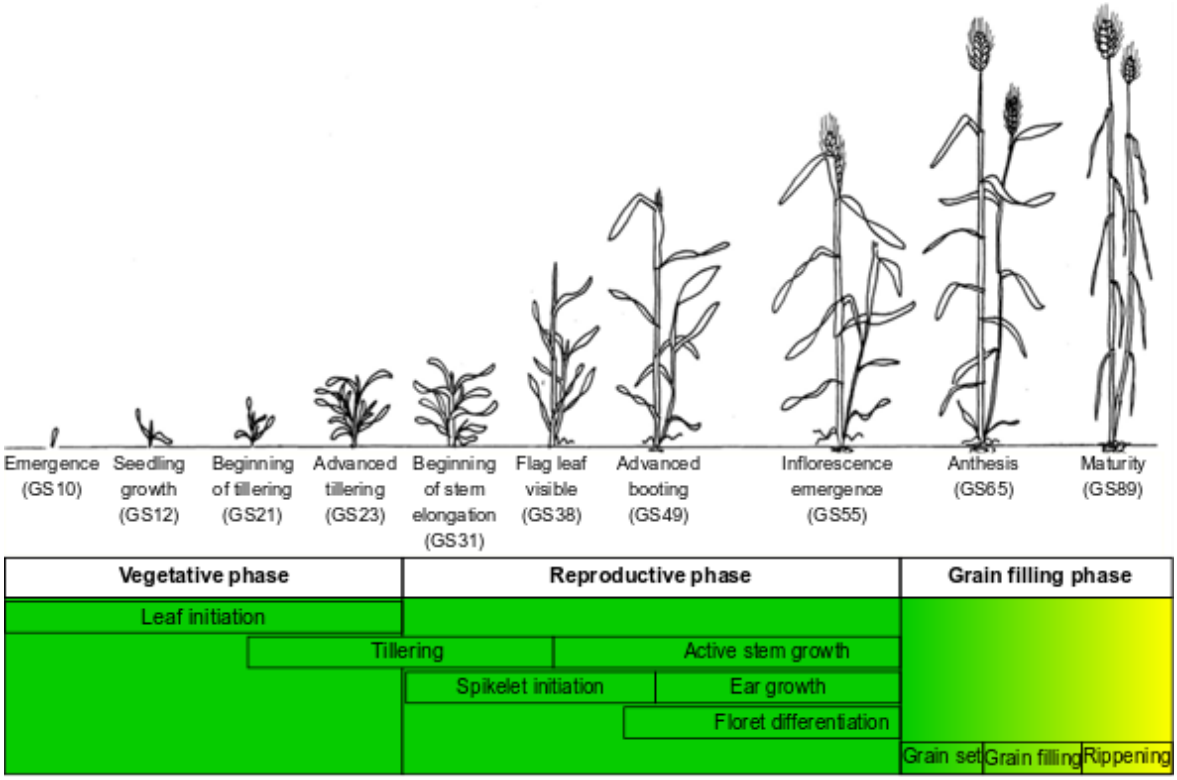

Figure 1.1. Major barley phenological events. Numbers in brackets correspond to growth stages (GS) according the Zadoks decimal scale. Image sourced from Royo and Villegas (2011) and diagram adapted from Sreenivasulu and Schnurbusch (2012).

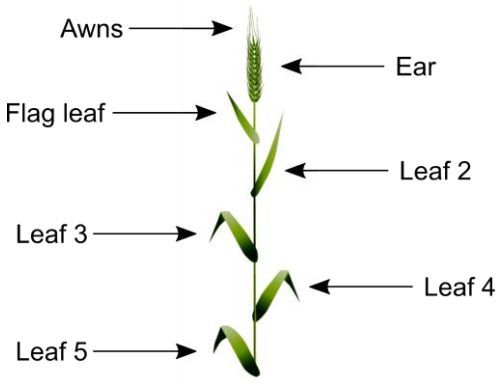

Figure 1.2. Leaf designations. Image sourced from AHDB Cereals & Oilseeds (2015).

The concurrence of tillering with stem elongation represents a point of major sink-based competition for assimilates, with the development of the youngest tillers sometimes aborting in response to the resources available (García del Moral et al.

1984). The canopy size (green area) of each plant is determined by the number and expansion of the individual leaves associated with the main shoot and all the tillers.

The appearance of the spikelet primordia (‘double ridge’) is the first visible sign of the initiation of the reproductive phase (Sreenivasulu and Schnurbusch 2012). Spikelet production and differentiation typically continues until awns are visible in the developing ear (GS49) marking the transition to the late reproductive phase. During the late reproductive phase, florets develop and differentiate culminating in grain set. However, many florets will abort before grain set due to competition for assimilates associated with sink strength, e.g., during stem extension (Sreenivasulu and Schnurbusch 2012; Kennedy 2015).

Anthesis, also known as flowering, is followed by a period of cell division and rapid water accumulation in the grain endosperm (grain set) (Nicolas et al. 1985). Subsequently, water accumulation stabilises and gives way to a profuse increase in dry matter corresponding to the deposition of starch (grain filling) (Slafer et al. 2009; Kennedy 2015). Leaf and ear post-anthesis photosynthesis, and remobilisation of stem reserves supply the demands of assimilates of the growing grains (Serrago et al. 2013). After grain filling, dry matter content increases at a lower rate and water content decreases sharply (grain ripening) (Slafer et al. 2009). This loss of water prepares the seed to enter into a quiescent state which marks the end of grain growth (Bewley et al. 2013).

From a yield viewpoint, the barley cycle can be simplified into two major characteristic phases. The first one incorporates emergence to anthesis and sets the structures for resource capture (canopy) and grain formation (potential grain-bearing tillers). The second phase, goes from anthesis to ripening and centres on the production of photoassimilates and mobilisation of reserves to the grain (Nicolas et al. 1985; Newton et al. 2011). Accordingly, it is understood that the former phase determines the number of grains per m^2 whereas the second phase determines the grain weight; both of these parameters together form the grain yield.

1.2.3 Barley pathogens

Biotic stresses are mostly represented by pests, weeds, fungal and viral infections that can cause severe yield losses in barley (Oerke and Dehne 2004).

Among these threats, fungal pathogens constitute the biggest share, whose control heavily depends on non-sustainable fungicide mixtures (Oerke and Dehne 2004; Walters et al. 2012). Some of the most economically important barley pathogens and their characteristic symptoms are summarised in Table 1.1 and Figure 1.3.

1.3 The seed

1.3.1 Seed germination

Seed germination can be defined as the processes that begins with the uptake of water by the dry seed and ends when either the coleorhiza (in monocots plants) or the radicle (in dicotyledons) protrudes from the seed coat (Bewley et al. 2013). To initiate this process, imbibition must first take place under adequate conditions of humidity, temperature, oxygen and light. During the process of germination, the seed transitions from a predominantly anaerobic metabolism to an aerobic metabolism that finalises with the emergence of the radicle tissue, marking the competition of the germination *sensu stricto* and the initiation of the seedling growth (Bewley et al. 2013). The dynamic of water uptake by the seed typically describes a triphasic pattern under optimal conditions. Dry seeds typically have a moisture content between 5-15 %, a very negative water potential (between -50 and -350 MPa), and almost no metabolic activity (Bewley et al. 2013).

During the first few hours, water moves from the substrate (e.g., soil) towards the interior of the seed driven by the difference in water potential (phase I). This is an intense and rapid process that occurs even in dormant or non-viable seed (Bewley et al. 2013). In the next phase (phase II) water absorption occurs very slowly. The production of osmotically active substances in the cells (e.g., sugars, amino acids, and potassium ions) allows the continual accumulation of water in the endosperm. In the last period of phase II, activation of proton pumps allows further moisture gain and cellular expansion. This phase ends with the appearance of the radicle (phase III) (Bove et al. 2002).

Table 1.1. Common fungal diseases of barley.

Disease name	Scientific name	Trophic relationship	Epidemiological conditions	Yield losses[a]	Reference
Scald or rhynchosporium	*Rhynchosporium commune*	Hemibiotroph	Wet and cool conditions	Up to 45 % and grain quality reductions	Avrova and Knogge (2012)
Powdery mildew	*Blumeria graminis* f.sp. *hordei*	Obligate biotroph	Warm and high humidity	Up to 20 %	Dreiseitl (2014)
Ramularia leaf spot	*Ramularia collo-cygni*	Endophyte to necrotrophic	Wet weather, prolonged leaf wetness	Up to 30 % and grain quality reductions	Walters et al. (2008)
Net blotch	*Pyrenophora teres* f. *teres* and *P. teres* f. *maculate*	Necrotroph (f. *teres*) and hemibiotroph (f. *maculate*)	Cool, wet and humid weather	Up to 40 %	Liu et al. (2011)
Yellow rust	*Puccinia striiformis*	Obligate biotrophic	Cool and wet	Up to 30 %	Brown et al. (2001)

[a]Estimated yield losses in susceptible cultivars based on published information.

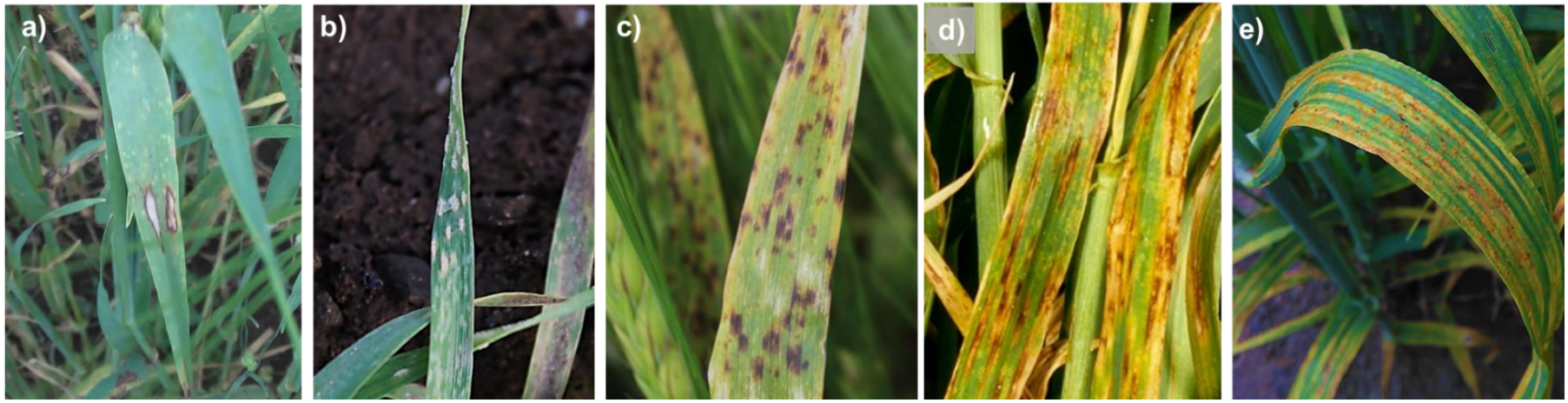

Figure 1.3. Characteristic disease lesions of a) rhynchosporium, b) powdery mildew, c) ramularia leaf spot, d) net blotch and e) yellow rust. Pictures c) and d) sourced from Bayer UK (https://cropscience.bayer.co.uk/threats/diseases/cereal-diseases/).

In phase III, rapid water absorption is reactivated, which is helped by the splitting of the integument that surrounds the seed. Morphologically, this is a phase of active growth of the radicle and also gives rise to the appearance of the cotyledon(s), which ultimately concludes the emergence of the new seedling. Storage reserves are actively mobilised from the endosperm to the cotyledon(s) to ensure the establishment of the new seedling. If imbibition is interrupted during phase II, the seed can be dried back to its original moisture without damage; however, phase III is irreversible. The duration of each phase varies widely depending on the species and seed characteristics (e.g., size, content of hydratable substrates, permeability of the seed coat), as well as external conditions such as temperature, moisture content or the composition of the soil.

1.3.2 Seed priming

At sowing, seeds encounter a number of physical constraints in the seedbed, such as soil crusting and insufficient water content; and biotic stresses, such as prolonged exposure to soil-borne pathogens that endanger crop establishment (Finch-Savage and Bassel 2016; Lamichhane et al. 2018). In this environment, rapid germination and uniform emergence are crucial for the successful establishment of the vulnerable seed (Ashraf and Foolad 2005). Seed priming is a pre-sowing treatment consisting of hydrating seeds to trigger the initiation of germinative metabolism (phases I and part of phase II) but preventing the completion of germination (phase III) (Figure 1.4) (Paparella et al. 2015). This allows preservation of desiccation tolerance so that seeds can be stored until sowing. When primed seeds are sown, the imbibition and lag phases pass more rapidly, increasing the likelihood of successful establishment. Among other strategies, seed priming can be an effective agronomic practice to enhance establishment under numerous stresses such as salinity, drought or suboptimal temperatures (Paparella et al. 2015; Ibrahim 2016; Wojtyla et al. 2016).

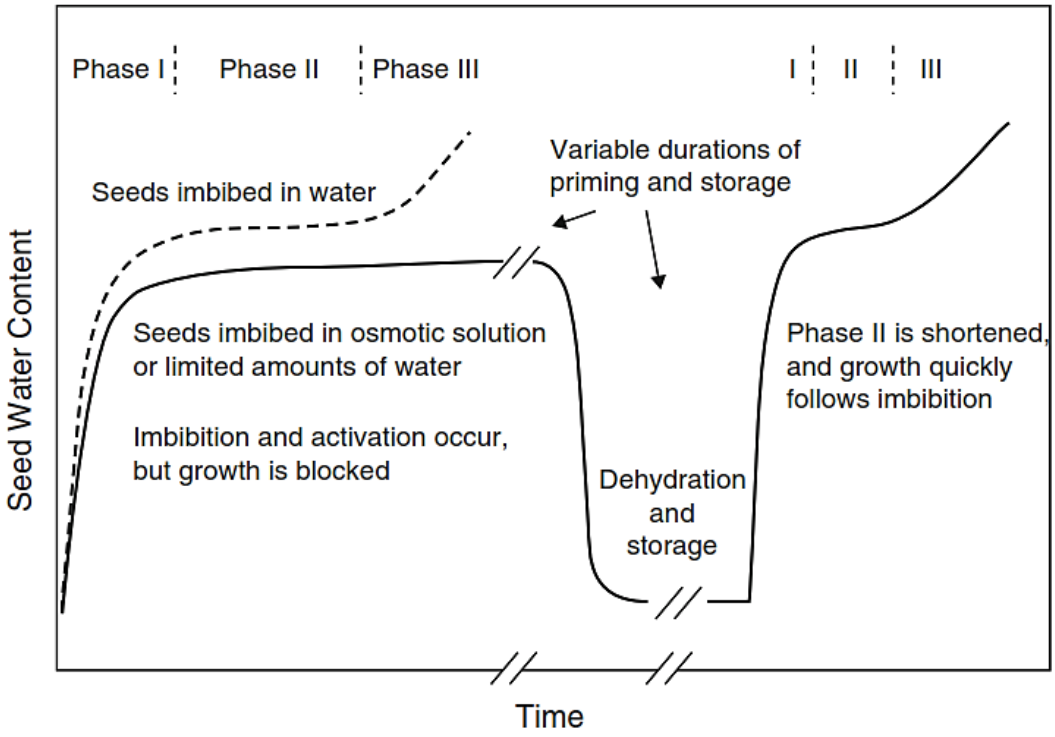

Figure 1.4. Schematic representation of the three different physiological phases of metabolism that occur during seed germination. Sourced from Bewley et al. (2013).

Many different methods for priming seeds have been developed (Table 1.2), which are classified depending on the media used and the way radicle protrusion is prevented. Irrespective of the priming method employed, the most commonly reported benefits from this practice are hastened and synchronous emergence (especially when sown under suboptimal temperatures), and often provide other benefits such as improved vigour or increased competitiveness against weeds and pathogens (Ashraf and Foolad 2005; Paparella et al. 2015; Lutts et al. 2016). Priming methods can be further improved by adding additional agents to the priming medium such as macro/micronutrients ('nutripriming'), beneficial microorganisms ('biopriming'), hormones ('hormopriming') or disinfectants ('chemopriming') (Ashraf and Foolad 2005; Paparella et al. 2015).

Table 1.2. A summary of seed priming methods based on published information.

Seed Priming type	Method	Prevention of radicle emergence	Medium	Control temp.	Aeration	Drying[a]	Cost[b]	Waste generated / health hazards[c]	Reference
Hydropriming	Seed immersion in free water	Insufficient soaking time	Water	Yes/no	Yes/no	RD	++/+	None / none	Ashraf and Foolad (2005)
'On-farm' seed priming (Hydropriming)	As above	As above	Water	No	No	SD	-	As above	Harris (2006)
Osmopriming	Seed immersion in osmotically negative solutions	Limited imbibition	PEG, mannitol	Yes	Yes	RD	+++++	Disposal of osmotic solutions / none	Ashraf and Foolad (2005)
Halopriming (Osmopriming)	As above	As above	Inorganic salts	Yes	Yes	RD	++++	As above	Parera and Cantliffe (1994)
Solid matrix priming	Seed incubation in wet substrate	Limited water available	Vermiculite, calcinated kaolin	Yes	Yes/no	RD	+++	Disposal of matric material / fine dust	Parera and Cantliffe (1994)
Drum-priming	Seed incubation in rotating drums	Insufficient moisture available	Water vapour	Yes	Yes/no	RD	+++	None / none	Rowse (1996)
Drum-priming (with water)	As above	Insufficient water available	Water	Yes	Yes/no	RD	++	As above	Ashraf and Foolad (2005)

[a]Drying after treatment: SD, surface dried, RD, re-dried to original moisture.
[b]Priming medium, aeration equipment and energy for controlling temperature, removing priming medium from seeds and seed drying system costs: scale from very costly to marginal cost (+++++, ++++, +++, ++, +, -).
[c]Waste generated or health hazards derived from performing the priming activity.

With the exception of hydropriming, the methods shown in Table 1.2 are commercially performed by seed companies with highly specialised equipment and customised protocols for each species and cultivar. However, due to the high costs associated with these technologies, seed priming is mostly restricted to expensive horticultural and flower seeds and not routinely performed for cereal grains despite their known benefits (Farooq et al. 2008; Paparella et al. 2015). Although hydropriming methods can deliver similar or even better benefits than the other methods of seed priming (e.g., Caseiro et al. (2004); Farooq et al. (2008); Sharma et al. (2014)), they are not routinely carried out in commercial settings. The intense hydration of this method may cause osmotic shock and lead to uneven priming effects and is thus, not up to the high standards of the seed industry.

1.4 'On-farm' seed priming in the developing world

'On-farm' seed priming differs from other priming strategies as it simply consists of soaking seeds in water for a number of hours (usually overnight), and so only water and a receptacle are needed (Harris 2006). Seeds are subsequently surface dried (to allow limited storage) and sown soon after. The seed remains partially hydrated so that it can attenuate the effects of a drying seedbed and, subsequently, reduce the risk of crop failure. The affordability and simplicity of this method can bring the benefits of seed priming technology to farmers, and has been named "on-farm" seed priming to differentiate it from the energy-intensive commercial seed priming methods (Harris et al. 1999).

'On-farm' seed priming qualifies as one more effort within a set of measures with barely no financial cost, such as the smart-use of farmyard manure or water harvesting systems, and could contribute as a first step for intensifying agriculture and, subsequently, food security in rain-fed systems of developing countries (Aune and Bationo 2008). Much social-scientific work has been carried out over the past 20 years to develop and promote the adoption of 'on-farm' seed priming among smallholder farmers of marginal areas of south Asia and southern Africa (Harris et al. 1999, 2001b, a; Musa et al. 2001; Virk et al. 2006), which can be split into two distinct fields of research. The first one is primarily focussed on fundamental research for the determination of the 'safe limits', the soaking duration to ensure the radicle does not emerge before sowing, and therefore takes into account potential

unforeseeable delays in sowing (Harris 2006). These ‘safe limits’ have been obtained through *in-vitro* (germination and seedling testing) and research-managed field trials for the most common tropical and sub-tropical crop species (Harris 2006). The second field of research has focussed on implementation and dissemination of ‘on-farm’ seed priming in subsistence agriculture of the most marginal areas of the world (Harris et al. 1999, 2001b, a; Musa et al. 2001; Virk et al. 2006). This work has included ‘know-how’ workshops and participatory farmer-led trials for implementation and, surveys and focus group discussions for gathering farmers’ impressions and promoting dissemination among them (Harris et al. 1999, 2001b). The results from this participatory work have generally shown a positive response from farmers as ‘on-farm’ seed priming was perceived as an effective intervention to prevent crop failure (Harris et al. 2001a). The most commonly reported benefits were earlier emergence and higher yields, but also other indirect benefits such as improved competition with weeds, due to more complete establishment; or more straw production, which can be used for animal feed (Harris et al. 2001a, b).

Despite a number of positive reports, ‘on-farm’ seed priming adoption and dissemination have been discontinued (Harris et al. 1999; Sime and Aune 2018). A lack of support from policymakers and institutions (e.g., extension systems) and ending participatory programs before the technique is thoroughly taken up by social networks have been identified as the major impediment to greater uptake (Sime and Aune 2018). At a practical level, the lack of adequate information about ‘on-farm’ seed priming has also discouraged its use (Sime and Aune 2018). Among farmers who have used the technique, there are reports of using soaking times below the ‘safe limits’, or being unaware of the ‘safe limits’ and, therefore, local farmers have had varying degrees of success with this technology (Harris et al. 1999, 2001a; Rashid et al. 2006; Virk et al. 2006). Some farmers have soaked seed before sowing (not necessarily aware of the concept of seed priming), but only under adverse circumstances, e.g. especially dry years, or when re-sowing in an effort to try and ‘catch up’ with the rest of the crop (Harris et al. 1999, 2001a). Therefore, in the places where it could be most useful, ‘on-farm’ seed priming is currently being underexploited by resource-poor farmers.

Given the increasingly unpredictable weather in temperate climates and escalating demand for sustainable intensification measures, it is conceivable that

‘on-farm’ seed priming may also be of use in conventional or organic agriculture of the developed world. Currently, ‘on-farm’ seed priming has not been adopted in Europe, where growers mainly use non-primed seeds, or seed priming technologies that are both energy-intensive and expensive for high value seeds. Therefore, there is a need to determine whether ‘on-farm’ seed priming can fill the gap for lower value seeds such as grains and intensify cereal production in a European context. Importantly, there is evidence that crops grown from ‘on-farm’ seed primed seeds can exhibit a certain degree of disease resistance (Musa et al. 2001; Rashid et al. 2004a; Harris et al. 2005). Thus, given the dependence of European agriculture on pesticides to control crop disease, the benefit of ‘on-farm’ seed priming for conferring tolerance or resistance could be incorporated into a more sustainable integrated disease management strategy.

1.5 Research rationale, aims and objectives

There is an urgent need to intensify food production systems globally in order to tackle world hunger. Central to this need is the requirement to reduce our reliance on synthetic agronomical inputs, and this can only be achieved through integrating inexpensive SI strategies into current agricultural systems. Seed germination and emergence are the most vulnerable stages in the crop cycle so that sustainable strategies with potential to alleviate unfavourable seedbed conditions are crucial to ensure that yield potential is not restricted early in the season. Industrial seed priming is an effective strategy, but its high economic and resource cost is a major drawback. ‘On-farm’ seed priming offers a low-cost alternative; however, it is commonly underused in the developing world, and it has never before been evaluated in conventional European agriculture where there may exist opportunities for its exploitation.

Therefore, the overarching aim of this thesis is to determine the potential contribution of ‘on-farm’ seed priming to increase food production in the developing world, and to assess the effectiveness of ‘on-farm’ seed priming to sustainably intensify barley production in conventional agricultural systems of the UK and Europe.

This aim will be addressed through the following objectives:

1. Quantify the potential of ‘on-farm’ seed priming to increase crop production relative to current farmers’ practices in the developing world. These findings will provide governmental institutions and policymakers in developing countries with the evidence needed to promote widescale adoption (Chapter 2).

2. Develop methods for optimisation of ‘on-farm’ seed priming of barley. These findings will allow rapid and economic optimisation and, thus, a fuller exploitation of ‘on-farm’ seed priming (Chapter 3).

3. Determine whether ‘on-farm’ seed priming can intensify spring barley production in conventional systems. These findings will allow an assessment of the potential for exploitation of ‘on-farm’ seed priming in conventional agricultural systems of Europe (Chapter 4).

4. Assess whether ‘on-farm’ seed priming can enhance host defences of winter barley in a European conventional agricultural system. These findings will provide data for the incorporation of ‘on-farm’ seed priming into a more sustainable integrated disease management strategy (Chapter 5).

Chapter 2: Quantifying the potential of ‘on-farm’ seed priming to increase crop performance in developing countries

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2.1. Abstract

Low-input agriculture in marginal areas of developing countries faces considerable challenges during crop development. A key stage in crop growth is seed germination, which is often constrained by abiotic factors such as low water potential, high temperatures and soil crusting, which can result in poor establishment. This is exacerbated by low soil fertility, salinity, drought, pests and diseases, which ultimately leads to reduced yields. Over the last 20 years, the potential of 'on-farm' seed priming, a traditional, low-cost technique, consisting of soaking seeds in water prior to sowing, has been applied to different crops and conditions with varying degrees of success. To understand the significance of this potentially transformative agronomic strategy, we have conducted a global meta-analysis of on-farm seed priming by quantifying (i) the rate of emergence, (ii) final emergence and (iii) total yield from 44 published papers on 17 crops across 10 countries. Our results show that on-farm seed priming has a significantly positive effect on crop performance: seeds emerge 22 % faster, with an increased final emergence of 11 %, with total yields 21 % higher than conventionally sown seeds. Furthermore, sub-group analyses demonstrated that on-farm seed priming is more advantageous under stressful abiotic conditions with case studies categorized as being either 'nutrient deficient', 'salinity-stressed' or 'dry climates' gaining the highest yield improvements (22 – 28 %). On-farm seed priming can be particularly beneficial to resource-poor farmers working in low-input agricultural systems where yield potential is limited by intrinsically stressed agronomic environments. Here, we demonstrate for the first time that on-farm seed priming is perfectly adapted to local situations in developing countries. Our results provide the evidence that on-farm seed priming could be effectively adopted by resource-poor farmers as a strategy to increase food security in some of the most marginal agricultural areas.

2.2. Introduction

Low-input agriculture in marginal and semi-arid areas of developing countries encounters many challenges that limit yield potential and thus restricts food security (Tittonell and Giller 2013; Aune et al. 2017). This is further intensified by predicted climate change scenarios such as increasingly unpredictable rainfalls and extreme

temperatures (Knox et al. 2012). For example, in semi-arid agricultural systems, important physical constraints in the seedbed, such as low water potential and soil crusting, have frequently been identified as the most significant issues for successful crop establishment (Townend et al. 1996; Tisdall 1996; Nabi et al. 2001; Passioura and Angus 2010). Tillage, fertilizers and amendments of the seedbed, together with timely irrigation, may ameliorate some of these constraints, although are often unaffordable or not accessible to smallholder farmers (Chianu et al. 2012; Tittonell and Giller 2013; Tonitto and Ricker-Gilbert 2016). Therefore, inexpensive and sustainable strategies with the potential to alleviate unfavourable conditions and reduce input (e.g., cover crops, water harvesting or organic fertilizer) are becoming more relevant for ensuring food security in semi-arid agro-ecosystems (Branca et al. 2013).

Over the past three decades, there has been a renewed interest in a traditional agronomic technique known as 'on-farm' seed priming, in part because of its simplicity and low-cost (Murungu et al. 2004a; Rashid et al. 2006). 'On-farm' seed priming is a form of hydro-priming, which consists of soaking seeds in water for a number of hours, usually overnight, surface drying them (to allow limited storage) and sowing soon after (Figure 2.1) (Harris 1996). Prior soaking of seeds in water decreases the time needed for seed imbibition in the soil after sowing; thus, 'on-farm' seed priming shortens the exposure of the seed to adverse soil conditions such as limited soil moisture (Harris et al. 2001a). 'On-farm' seed priming technology has been tested in a wide variety of crops and environmental conditions, and has been extensively developed through participatory trials with local farmers (Harris et al. 1999; 2001a; Rashid et al. 2006). Reports from participatory workshops and research-managed trials have largely agreed that crops grown from on-farm primed seeds emerge faster, obtain higher plant density and vigour, reach flowering and harvest more rapidly, and ultimately, result in higher yields compared with non-primed crops (Harris 1996; Harris et al. 1999; 2001a; 2001b; Murungu et al. 2003, 2004b; Farooq et al. 2008). However, the extent of these benefits varies widely even under similar contexts; for example, yield improvement of chickpea ranged from 25 to 67 % and 4 to 35 % in two different villages in India (Harris et al. 1999). There have also been cases where soaking seeds has turned out to be counterproductive,

e.g., for cotton (Murungu et al. 2004b), barley (Rashid et al. 2006), pearl millet (Aune and Ousman 2011) and sesame (Ousman and Aune 2011).

Figure 2.1. 'On-farm' seed priming steps carried out with maize seeds in Kenya: a) Pouring seeds into buckets, b) soaking seeds, and c) and surface drying after priming. d) Effect on emergence of wheat in Pakistan: non-primed (left) vs. primed (right). (Photos courtesy of H. Wainwright and A. Rashid).

The most important aspect of 'on-farm' seed priming is the duration of the soaking, which must be calculated for each crop species, and even for each variety or cultivar of crop (Harris 2006). Exceeding a 'safe limit' of soaking will trigger premature germination, which could lead to damage of the radicle during sowing, or if seeds are left in the priming water for too long, or not surface dried properly, they will begin to rot (Harris 2006). Although farmers have some knowledge of the advantages of soaking seeds prior to sowing (Harris et al. 1999, 2001a), it is often only carried out on seeds for re-sowing in order to 'catch up' with the rest of crop and is rarely used as a routine practice. In general, farmers who have used on-farm seed priming are unaware of safe limits and therefore have had varying degrees of success or failure with this method (Harris et al. 1999, 2001a).

To date, only narrative reviews about on-farm seed priming have been published (Ashraf and Foolad 2005; Harris 2006); therefore, a more systematic approach, such as meta-analysis, is needed to quantitatively review this simple technology in terms of increased crop establishment and production. Meta-analysis is a powerful synthesis tool that is being increasingly adopted in agro-ecological disciplines (e.g. Tonitto and Ricker-Gilbert 2016), and using this approach will allow a large number of independent on-farm seed priming case studies to be objectively analysed across different crop types and environments. A better understanding of the potential of on-farm seed priming, and in which environments it could be most usefully promoted, could provide governmental institutions and policymakers in developing countries with the evidence to promote its adoption as recommended practice. Therefore, the overarching aim of this chapter was to quantify the effect of 'on-farm' seed priming compared to conventional sowing and identify the context where it can best be applied. Specifically, our objectives were to quantify the effect of 'on-farm' seed priming on crop performance (speed of germination, final emergence and yield) and evaluate the impact of climate, crop type and common yield-limiting factors on the final outcome of crops grown from on-farm primed seed.

2.3. Material and methods

2.3.1. Sources of data

A literature search was carried out in 'Web of Science Core Collection' on 15 November 2017 using the key-words: 'on farm seed priming', 'on station seed priming', 'pre-sowing seed soak*' or 'hydro*priming', which resulted in a total of 293 articles. Titles and abstracts were screened and unrelated papers, or studies focussed on tree seeds were discarded. The full text of the remaining papers was examined and had to meet the following criteria: (1) The study had to contain a dry seed sample (control) and primed seed samples (treatment) consisting of seeds submerged in water with no additional oxygenation, and (2) seeds had to be surface dried or partially dried after priming (maximum air-drying duration of less than 24 h). Artificial drying methods such as ovens or air-conditioned cabinets and seeds re-dried to their original moisture, regardless of the methods used, were not included. Other priming strategies, e.g. seeds placed between filter paper and saturated jute mat, were also excluded due to the confounding effects of matric potential. We

excluded 141 articles that did not match these requirements. In addition, studies performing other types of seed treatments (19), not containing or missing data (15), lacking or giving ambiguous description of priming (8) and reviews (5) were excluded, and a further 23 papers without full-text, and five more because the same data had been used in several publications, were also excluded. Six additional papers were identified in the reference list of one of the selected papers, which gave a total of 44 valid papers available for meta-analysis (Table 2.1).

For each publication, three variables were recorded for both control and primed treatments: (i) final emergence, (ii) time to 50 % emergence and (iii) yield (i.e. the most common unit of yield for each crop, e.g. grain for cereals, pods for legumes) giving three datasets. The mean and the number of paired observations (n) contributing to that mean value were recorded, e.g. the experimental design of (Harris et al. 2005) consisted of two cultivars of chickpea with three replications during two seasons, i.e. n = 12. When available, standard deviation (SD), standard error (SE) or standard error of the difference in mean (SED) were also collected as a measure of the variance. Mean and variation values from published graphs were extracted taking a snapshot of the figure and scaling the axes with WebPlotDigitizer Version 3.10 (Rohatgi 2010) to obtain numerical values. In addition to the statistical data, any characteristics that may have influenced the outcome of the priming treatment and thus could potentially explain heterogeneity in effect size (moderator variables) were also recorded.

Table 2.1. Data sources reviewed in the meta-analysis. E_{50}: time to 50 % emergence. FE: final emergence.

Author	Journal	Country	Crop	Response variables	Study type	Yield-limiting factor[a]	Climate zone[a]
Abdalla et al. (2015)	Agronomy-Basel 5 (4):476–490	Sudan	Sorghum, groundnut, sesame, and cowpea	FE and yield	Field	Nutrient-stressed	Semi-arid
Abro et al. (2009)	Pak J Bot 41 (5):2209–2216	Pakistan	Wheat	E_{50} and yield	Field	Salinity	Arid
Ahmad et al. (2013)	Int J Agric Biol 15 (4):791–794	Pakistan	Rice	E_{50}, FE, and yield	Field	Non-stressed	Arid
Ali et al. (2013)	Turk J Agric For 37 (5):534–544	Pakistan	Wheat	FE and yield	Field	Non-stressed	Arid
Ali et al. (2008)	Aust J Crop Sci 2 (3):150–157	Pakistan	Wheat and maize	Yield	Field	Non-stressed and nutrient-stressed	Semi-arid
Anwar et al. (2013)	Pak J Bot 45 (1):157–162	Pakistan	Rice	FE	Lab and field		
Ashraf et al. (2003)	Agronomie 23 (3):227–234	Pakistan	Pearl millet	E_{50} and FE	Lab		
Aune and Ousman (2011)	Exp Agr 47 (3):419–430	Sudan	Sorghum and pearl millet	FE and yield	Field	Nutrient-stressed	Arid
Aune et al. (2012)	Outlook Agr 41:103–108	Mali	Sorghum and pearl millet	Yield	Field	Nutrient-stressed	Semi-arid
Basra et al. (2011)	Int J Agric Biol 13 (6):1006–1010	Pakistan	Maize	E_{50} and FE	Pots		
Basu et al. (2014)	Indian J Agr Sci 74 (6):311–315	Bangladesh	Maize	FE	Field		
Chivasa et al. (2000)	Tanzanian J. Agric. Sci: 3, 103–112	Zimbabwe	Maize and sorghum	E_{50}	Pots		
Eyob (2009)	J Med Plants Res 3 (9):652–659	Etiopia	Korarima	FE	Pots		
Farooq et al. (2008)	J Agron Crop Sci 194 (1):55–60	Pakistan	Wheat	E_{50} and yield	Field	Non-stressed	Arid
Farooq et al. (2017)	Plant Physiol Bioch 111:274–283	Pakistan	Chickpea	FE	Pots		
Fattahi et al. (2011)	Hortic Environ Biote 52 (6):559–566	Iran	Dracocephalum kotschyi Boiss	E_{50} and FE	Pots		
Finch-Savage et al. (2004)	Field Crop Res 90 (2–3):361–374	UKb	Maize	E_{50} and FE	Lab and pots		
Ghassemi-Golezani et al. (2008)	Research Journal of Seed Science 1 (1):34–40	Iran	Chickpea	FE and yield	Field	Non-stressed	Semi-arid

Table 2.1. Continued.

Author	Journal	Country	Crop	Response variables	Study type	Yield-limiting factor[a]	Climate zone[a]
Harris (1996)	Soil and Tillage Research 40 (1–2):73–88	Botswana	Sorghum	E_{50} and FE	Lab and field		
Harris et al. (2005)	Aust J Agr Res 56 (11):1211–1218	India	Chickpea	Yield	Pots		
Harris et al. (1999)	Exp Agr 35 (1):15–29	India	Chickpea	Yield	Field	Nutrient-stressed	Semi-arid
Harris et al. (2001a)	Agr Syst 69 (1–2):151–164	India	Maize	Yield	Field	Nutrient-stressed	Semi-arid
Harris et al. (2001b)	Exp Agr 37 (3):403–415	India, Nepal and Pakistan	Wheat	E_{50} and yield	Lab and field	Non-stressed, nutrient-stressed, and salinity	Temperate, tropical, and arid
Harris et al. (2007)	Field Crop Res 102 (2):119–127	Pakistan	Maize	Yield	Field	Nutrient-stressed	Semi-arid
Harris et al. (2008)	Plant Soil 306 (1–2):3–10	Pakistan	Wheat and chickpea	Yield	Field	Nutrient-stressed	Semi-arid
Iqbal and Ashraf (2005)	J Integr Plant Biol 47 (11):1315–1325	Pakistan	Wheat	Yield	Field	Non-stressed and salinity	Arid
Iqbal and Ashraf (2010)	J Agron Crop Sci 196 (6):440–454	Pakistan	Wheat	FE and yield	Lab and field	Non-stressed and salinity	Arid
Islam et al. (2015)	Acta Physiol Plant 37 (8)	Pakistan	Wheat	E_{50}, FE, and yield	Pots	Non-stressed and salinity	Arid
Khanal et al. (2004)	Proc Micronutr South and South East Asia, Kathmandu, Nepal, pp 121-132	Nepal	Chickpea	FE and yield	Field	Nutrient-stressed	Temperate
Kumar et al. (2002)	Int Sorg Mill Newsl 43(1),:90–92	India	Finger millet	Yield	Field	Non-stressed	Temperate
Mani et al. (2013)	J Agrometeorol 15 (2):138–141	India	Wheat	Yield	Field	Non-stressed	Semi-arid
Marwat et al. (2007)	Pak J Bot 39 (5):1583–1591	Pakistan	Maize	Yield	Field	Nutrient-stressed	Semi-arid
Murungu et al. (2004b)	Exp Agr 40 (1):23–36	Zimbabwe	Maize and cotton	E_{50}, FE, and yield	Field	Non-stressed	Semi-arid

Table 2.1. Continued.

Author	Journal	Country	Crop	Response variables	Study type	Yield-limiting factor[a]	Climate zone[a]
Murungu et al. (2004a)	Field Crop Res 89 (1):49–57	Zimbabwe	Maize	E_{50} and FE	Field		
Murungu and Madanzi (2010)	Afr J Agr Res 5 (17):8	Zimbabwe	Wheat	E_{50} and FE	Field		
Musa et al. (2001)	Exp Agr 37 (4):509–521	Bangladesh	Chickpea	FE and yield	Field	Non-stressed	Tropical
Neamatollahi et al. (2009)	Not Bot Horti Agrobo 37 (2):190–194	Iran	Fennel	FE	Lab		
Ousman and Aune (2011)	Exp Agr 47 (3):431–443	Sudan	Groundnut, sesame, cowpea	FE and yield	Field	Nutrient-stressed	Arid
Rashid et al. (2004a)	Crop Prot 23 (11):1119–1124	Pakistan	Mungbean	FE and yield	Field	Nutrient-stressed	Semi-arid
Rashid et al. (2004b)	Exp Agr 40 (2):233–244	Pakistan	Mungbean	E_{50}, FE, and yield	Field	Nutrient-stressed	Semi-arid
Rashid et al. (2006)	Eur J Agron 24 (3):276–281	Pakistan	Barley	Yield	Field	Non-stressed, nutrient-stressed, and salinity	Semi-arid
Rehman et al. (2011a)	Int J Agric Biol 13 (5):786–790	Pakistan	Rice	E_{50} and yield	Field	Non-stressed	Arid
Rehman et al. (2011b)	Turk J Agric For 35 (4):357–365	Pakistan	Rice	E_{50}, FE, and yield	Field	Non-stressed	Arid
Virk et al. (2006)	Exp Agr 42 (4):411–425	India	Horsegram	E_{50}, FE, and yield	Lab and field	Nutrient-stressed	Temperate

[a]Data corresponding to response variable ‘yield’.
[b]Simulating semi-arid climate conditions in cabinets.

If a single publication presented several case studies, the mean effect was calculated (in order to minimize within-study dependence); however, if the moderators differed then, they were considered as independent case studies in the meta-analysis (Koricheva et al. 2013). In cases where several priming outcomes had a common control, the total number of replications of the control group was divided by the number of treatments to avoid overweighting. If papers presented results that had been carried out by distinct groups, e.g. the design of some of the on-station trials, which included both researcher-led and farmer-led experimental and participatory trials, these data were considered as independent. Although these observations cannot be considered fully independent, this approach is commonly used in both plant biology and ecology meta-analyses and allows greater statistical power (Castagneyrol and Jactel 2012; Mayerhofer et al. 2013; Shrestha et al. 2016). The resulting dataset contained 129 case studies derived from 44 papers, which covered 17 crops across ten countries.

2.3.2. Effect Size and Meta-Analysis

The natural log response ratio (*lnR*) of the experimental mean divided by the control mean was used as metric of treatment effect (Hedges et al. 1999):

$$lnR = \ln\left(\frac{X_e}{X_c}\right)$$

where X_e and X_c are the experimental and control mean. Given that more than 50 % of the case studies did not provide a measure of variance, case studies were weighted using nonparametric variance (V_{lnR}) (Adams et al. 1997):

$$V_{lnR} = \frac{n_e + n_c}{n_e * n_c}$$

where n_e and n_c are experimental and control number of paired observations respectively.

Bias-corrected bootstrapped 95 % confidence intervals based on 10,000 iterations were calculated for overall effect sizes (Adams et al. 1997) and represented as a percentage change relative to controls (%), transforming them back by (exp(*LRR*) − 1 × 100) for easier interpretation, where *LRR* is the weighted summary effect size across case studies. Overall effect sizes were considered

significant when their confidence intervals did not overlap (Gurevitch and Hedges 1999).

A random effects model was chosen because of the high variation expected between studies due to the diversity of crops and environmental factors. In addition, the aim of this study was to obtain mean effects that can be generalized to different scenarios, which is best done with random effects models (Borenstein et al. 2009).

To investigate the relationship between emergence and yield, pairs of effect sizes of 'time to 50 % emergence' and 'final emergence', and pairs of final emergence and 'yield' from the same case studies were analysed using time to 50 % emergence and final emergence as moderators, respectively. The influence of each moderator was assessed with F_M (test of moderator) by meta-regression using restricted maximum likelihood with Knapp-Hartung adjustment (Viechtbauer 2010; Inthout et al. 2014), assuming a fixed effect across levels and a random effect within levels (Borenstein et al. 2009). Given the importance of soil interactions, papers reporting laboratory-based experiments were omitted from these specific analyses. To further quantify the extent of yield benefits that can be ascribed to emergence, a hypothesized regression line where changes in final emergence are equal to increments in yield was compared against the weighted linear regression obtained from the meta-regression using linear hypothesis testing.

All calculations were conducted with *metafor* (Viechtbauer 2010), *car* (Fox et al. 2016) and *boot* packages (Canty and Ripley 2012) in R version 3.3.0 (R Development Core Team 2016).

2.3.3. Moderator variables

Sub-group analysis allowed further exploration of variables in terms of explaining variability and identification of possible trends (Borenstein et al. 2009). We considered levels within moderators to be significantly different from one another when their confidence intervals did not overlap (Gurevitch and Hedges 1999).

The effect of climate on total yield was accounted for by categorizing papers as either 'temperate', 'tropical' or 'dry' according to the Köppen-Geiger climate classification (Kottek et al. 2006) (Table S2.1). Dry climates were further subdivided

into ‘semi-arid’ or ‘arid’ to account for potential evapotranspiration as a function of temperature and cycle of precipitation (Kottek et al. 2006). For this purpose, the high-resolution Köppen-Geiger climate world map (http://koeppen-geiger.vu-wien.ac.at/present.htm) was loaded into Google Earth Pro (Wuthrich 2006) and the location of the case studies in each paper used to determine the climate group. When geographical coordinates were not reported, the location of the experimental station or the nearest city at which the study took place was used.

Based on yield-limiting factors, three agronomic scenarios were commonly identified across the case studies and used for evaluation of on-farm seed priming on yield. The first scenario included case studies where crops were grown without major nutrient and water limitations. The second scenario contained case studies where crops were grown under rain-fed conditions and low soil fertility was identified as a major constraint (by authors stating that there were low levels of the main macronutrients or other known nutrient deficiencies in the area). The third scenario contained case studies where salinity was identified as the main constraint or when trials were designed to test the effect of salinity. These scenarios were named as ‘non-stressed’, ‘nutrient deficient’ and ‘salinity stressed’, respectively. Case studies not mentioning or giving ambiguous descriptions about any of these factors were omitted for categorical analyses.

2.3.4. Dataset overview

Overall, our analysis comprised work conducted in 10 countries across the Middle East, South Asia and sub-Saharan Africa. The three most globally cultivated cereals, wheat (*Triticum aestivum*), maize (*Zea mays*) and rice (*Oryza sativa*), comprised 46 % of case studies, whilst 19 % of case studies included essential cereals common in semi-arid areas: sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum* and *Eleusine coracana*) and barley (*Hordeum vulgare*). Legumes, including chickpea (*Cicer arietinum*), mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*) and horsegram (*Macrotyloma uniflorum*), represented 21 % of the case studies. Cash crops, such as sesame (*Sesamum indicum*), cotton (*Gossypium hirsutum*) and groundnut (*Arachis hypogaea*), represented 11 % of case studies, and minor crops, fennel (*Foeniculum vulgare*), korarima (*Aframomum corrorima*)

and *Dracocephalum kotschyi* Boiss (the last two grown for their spice and medicinal properties) accounted for 3 % of case studies analysed.

The dataset of time to 50 % emergence was mainly characterized by case studies using small-scale trials (three to four replications) testing the response of varieties or cultivars to on-farm seed priming and, to a lesser extent, different soaking durations. The growing conditions included field trials (46 %), pots trials (25 %) and lab experiments (29 %); most case studies were carried out with monocots (83 %). The final emergence dataset encompassed small-scale trials and medium size trials repeated over two to three seasons. More than half of the case studies in this dataset were conducted in the field (61 %), with fewer laboratory (22 %) and pot trials (17 %). Monocot (56 %) and dicot species (44 %) were almost equally represented in this dataset. For the yield dataset (65 case studies), most of the experiments were conducted under field conditions (with only three pot trials), in both irrigated and in rain-fed plots. Over half of the case studies were carried out at research farms, commonly testing priming treatments on different cultivars or varieties over several seasons, averaging 15 experimental replications per study. The remaining 43 % of the case studies were mainly participatory trials carried out by local farmers following local practices and constraints. The average experimental replications for these case studies was 38, and the biggest study accounted for 108 trials of wheat across the state of Guajarat in India (Harris et al. 2001b).

2.3.5. Publication Bias and Sensitivity Analysis

Studies showing negative results are less likely to be published; therefore, effect sizes in meta-analyses could be overestimated (Gurevitch and Hedges 1999). Consequently, indirect methods such as rank correlation tests and funnel plots of effect size vs. variance are commonly used to detect bias (Gurevitch and Hedges 1999; Koricheva and Gurevitch 2014). We conducted Kendall's tau test, where significant correlation between effect size and corresponding sample size would indicate asymmetry in the funnel plot and therefore, potential publication bias (Begg and Mazumdar 1994; Viechtbauer 2010). However, no significant relationship between effect size and increasing number of replicates for any of the three datasets in our analysis was seen (Table S2.2). We also performed 'trim and fill' funnel plots

to detect potential missing studies. The trim and fill method is a funnel-based test that imputes values that would compensate for the most extreme values in one side of the funnel (Duval et al. 2000). In our meta-analysis, trim and fill imputed 12 and 15 potential missing case studies in time to 50 % emergence and yield datasets, respectively. In both cases, adjusted summary effects would further deviate from zero suggesting that our results may be conservative (Figure S2.1).

Sensitivity analyses are also important to determine the robustness of the results (Koricheva and Gurevitch 2014). Leave-one-out meta-analysis, i.e. recalculating summary effect size omitting the study with highest effect size for each variable and observing the deviation introduced by this modification, was performed to test robustness of the summary effects. The removal of the study with largest influence in the yield dataset (Harris et al. 2001b) increased the summary effect by 1.42 %. The study with the largest influence on final emergence was Finch-Savage et al. (2004), whose removal changed the summary effect by 1.89 %. Lastly, the study with biggest impact on the time to 50 % emergence dataset was Harris et al. (2001b), and its removal decreased the overall effect size by 6.04 %. In conclusion, we did not find evidence of publication bias in our datasets, and although the time to 50 % emergence dataset presented some sensitivity, all three datasets were suitable for meta-analysis.

2.3.6. Data availability

The dataset generated and analysed during the current study is available in the Stirling Online Repository for Research Data repository as Electronic Supplementary Material 1 (ESM1) at http://hdl.handle.net/11667/123.

2.4. Results and discussion

2.4.1. ‘On-farm’ seed priming: an inexpensive technology for increased food security

Our meta-analysis showed that on-farm seed priming has a significantly positive effect on crop performance, from nascence until harvest, relative to conventional (‘control’) seed sowing (Figure 2.2). Although there is substantial variation (ranging from −36 to −7 %), on-farm seed priming significantly decreases the time to

emergence by 22 % compared with non-primed seeds. On average, the number of plants emerged increased by 11 %. Ultimately, yields increased by 21 % compared with non-primed seeds, and only six out the 65 case studies reported negative effects on yield (ESM1).

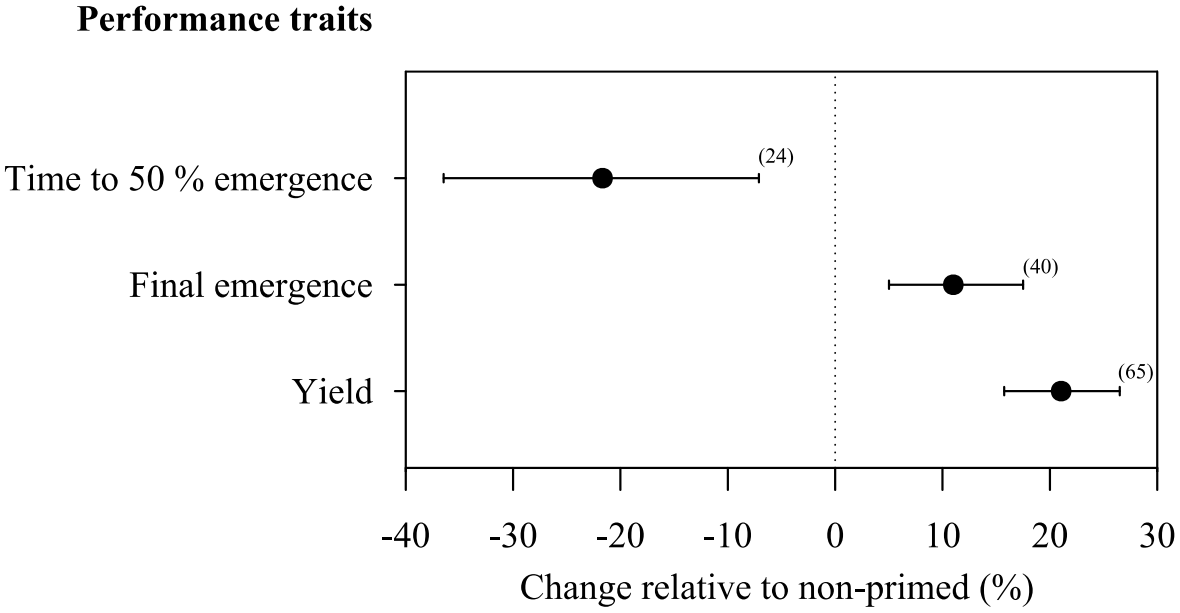


Figure 2.2. Summary analyses of the response of crops to 'on farm' seed priming. Numbers in parentheses indicate number of case studies. Error bars represent back-transformed 95 % bootstrap CIs.

Improved crop performance following on-farm seed priming can have important implications for smallholders' food production. Higher yield is often accompanied by higher straw biomass, which is especially remunerative in mixed crop-livestock systems. Enhanced plant density reduces costs and the labour needed for re-sowing, and can also increase the willingness of farmers to invest in fertilizers, as the risk of plant stand failure is lower. Faster emergence typically results in plants reaching flowering and harvest stages earlier (e.g. by several weeks), giving farmers more labour flexibility, for example, by facilitating more optimal sowing for the subsequent crop or including an extra crop in rotation systems, or even by allowing migration for off-season work (Harris et al. 1999, 2001a; Virk et al. 2006). Furthermore, the benefits are not restricted to the traits accounted for in our data, as faster development combined with the improved vigour and more uniform emergence in crops from on-farm primed seeds may save labour allocated to weeding. Although it is tempting to suggest that these benefits may increase net

incomes, additional costs such as extra fertilizer or extra costs associated with harvesting, processing and storing greater yields, together with access to markets, will determine the final return from adopting on-farm seed priming.

2.4.2. Relationships between early growth and yield on crops grown from ‘on-farm’ primed seeds

To further investigate the relationships between rate of emergence, crop establishment and yield, we conducted separate analyses of the effect of time to 50 % emergence on final emergence and the effect of final emergence on yield of crops from ‘on-farm’ primed seeds relative to non-primed. Final emergence versus time to 50 % emergence showed that in general, quicker emergence conferred by on-farm seed priming relative to non-primed seeds produced a higher number of successfully emerged seedlings (Figure 2.3a). Although this relationship was significant ($P < 0.01$), it must be interpreted with caution due to the relatively small number of case studies. Meta-regression of yield versus final emergence (relative to crops from non-primed seeds) showed a positive relationship (Figure 2.3b), although this relationship was not found significant. We found no difference between the hypothesized line and the meta-regression line ($P > 0.05$), which demonstrates that higher yields are proportional to improvements in emergence. However, in over two-thirds of the case studies, improvements in yield were proportionally higher than the expected gain due solely to improvement in final emergence. This suggests that increments in yield due to ‘on-farm’ seed priming are not only a consequence of rapid and more prolific emergence, but that additional benefits may persist long after emergence.

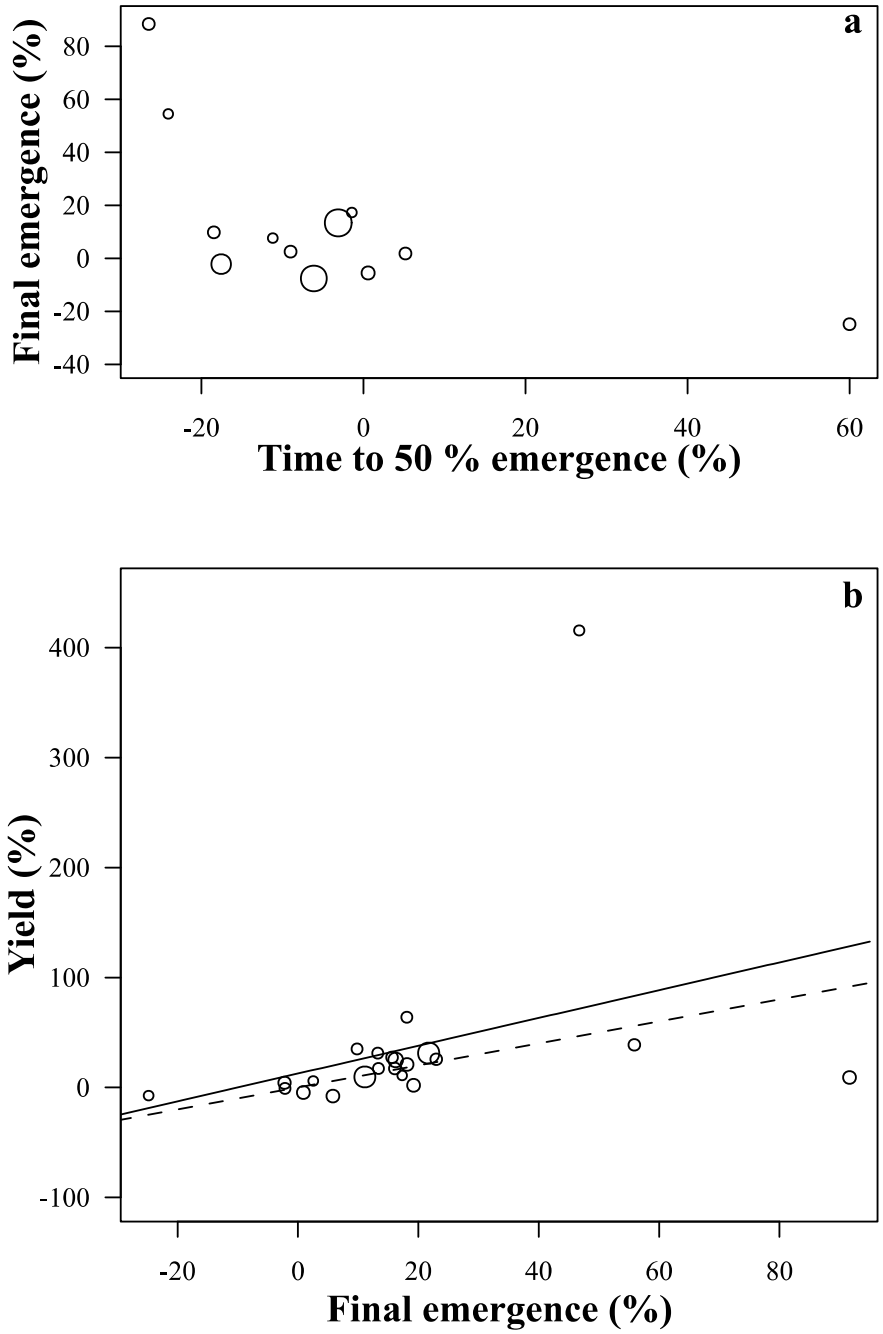


Figure 2.3. a) Relationship between final emergence and time to 50 % emergence relative to crops from non-primed seeds (n = 12). b) Relationship between field and final emergence relative to crops grown from non-primed seeds. Solid line represents the weighted model regression line and dotted line represents the hypothesized regression line where changes in final emergence cause equal changes in yield (n = 22). Bubble size represents the weight of each study in the meta-regression.

Rapid emergence is crucial for the vulnerable seedling to avoid abiotic and biotic stresses and ensure high crop establishment (Gardarin et al. 2016). ‘On-farm’ seed

priming facilitates rapid emergence by accelerating germination through two complementary mechanisms. Firstly, it ensures water availability and the successful completion of phase I (the imbibition phase) prior to sowing, rather than relying on the seed imbibing soil moisture in the field where the water supply can be restricted or discontinuous (Wojtyla et al. 2016). Throughout the imbibition phase, both mechanical and biochemical changes, e.g. embryo enlargement, respiration, protein synthesis and DNA repair, are initiated (Gallardo et al. 2001; Weitbrecht et al. 2011; Steinbrecher and Leubner-Metzger 2017). All these processes prepare the seed for cell elongation (phase II, the lag phase); therefore, 'on-farm' primed seeds are developmentally more advanced than dried seeds, resulting in a 'head start of germination' (Chen and Arora 2013). Secondly, 'on-farm' primed seeds are only externally dried so that, once in the field, seeds need to absorb less water from the soil to complete phase III (the post-germination phase) when the radicle emerges from the seed coat. Furthermore, it has been reported that seed soaking enhances the production of the enzyme α-amylase (Ashraf and Foolad 2005; Farooq et al. 2017), which plays a crucial role in starch mobilization and provides the embryo with carbohydrates for respiration during germination and seedling growth (Ashraf and Foolad 2005; Farooq et al. 2017). As a result, seedlings from 'on-farm' primed seeds have more developed roots before the common limiting factors such as declining soil moisture, crust formation and/or high salinity prevent successful emergence.

Our results suggest that the gains in yield due to 'on-farm' seed priming can be mainly attributed to enhanced emergence, i.e. rapid emergence leads to better crop establishment, which is conducive to higher yields. However, advanced establishment may also be coupled with higher vigour of individual plants, which is translated into significantly more tillers, more fruits (cobs/panicles/pods) per plant, greater number of grain and 1000-grain weight, or straw yield (Harris 2006; Rashid et al. 2006; Harris et al. 2007; Farooq et al. 2008). In addition to these physiological benefits, other circumstantial benefits are frequently observed, for example, earlier maturation decreases crop exposure to end of season drought, disease and pest attacks (Harris et al. 2001a; Rashid et al. 2006). It is also likely that seed priming exerts important metabolic changes during early plant growth that are able to persist until later stages of development (Ashraf and Foolad 2005; Chen and Arora 2013);

for example, there is evidence for enhanced disease resistance (Musa et al. 2001; Rashid et al. 2004a; Harris et al. 2005) or drought tolerance (Wojtyla et al. 2016).

2.4.3. What modulates the 'priming' response?

It is important not only to identify the context where 'on-farm' seed priming can best be applied, but also understand the potential situations where it can be counterproductive. Therefore, a subgroup analysis of moderators was conducted to examine potential factors that influence the effect of seed priming.

Climate

It is clear that yield benefits are more evident under low and unpredictable rain conditions. The largest response to 'on-farm' seed priming was seen in areas with dry climates (Figure 2.4a) with significantly higher yields for both arid (22 %) and semi-arid (28 %) climates compared to temperate climates (11 %). Variation in yield between seasons due to 'on-farm' seed priming has been frequently attributed to rainfall profiles, with greater yield increments commonly reported during rainy seasons with limited precipitation (Rashid et al. 2006; Virk et al. 2006; Ousman and Aune 2011). Low soil moisture and high evapotranspiration can slow and interrupt imbibition, which is conducive to emergence failure (Harris 1996); however, 'on-farm' seed priming can offset a lack of soil moisture, as seeds have already imbibed water prior to sowing.

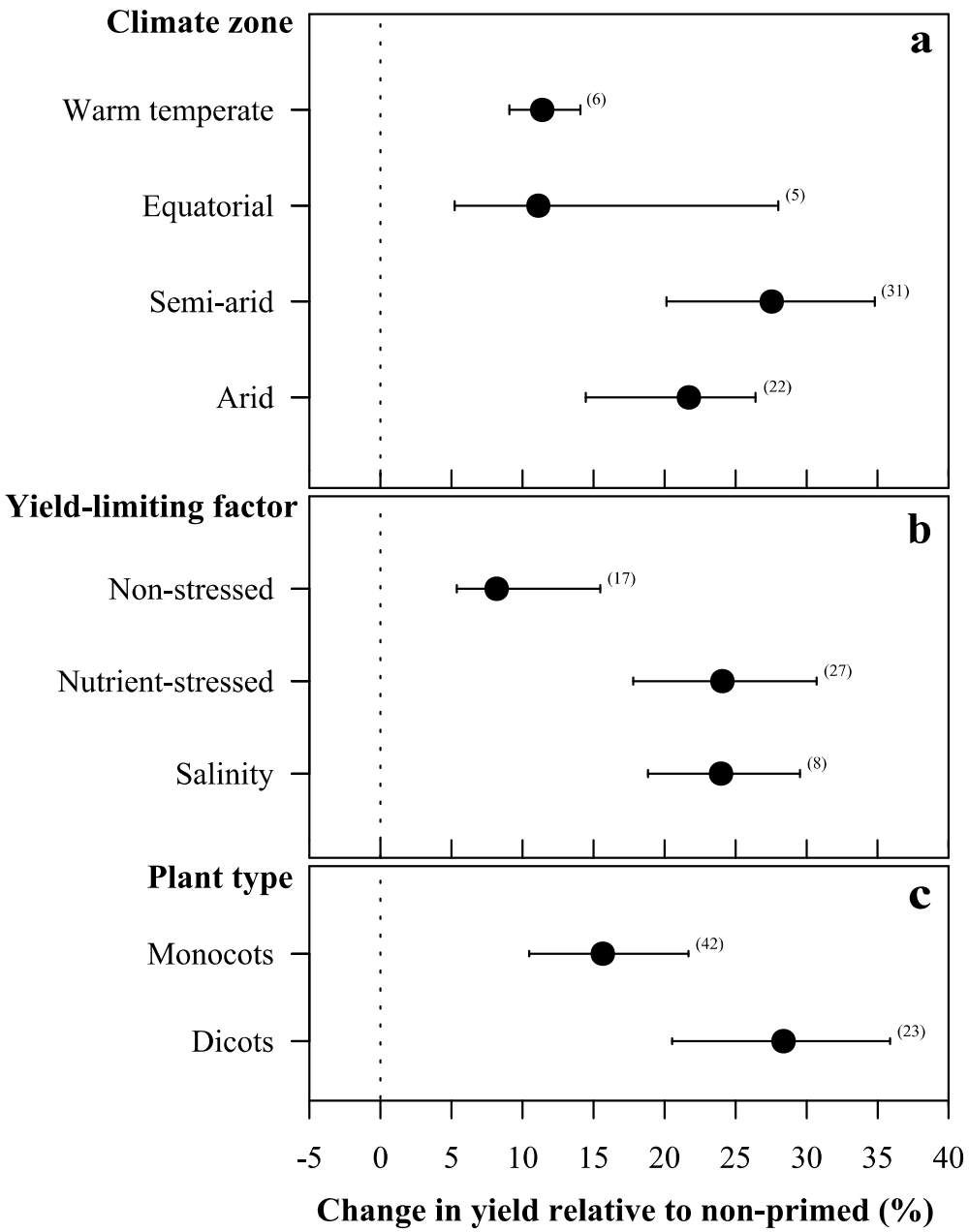


Figure 2.4. Sub-grouped summary effect sizes and 95 % CIs for priming effect on crop yield. Comparisons among (a) levels of climate, (b) levels of yield-limiting factors and (c) levels of plant type. Numbers in parentheses indicate number of case studies. Error bars represent back-transformed 95 % bootstrap confidence intervals.

Importantly, in crust-prone soils, if rainfall occurs before emergence, shoots from 'on-farm' primed seeds could be mechanically impeded, whilst the later emerging non-primed seedling may find more favourable soil strength (Murungu et al. 2004a). Equally, if rainfall is considerably delayed after sowing, seedlings from 'on-farm' primed seeds may be damaged as germination has already been initiated and a lack of water could kill the developing seedling, whereas non-primed seeds will not

initiate germinate until the rain comes (Murungu et al. 2003; Rashid et al. 2006). However, the occurrence of these events seems to be very rare (Murungu et al. 2003; 2004a), and our data at emergence stage is consistent with the yield subgrouping, i.e. showing the higher benefits under dry climates (ESM1).

The interaction between soil temperature and 'on-farm' seed priming, however, is less clear. Primed maize seed is more sensitive to elevated temperature under both dry and wet soil conditions (Finch-Savage et al. 2004; Murungu et al. 2004b). For the former, internal seed moisture may induce heat stress by acting as a thermal conductor in soils of higher temperatures, while wet soils may exacerbate prolonged hypoxia (Finch-Savage et al. 2004). Conversely, late sown wheat and chickpea plants from 'on-farm' primed seeds have shown increased tolerance to chilling temperatures (Farooq et al. 2008, 2017), possibly due to enhanced carbohydrate supply to the germinating embryo, which together with an accumulation of trehalose, can protect proteins and membranes from oxidative damage under abiotic stress.

Yield-limiting factors

Figure 2.4b shows that crops from 'on-farm' seed grown under 'salinity stress' or in nutrient deficient soils had significantly higher yields compared to crops from 'on-farm' seed grown under non-stressed environments (approximately 16 % difference). In saline environments, germination is delayed or inhibited through reduced water availability and/or accumulation of toxic Na^+ and Cl^-. However, primed seeds are already hydrated and therefore less subjected to these constraints (Ibrahim 2016; Savvides et al. 2016). Importantly, case studies growing crops in conditions defined as non-stressed were mostly from research-managed trials using fertilizers and pesticides, whilst case studies grouped as nutrient deficient were mainly from farmer-managed trials with limited access to fertilizers and pesticides, and therefore more accurately reflect resource-poor farming conditions in marginal areas. These data indicate that 'on-farm' seed priming can compensate, to some extent, for low-yielding environments and the lack of inputs that would further limit yields. Under low fertility environments, the quicker development of seedlings from 'on-farm' primed seeds allows greater uptake from fertilizers, before nutrients are leached from the soil surface or become volatized (Harris et al. 2001b; Rashid et al. 2006).

Declining soil fertility together with limited access to affordable mineral fertilizers is a major constraint for achieving optimal yields in marginal areas of developing countries (Chianu et al. 2012). However, low-cost strategies that combine 'on-farm' seed priming with low amounts of inorganic fertilizers have been carried out to alleviate nutrient deficiencies with promising results (Aune and Ousman 2011; Ousman and Aune 2011). 'On-farm' seed priming in combination with micro-dosing, i.e. application of small amounts of fertilizer in the planting pocket, demonstrated greater fertilizer use efficiency than micro-dosing alone for all the crops tested (Aune and Ousman 2011; Ousman and Aune 2011). Small amounts of micronutrients added to the water used for 'on-farm' seed priming, e.g. $ZnSO_4$, can also be highly cost-effective (Harris et al., 2007; 2008).

Plant type

'On-farm' seed priming of all the major tropical crops produces similar or greater yields than traditionally sown crops in almost all cases (ESM1). However, decreased performance following 'on-farm' seed priming has also been occasionally reported for barley (Rashid et al. 2006), pearl millet (Aune and Ousman 2011), rice (Rehman et al. 2011), sesame (Ousman and Aune 2011), maize (Ali et al. 2008), wheat (Islam et al. 2015) and cotton (Murungu et al. 2004b); although for each of these crops, there are also studies showing an increased performance (e.g. Harris et al. (2007); Farooq et al. (2008); Rashid et al. (2006). Importantly, negative results are rarely attributed to the incompatibility of priming with the crop, but rather to untimely adverse environmental conditions. The largest yield loss due to 'on-farm' seed priming was 8 % for pearl millet in a series of on-station trials; however, in this study, the farmer-managed replicates registered a 30 % increase in yield (e.g. Harris et al. (2007); Farooq et al. (2008); Rashid et al. (2006). Therefore, we have found no consistent evidence of negative interactions between specific crops and 'on-farm' seed priming, which suggests that this is therefore a safe practice for all crop species trialled so far.

The effect of categorising case studies by plant type on total yield is shown in Figure 2.4c. On average, the yield increase of cereals (monocots) was 13 % less than dicots. Dicot plants, broadly represented by legumes with 18 out of 23 case studies, responded better to 'on-farm' seed priming averaging a 28 % yield increase.

This is in line with our final emergence data where greater effect sizes generally belonged to dicotyledonous crops (ESM1). Cereals were commonly grown with irrigation or during the rainy season, whilst legumes were sown as a component of the rotation after cereals in the post-rainy season or in fallow lands that were unsuitable for the main crop. In these marginal contexts, the benefit of seed being hydrated prior to sowing leads to more rapid emergence and establishment.

We cannot conclude from our data whether specific crops are more responsive to 'on-farm' seed priming than others; however, 'on-farm' seed priming may facilitate the use of legumes into rotational and intercropping systems. Currently, in both rotational and intercropping systems, the adoption of legumes is largely discouraged due to poor establishments of the legume component. In rotation, legumes are commonly grown utilizing residual soil moisture remaining during the dry season, and with no additional fertilization, whilst in intercropping systems, the planting of a legume companion is delayed in order to avoid shading and competition (Masvaya et al. 2017). Therefore, 'on-farm' seed priming may ameliorate these unfavourable planting conditions and boost the benefits of cereal-legume cropping systems, e.g. by improving soil fertility and providing an additional income.

2.5. Conclusion and perspectives

In developing regions of the world, tackling yield reductions due to both natural and socio-economic constraints, e.g. increasingly unpredictable rainfalls, declining soil fertility and limited access to inputs and resources, requires inexpensive and sustainable strategies to ensure food production and self-sufficiency. This is the first study quantifying the potential of 'on-farm' seed priming for sustainably increasing food production at a global scale, and our results have shown that it is a valid approach to closing yield gaps. The literature considered in our meta-analysis encompassed a representative number of agro-environments where 'on-farm' seed priming can be practiced and gives us the basis to draw the following conclusions.

'On-farm' seed priming attenuates the negative effects of adverse planting conditions, and low inputs, by facilitating rapid and enhanced crop establishment that may also result in improved individual plant performance. These effects are

more evident in semi-arid and arid regions and, given that millions of hectares in dry climates are experiencing yield reductions, these findings could have important implications. Our results have also highlighted that crops grown in marginal lands can especially benefit from this intervention. This is particularly important for farmers with limited access to mineral fertilizers where to a large extent an input of N is dependent on N_2 fixed by legumes.

'On-farm' seed priming can be seen as a starting point towards sustainable intensification in marginal areas of the developing world. This technology requires very few resources and technical knowledge, and its benefits would be compatible with a range of other sustainable strategies such as smart use of farmyard manure, micro-dosing and water harvesting practices. Therefore, our results provide the evidence needed to encourage governmental institutions and policymakers in developing countries to promote the adoption of 'on-farm' seed priming as recommended practice.

Chapter 3: Optimisation of ‘on-farm’ seed priming soaking times for barley (*Hordeum vulgare* L.)

3.1. Abstract

A traditional and low-cost technique named ‘on-farm’ seed priming is increasingly being recognised as an effective approach to maximise crop establishment. It consists of anaerobically soaking seeds in water before sowing resulting in rapid and uniform germination, and enhanced seedling vigour. The extent of these benefits depends on the duration soaking time, which must be long enough to allow pre-germinative metabolism to be arrested but prevent radicle emergence. Current determination of optimal soaking time by germination assays and mini-plot trials is resource-intensive, as it is species/genotype- and seed quality-specific, and only provides retrospective information of its effectiveness. Therefore, this study aimed to determine the potential of seed respiration rate (an indicator of metabolic activity) and seed morphological changes during barley priming as predictors of the priming benefits and, thus, facilitate determination of optimal soaking times. A series of germination tests revealed that germination speed is mostly attributable to rapid hydration of embryo tissues rather than to pre-germinative advancement as the greatest gains occurred before the resumption of metabolic activities. Germination uniformity, however, was not significantly improved until seed were primed for at least 8 h, i.e. after a first respiration burst was initiated, suggesting the occurrence of key metabolic activities at this stage with effects on the rate at which the germination programme proceeds. The maximum seedling vigour was attained when the priming process was stopped just before the beginning of the differentiation of embryonic axes (20 h) after which vigour began to decrease (‘over-priming’). The onset of embryonic axes elongation was preceded by a second burst of respiration, which can be used as a marker for priming optimisation. Thus, monitoring of seed respiration provides a rapid and inexpensive alternative to the current practice. The method could easily be implemented for determining the optimal soaking times of other cereal seeds and carried out by research agricultural institutions to provide recommended optimal soaking times for common cereal varieties within a specific region.

3.2. Introduction

Seed germination involves an array of coupled morphological and respiratory changes that make up three distinct phases each of which are characterised by the

dynamics of water uptake. Germination commences with ‘imbibition’ (phase I), a profuse uptake of water by the dry seed and a gradual increase of seed size, although this phase is associated with no or little metabolic activity (Bewley et al. 2013). This is then followed by the onset of seed respiration as a result of the resumption of pre-germinative activity, primarily attributed to the activation of mitochondrial energy production, which has been associated with the resumption of phosphorylation to produce ATP (Botha et al. 1992; Ma et al. 2017). Subsequently, the ‘lag’ phase (phase II) involve intense metabolic activity (including the transcription and translation of new genes) and stabilisation of water uptake and respiration rate takes place (Bove et al. 2002). Lastly, active mobilisation of reserves to the growing embryo causes another profuse increase of seed respiration and demand for water uptake, leading to the emergence of the radicle through the seed coat, which marks the end of germination sensu stricto and the beginning of seedling growth (‘post-germination’ or phase III) (Bove et al. 2002; Bewley et al. 2013).

‘On-farm’ seed priming is a farmer-managed type of seed treatment that differs from industrial priming strategies as it simply consists of anaerobically soaking seeds in water for a number of hours prior to sowing (Harris 2006). Seeds are subsequently surface-dried for 1-2 hours (to avoid clumping) and sown soon after. Once sown, seeds spend significant amounts of time absorbing water from the soil. However, by controlling the transition through the germination phases, i.e. allowing seeds to undergo the pre-germinative phases I and II but preventing the start of phase III, ‘on-farm’ primed seed retains the benefits of pre-germinative advancements and, concurrently, preserving desiccation tolerance (Harris 2006; Bewley et al. 2013). Subsequently, this can lead to quicker emergence and enhanced seedling vigour (and ultimately yield) when the primed seed is sown in the field as demonstrated for a range of crops (Carrillo-Reche et al. 2018). Importantly, to fully exploit this method of seed priming, the safe limit (the maximum length of time that seeds can be soaked without germination taking place before sowing) for each crop and cultivar first needs to be determined. However, the optimal duration for soaking seeds (in terms of yield benefits) is not necessarily the same as the safe limit, e.g. priming seeds to the safe limit could lead to seeds biochemically arrested at a very advanced stage in the transition from phase II to phase III (Salimi and Boelt 2019). Therefore, as seed soaking times are specific to

each crop species/genotype or even seed quality, the major obstacle for the determination of optimal ‘on-farm’ seed priming protocols is the large number of trials needed (Paparella et al. 2015; Salimi and Boelt 2019; Forti et al. 2020).

Currently, optimal ‘on-farm’ seed priming times have been determined for a range of crops by testing different seed soaking times (usually on moist filter paper) followed by sowing in mini-plot trials at research stations (e.g., Harris et al., 1999; Rashid et al., 2004, 2006; Virk et al., 2006). However, this process is resource-intensive and information on the soaking times from these trials are limited to the specific crop variety and trial conditions; published or recommended soaking times therefore tend to be conservative, and are likely to compromise any yield benefits that would have been gained from utilising ‘on-farm seed priming. Thus, farmers performing ‘on-farm’ seed priming have used conservative soaking times, for simplicity commonly “overnight”, despite this most likely being far from the optimum (Harris 2006). Consequently, there is a need for the development of cost-effective methods to rapidly determine optimal soaking times for ‘on-farm’ seed priming. For example, increases in respiration at the end of phase II are associated with the initiation of starch metabolism and have been used to predict seedling vigour of different species and cultivars (Patanè et al. 2006; Patanè and Avola 2013; Wang et al. 2016). Therefore, detecting indicators of seed metabolic changes (as the flux of either O_2 uptake or CO_2 release) during seed soaking could provide a useful marker for the optimisation of ‘on-farm’ seed priming. Using barley as a model crop, this chapter aimed to determine: a) whether seed morphology and/or seed respiration changes can be used to detect metabolic changes that occur during ‘on-farm’ seed priming; and b) whether changes in morphology and/or respiration are associated with optimal soaking times and, thus, can be used as a marker for optimising the duration of ‘on-farm’ seed priming.

3.3. Material and methods

3.3.1. Plant material and priming treatments

Barley (Hordeum vulgare L.) cultivars Concerto (Limagrain, Rothwell, UK) and RGT Planet (RAGT Seeds, Ickleton, UK) were chosen for this study as they represent a benchmark variety of spring barley in the UK and a modern elite cultivar

respectively, although they are more correctly representative of genotype x environment x management differences as genotype represents only one factor in seed batch comparisons. The priming treatments applied in all experiments consisted of seeds soaked in distilled water (1:6 (w/v)) in 100 mL plastic pots, at 20 °C in the dark. After treatment, seeds were allowed to air-dry on paper towel for an hour (unless specified otherwise). In all cases, non-primed dry seeds were used as controls.

3.3.2. Effect of 'on-farm' seed priming soaking times on germination

Soaking times and moisture content determination

Samples of 150 seeds were soaked for either 4, 8, 12, 16, 20, 24 or 28 h (28h was established as the upper limit as it was when the coleorhiza tip became visible for some seeds) in triplicate for each soaking time. A subsample of unsoaked seeds were oven dried at 103 °C for 17 h to determine initial moisture content (Mci). The soaked samples were weighed before and after each soaking time to determine final moisture content (Mc), which was calculated as:

$$Mc = \frac{Wi \ * Mci + \Delta W}{Wf}$$

where *Wi* and *Wf* are seed weight before and after drying respectively, and ΔW the difference between *Wf* and *Wi*.

Respiration measurements

Immediately after soaking, the concentration of CO_2 released by the seeds was measured with an EGM-4 CO_2 gas analyser (PP Systems, Amesbury, Massachusetts, USA). Plastic pots containing soaking seeds were hermetically closed with a lid connected to the infrared analyser through inlet and outlet tubing, in order to create a closed system to monitor the flux of [CO_2]. The net CO_2 flux was calculated as the increment within 1 min (average of three sequential readings representing one replicate) prior to allowing CO_2 to accumulate within the tubing system for 15 s (modified from Patanè et al. (2006)). Seed respiration rates (SRR), expressed as µmol CO_2 s^{-1} g^{-1} seed DW (dry weight), for each soaking time were calculated as follows:

$$SRR = \left(\frac{\Delta CO_2}{\Delta t}\right) x \left(\frac{V}{RT}\right)$$

where $\Delta CO_2/\Delta t$ (µmol CO_2 s^{-1}) is the change in CO_2 concentration over the measurement time; V (m^3) is the total volume of the system (volume of priming pot, tubing and gas analyser); R (kPa m^3 mol^{-1} K^{-1}) is the ideal gas constant; and T (K) is the temperature in the incubator.

Histological observations

To examine the morphology changes over time, seeds were transversally sectioned with a razor blade after each soaking time. Seed embryo structures were observed under a stereomicroscope (magnification x 9, Leica GZ6) and photographed using a digital camera (Nikon Coolpix 950).

Germination test

One hundred seeds per soaking time were placed over four sheets of paper towel covered with another two sheets previously moistened with 30 ml of sterile distilled water in plastic containers (304 x 216 x 55mm) with lids, and incubated at 20 ºC in darkness for 72 h. Seeds were counted as having germinated when the radicle length was greater than 2 mm. In order to accurately determine germination dynamics, counts were made every 2 h from the start of germination until cumulative germination was above 75 %. After 72 h, remaining germinated seeds were counted. Each soaking time and germination assay were carried out three times.

Desiccation tolerance test

To simulate a delay before “sowing”, the same soaking times were repeated (as above) and allowed to air-dry on paper towel for 30 days at 20 °C in the dark, and then germination tests carried out as described above.

3.3.3. Effect of ‘on-farm’ seed priming soaking times on seedling vigour

Based on the Germination test results, soaking times of 16, 20 and 24 h were selected for seedling vigour testing. A standard International Seed Testing Association (ISTA) cold test (Hampton and TeKrony 1995) was carried out, where seeds sown in vermiculite in three seed tray inserts (60 cells per tray). All treatments were equally present in each trait and their position was randomised within each

tray. Trays were watered to reach 80 % saturation, covered with aluminium foil to avoid evaporation, and kept at 10 °C in the dark. This setup provided high water availability, good aeration of the substrate and low temperature to minimise any potential head-start related to seed water content. In all cases, un-soaked seeds were also sown as a positive control. After seven days, the trays were uncovered and moved to a growth chamber at 20 °C, 12 h photoperiod and 70 % relative humidity for 5 days. Each tray was watered with 75 mL of distilled water every other day and emergence recorded daily. After 5 days, seedlings were removed from the inserts and categorised as either healthy (viable enough to turn into a healthy plant), or abnormal, e.g. damaged, or deformed or decayed as a result of infection (Figure 3.1 for illustration of abnormality criteria). All healthy seedlings were dried at 110 °C for 17 h to obtain dry weights. The experiment was repeated three times.

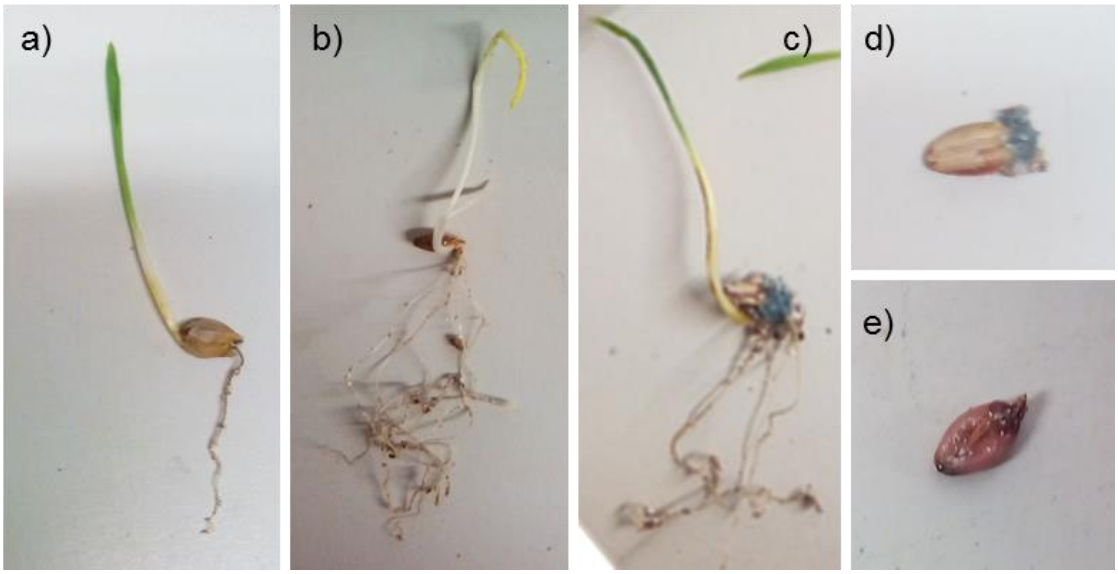


Figure 3.1. Evaluation criteria for seedling abnormalities. a) Damaged seedling missing side roots, b) seedling with a deformed etiolated shoot, c) decayed seedling presenting a fungal infection around the seed coat; d) un-germinated seed due to a primary infection around the germ; and e) non-viable seed.

3.3.4. Data analysis

Indices for time to 50 % germination (G_{50}), time to 50 % emergence (E_{50}), uniformity (U), calculated as the time interval between 25% and 75% of seeds to germinate/emerge, the percentage of total germinated seeds (%TG), and the percentage of healthy emerged seedlings (%TE) were calculated using the

'Germinator' tool (Joosen et al. 2010). Effect of cultivar (Cv), soaking time (Tr) and their interaction on germination variables were assessed by analysis of variance (ANOVA) and emergence variables by linear mixed-effects model (LMM), with experiment repetitions as a random term, in R version 3.3.0 (R Development Core Team 2016). Assumption of normality and homoscedasticity of variances were checked by QQ-plots and residuals against fitted value plots respectively. When these assumptions were not met, data was transformed. G50 data from Germination test were square root transformed and continuous proportional data, i.e., percentage of germination (%TG) and percentage of germination (%TE) were arcsine transformed to approximate normality. Post-hoc Fisher's LSD tests were performed to separate significant differences at P values < 0.05 with predictmeans package (Luo et al. 2014). P values were adjusted to avoid Type I errors (false positives) using the Benjamini–Hochberg correction (Waite and Campbell 2006). Means for significant main effects are presented based on the highest order of factorial combination that was significant in the ANOVA or LMM.

In order to investigate the relative contribution of initial moisture content and advancement of germination to speed of germination at each germination phase, moisture content (Mc) and cumulative CO_2 (ΣCO_2) at the moment of sowing were used as predictors of G_{50}. Data from both cultivars were pooled for this test. Relative importance of predictor variables and their bootstrapped 95% confidence intervals were calculated with the *relaimpo* package (Grömping 2006) in R. Absence of collinearity between the two variables was verified by variance inflation factor.

3.4. Results

3.4.1. Changes in seed morphology and respiration during 'on-farm' seed priming

Barley seeds showed clear morphological differences indicative of the transition from one germination phase to another (Figure 3.2). After the first 4 h of imbibition the wetting of the embryonic tissues was visually evident. This was reflected in moisture content as almost half of the total water absorbed occurred within the first 4 h of soaking, which is characteristic of the phase I "imbibition" stage (Figure 3.3a). From 4 h to 20 h, no major morphological changes were observed, although the overall seed size increased gradually concurrent with a progressive increase in

moisture content. Typically, differentiation and expansion of the embryonic axis began at 24 h, accompanied by seed coat loosening and wetting of the endosperm. At 28 h, emergence of the coleorhiza tip through the micropylar was visually distinguishable for most of the seeds. Soaking times beyond 28 h did not result in further visual morphological development of the seed and only marginal increments in moisture content.

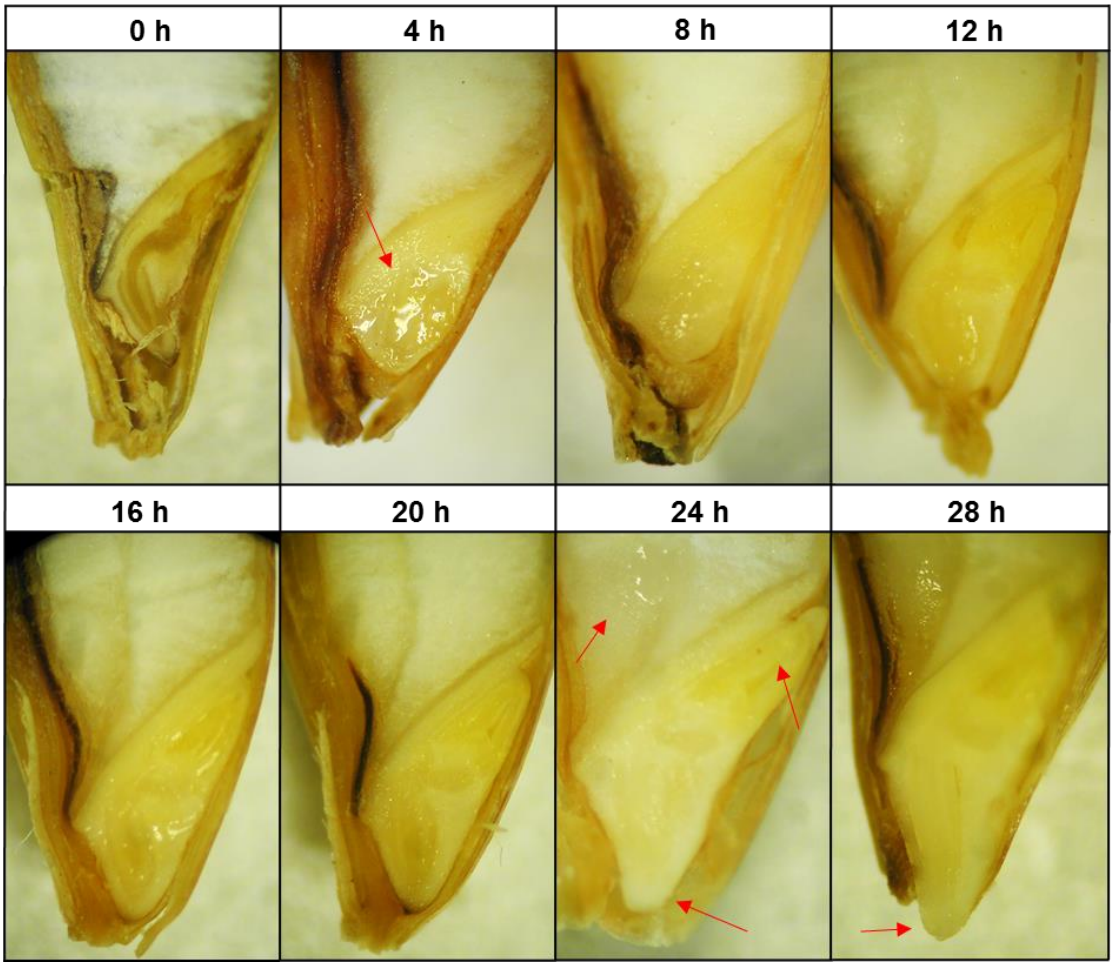


Figure 3.2. Structural morphology of barley seeds at the end of each soaking time. Transversal embryo observation by stereomicroscopy. From left to right, red arrows show wetting of the germ, wetting of the endosperm, expansion of the coleorhiza, expansion of the coleoptile and emergence of the radicle tip.

The initiation of respiration about 4 h after imbibition marked the primary activation of germinative metabolism (Figure 3.3b). The onset of respiration was followed by a steep rise in respiration until about 16 h where the rate of respiration became constant. This plateau, characteristic of the phase II “lag” stage, was punctuated by a second release of CO_2 after 20 h of soaking, which corresponds

with the major morphological changes at 24 h (Figure 3.2). This burst of respiration declined by 28 h, and soaking times beyond this did not result in further increases of water content or seed respiration that typically mark the onset of phase III.

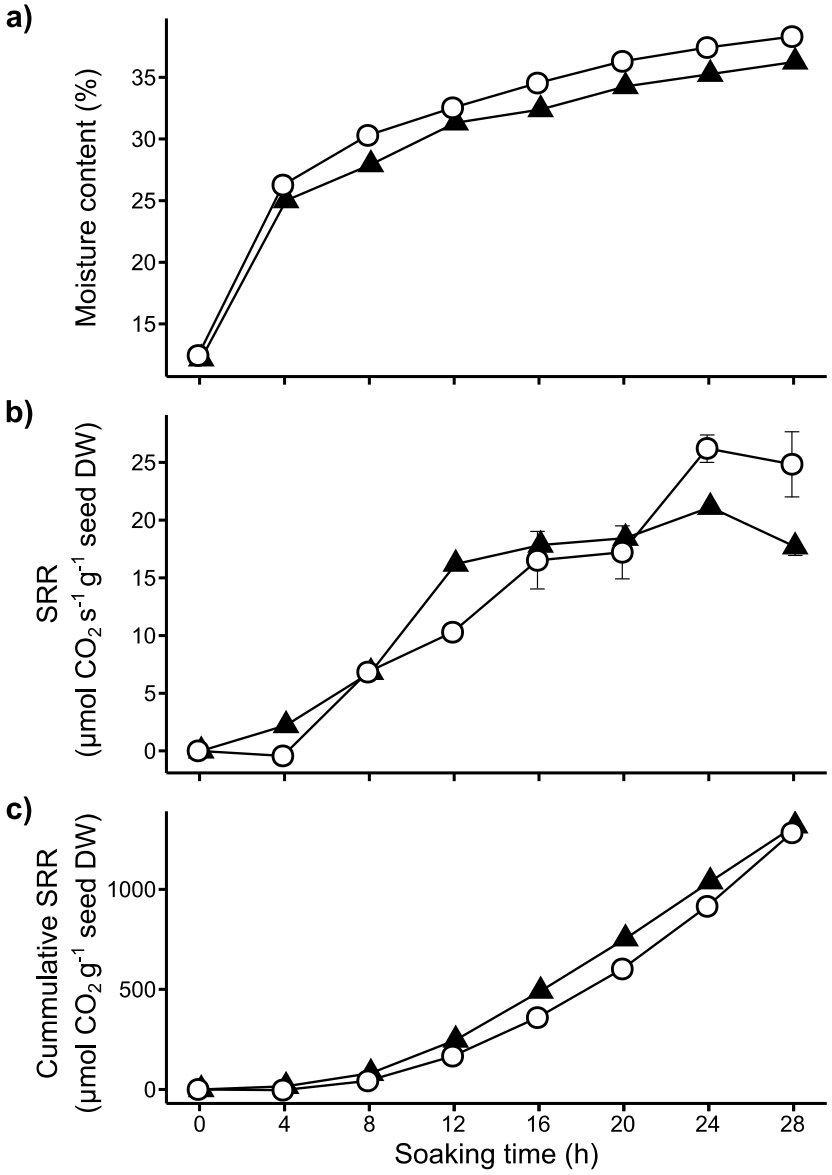


Figure 3.3. The effects of 'on-farm' seed priming on, (a) seed moisture content, (b) seed respiration rate and (c) cumulative respiration at specific intervals for Concerto (open circles) and RGT Planet (closed triangles) barley seeds. Vertical bars show ± SE (only if the SE is greater than the symbol size).

Respiration curves for both cultivars showed a similar triphasic-like shape with some disparity in the initiation in respiration (Figure 3.3b), i.e., the onset of cultivar-specific respiration. For RGT Planet, this occurred within the first 4 h of soaking, whereas for Concerto this happened after 4 h. Cumulatively, although RGT Planet

had earlier metabolism, both cultivars had released similar amounts of CO_2 by the end of the experiment (Figure 3.3c). This cumulative respiration was later used as a proxy of seed germination advancement (ΣCO_2).

3.4.2. Effect of different soaking times on germination parameters

Germination tests were carried out to determine the most promising soaking times for each cultivar. There was a significant interaction between cultivar and soaking time ($P < 0.001$) in time to 50 % emergence. Longer soaking times reduced the time to 50 % germination, although the residual increment after each soaking interval decreased progressively to a minimum between 24 and 28 h (Table 3.1). For both cultivars, the most effective durations were ≥ 16 h. In terms of uniformity of germination, soaking time but not cultivar had a significant effect ($P < 0.001$). Soaking times greater than 4 h significantly improved uniformity, with 16 h being the most effective soaking duration for both cultivars (Table 3.2). However, regarding %TG, there was no soaking time effect ($P = 0.13$) but cultivar effect ($P < 0.001$) with Concerto having 7 % more than RGT Planet. Overall, soaking times exerted very similar effects on germination parameters of both cultivars, thus, based on these results, soaking times of 16 h, 20 h and 24 h were selected for the subsequent seedling vigour tests. Although 28 h soaking time achieved similar values to those of the selected soaking times, it was considered excessively long as the coleorhiza tip was visible in some seeds, indicative of ‘over-priming’ (liable to loss of vigour, desiccation and damage during sowing).

Table 3.1. Effect of seed priming on time to 50 % germination (G_{50}). Values followed by different letters within a column (for each cultivar), differ significantly from each other (LSD test; $P < 0.05$).

Cultivar (Cv)	Soaking time (Tr)	G_{50} (h)[1]
Concerto	0 h	36.9 (6.07[a])
	4 h	22.8 (4.77[b])
	8 h	21.4 (4.62[c])
	12 h	20.8 (4.56[c])
	16 h	18.9 (4.34[d])
	20 h	18.6 (4.31[d])
	24 h	18.0 (4.24[e])
	28 h	17.7 (4.21[e])
RGT Planet	0 h	34.5 (5.87[a])
	4 h	23.9 (4.89[b])
	8 h	20.8 (4.56[c])
	12 h	20.7 (4.55[c])
	16 h	19.8 (4.45[d])
	20 h	19.0 (4.36[e])
	24 h	16.7 (4.09[f])
	28 h	16.1 (4.02[g])
$LSD_{Cv \times Tr}$		(0.06)
df		32

LSD: least significant differences for the interaction; df: degrees of freedom for the residual term.
[1]Back-transformed means and means on the transformed scale (between brackets).

Table 3.2. Effect of seed priming on uniformity of germination (U) and total germination (%TG). Values followed by different letters within a column (for each main effect), differ significantly from each other (LSD test; $P < 0.05$).

Main effects	Levels	U (h)	%TG[1]
Cultivar (Cv)	Concerto	3.74	98.3 (1.44[a])
	RGT Planet	3.72	91.2 (1.27[b])
Treatment (Tr)	0 h	4.70[z]	95.4 (1.36)
	4 h	4.56[z]	96.4 (1.38)
	8 h	3.55[y]	96.0 (1.37)
	12 h	3.56[y]	95.9 (1.37)
	16 h	3.08[y]	96.4 (1.38)
	20 h	3.42[y]	94.8 (1.34)
	24 h	3.59[y]	94.0 (1.32)
	28 h	3.40[y]	93.9 (1.32)
LSD_{Cv}		0.24	(0.03)
LSD_{Tr}		0.48	(0.06)
df		32	32

LSD: least significant differences for the interaction; df: degrees of freedom for the residual term.

[1]Back-transformed means and means on the transformed scale (between brackets).

The proportional contribution of moisture content (expressed as the moisture content at sowing) and germination advancement (expressed as accumulated CO_2 at the moment of sowing) to time to 50 % germination was resolved through linear regression for each phase (Table 3.3). At imbibition, 97 % of the total variability was explained by the model and showed that reductions in time to 50 % germination can be largely ascribed to the moisture content rather than cumulative CO_2 (90 % vs. 7 %) (Figure 3.4). However, this situation was reversed during the lag phase as cumulative CO_2 contributed 1.5-fold more than moisture content to the total explained variance (87 %).

Table 3.3. Linear regression coefficients of time to 50 % emergence (G_{50}) as response variable and, moisture content (Mc) and cumulative CO_2 (ΣCO_2) as explanatory variables. R^2 is the coefficient of determination; and RSE is the residual standard error.

Germination phase	Equation	R^2	RSE	*P* value
Imbibition	$G_{50} = 47.04 - 0.923\ Mc - 0.007\ \Sigma CO_2$	0.97	1.17	< 0.001
Lag	$G_{50} = 22.77 - 0.052\ Mc - 0.003\ \Sigma CO_2$	0.87	0.63	< 0.001

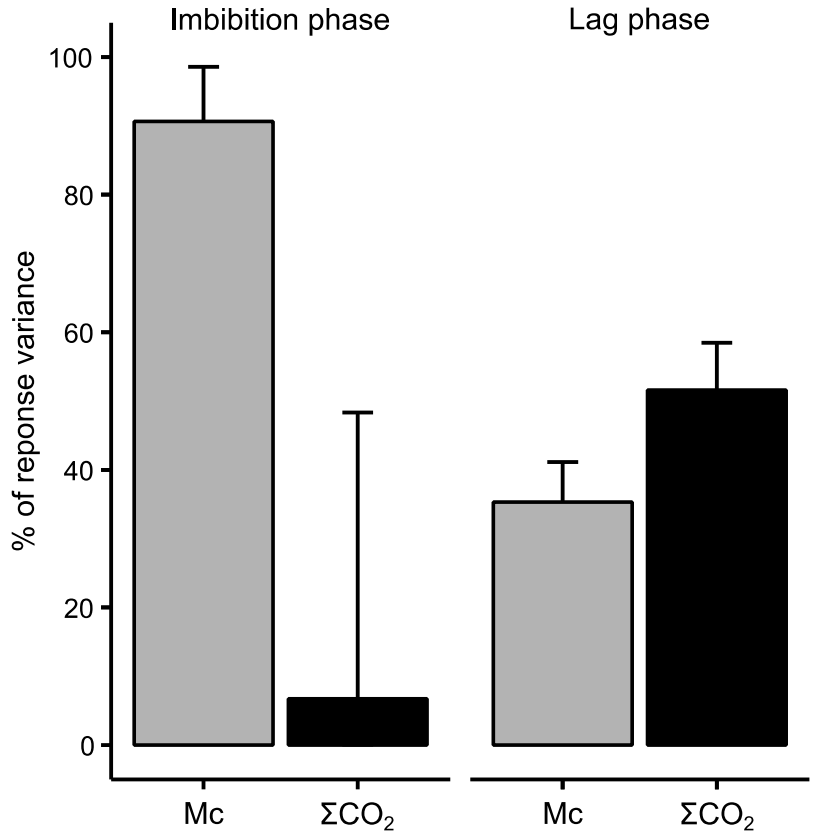


Figure 3.4. Percentage of variance explained by moisture content (Mc) and Cumulative CO_2 (ΣCO_2) to time to 50 % germination during phase I "imbibition" and phase II "lag". Vertical bars show 95% bootstrap confidence intervals.

3.4.3. Vigour: optimization of soaking times and desiccation tolerance

In order to assess the effect of cultivar and treatment on timing of germination, a cold test was designed that would attribute potential changes in biomass to greater vigour rather than initial water content at sowing. No differences in emergence of healthy seedlings were found in relation to cultivar (P = 0.12), treatment level (P = 0.80), or their interaction (P = 0.73) indicating that seed viability remained unaffected

under prolonged exposure to soaking and high moisture (Table S3.1). Similarly, no significant differences for time to 50 % emergence were found among soaking times and control (P = 0.49); therefore, the experimental design was effective for counteracting the effect of initial moisture content (Table S3.1).

In contrast, both main effects significantly affected biomass but not the interaction, indicating that the effect of soaking time was similar for both cultivars (Figure 3.5). Soaking for 20 h produced the highest amount of biomass of all soaking times and was significantly higher than seeds soaked for 16 h (P < 0.01) and 24 h (P < 0.05). Based on these results, 20 h was considered the optimum soaking time for both cultivars.

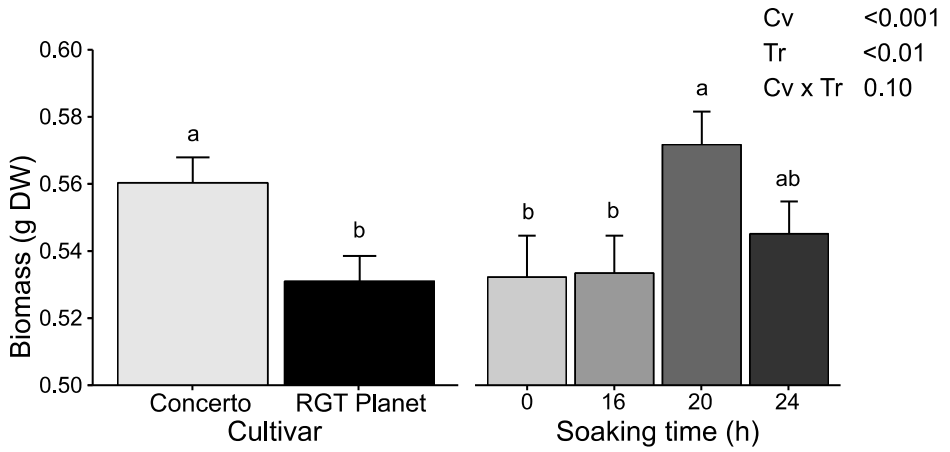


Figure 3.5. Average dry weight of seedlings at the end of the cold test. Linear mixed-effects model P values are for factor cultivar (Cv) and soaking time (Tr). Bars with different letters differ significantly according to LSD test (P < 0.05). LSD_{Cv} = 0.02; LSD_{Tr} = 0.02. Vertical bars show the mean + SE.

Analysis of variance for the effect of desiccation on time to 50 % emergence showed significant differences for cultivar and soaking time (P < 0.001) but not for the interaction (P = 0.94). The seeds of RGT Planet were more affected than Concerto by the 30-day desiccation period (Table 3.4). For both cultivars, seeds soaked for 24 and 28 h needed significantly longer to attain 50 % of germination compared with the rest of the soaking times. Soaking for 8 h yielded the shortest time to 50 % emergence and 28 h soaking the longest time. Differences in total germination were due to cultivar effect (P < 0.001), where again RGT Planet was more sensitive to desiccation. No significant differences among soaking times (P = 0.27) or the interaction (P = 0.40) were found (Table 3.4). Comparison of time to 50

% germination and total germination of (unsoaked) control treatments relative to the corresponding control showed a negative effect in germination performance that was attributable to storage conditions (i.e. 30 d at 20 °C). These effects were most apparent for RGT Planet with +26.4 and -4.5 % change in time to 50 % germination and total germination respectively; whilst the effect for Concerto was minor, +1.8 and -0.7 % respectively.

Table 3.4. Effect of desiccation after different soaking times on time to 50% germination (G_{50}) and total germination (%TG). Values followed by different letters within a column (for each main effect), differ significantly from each other (LSD test; $P < 0.05$).

Main effects	Levels	G_{50} (h)	%TG[1]
Cultivar (Cv)	Concerto	38.3^{b}	98.3 (1.44^{a})
	RGT Planet	44.2^{a}	91.2 (1.27^{b})
Treatment (Tr)	0 h	40.6^{xy}	95.4 (1.36)
	4 h	40.1^{xy}	96.4 (1.38)
	8 h	39.6^{x}	96.0 (1.37)
	12 h	40.6^{xy}	95.9 (1.37)
	16 h	40.9^{xy}	96.4 (1.38)
	20 h	41.5^{y}	94.8 (1.34)
	24 h	43.3^{z}	94.0 (1.32)
	28 h	43.4^{z}	93.9 (1.32)
LSD_{Cv}		0.2	(0.03)
LSD_{Tr}		1.3	(0.06)
df		32	32

LSD: least significant differences for the interaction; df: degrees of freedom for the residual term
[1]Back-transformed means and means on the transformed scale (between brackets).

3.5. Discussion

3.5.1. Seed respiration as a tool for detecting the activation of metabolic processes during ‘on-farm’ seed priming

The present work has shown that monitoring of CO_2 flux patterns is a reliable tool for detecting key germination events during ‘on-farm’ seed priming. As under regular germination conditions, barley respiration during priming describes a

triphasic curve where the transition from one germination phase to another is marked by a burst of seed respiration, providing useful information on the timing of metabolic changes that occur during the course of priming. The highest biomass for both cultivars was attained in seeds primed for 20 h, which morphologically, corresponds with stopping the priming process just before the elongation of embryo tissues into coleoptile and coleorhiza; and before the second burst of CO_2 flux. Therefore, both seed morphology and CO_2 flux patterns can be used as a marker for ‘on-farm’ priming optimisation.

Unlike regular germination, the continuation of phase III beyond its initiation is impeded during ‘on-farm’ seed priming, and longer soaking times do not result in further development of the coleorhiza tip nor a sharp increment of water uptake. Due to the hypoxic conditions within the seed, the energy demands for early barley seed development are mostly provided through oxygen-independent metabolic pathways, e.g. glycolysis and alcohol fermentation (Østergaard et al. 2004; Zhang et al. 2004). CO_2 is released as a waste product of mitochondrial phosphorylation for ATP production and stabilised when oxygen is depleted during the lag phase (Rosental et al. 2014; Ma et al. 2017). However, in late phase II, the further development of the embryo requires oxygen-dependent cycles such as tricarboxylic acid (TCA) that are more efficient for active mobilization of storage reserves (38 mol ATP vs. 2 ATP per mol of glucose) and cannot be fulfilled by anaerobic respiration alone (He et al. 2015; Ma et al. 2017). When exogenous O_2 is available, a profuse second burst of CO_2 flux, attributable to TCA taking place in the newly synthesised mitochondria and mobilisation of reserves, is followed by the appearance of the coleorhiza tip and more water uptake (Bewley et al. 2013; Ma et al. 2017). However, this second burst declines soon after and is not followed by an increase of water uptake under the hypoxic conditions imposed by ‘on-farm’ seed priming. Although respiration remains active, possibly through fermentation and the NO cycle (Ma et al. 2016), further root development is impeded.

Sectioning and observation of seed morphology seems useful for detecting the beginning of phase III, which corresponds with the elongation of the coleoptile and coleorhiza tissues in the embryo, but not for other metabolic processes. As observed for other cereal seeds, although some enlargement of the seed size throughout

soaking could be seen by eye, actual changes in seed structures are minimal even under the microscope until phase III (An and Lin 2011; He et al. 2015).

Cultivars showed distinct seed vigour from one another, although this was not only due to genotype differences but also likely to differential seed quality (as manifested by the notable deterioration of RGT Planet germination performance after a storage period under unfavourable conditions). However, both cultivars performed similarly with an optimal soaking time of 20 h, suggesting that seed vigour and/or seed quality have minor influence in soaking times. Although it is tempting to generalise that 20 h is the optimal soaking time for barley, it is still to be elucidated the extent to what seed vigour and/or seed quality components can influence priming soaking times. Seed phenotypical characteristics (e.g. seed coat, grain composition and size), ageing and the make-up of the maternal tissues are known to alter the germination process and, by extension, likely to affect seed priming soaking times (Finch-Savage and Bassel 2016; Salimi and Boelt 2019).

3.5.2. Mechanistic of the priming benefits: Timing and contribution of its drivers

In order to better leverage ‘on-farm’ seed priming, it is critical to understand the timing and contribution of the two main drivers for rapid germination: 1) a hydrated seed, and 2) being developmentally more advanced than dry seeds at the moment of sowing. Much of the moisture content of a germinating seed is gained within the first few hours of imbibition, and the rapid germination of ‘on-farm’ primed seeds can be mainly ascribed to the rapid hydration of internal tissues rather than to the germination advancement gained during the soaking time. In this study, few hours of soaking (~4 h) were sufficient to dramatically reduce the time for germination relative to dry seeds (35 % out of the 53 % average total gain), after which residual gains from longer soaking times were gradually ascribable to developmental advancement. Longer soaking times (≥8 h) are needed to significantly enhance uniformity of germination, after which no further improvements in uniformity are attained. This suggests the occurrence of metabolic changes at early lag phase which completion ensures that all seeds have reached, by way of checkpoint, a common stage in the germination programme.

It follows from the above discussion that simply soaking for several hours, e.g. 8 h as equivalent to the “overnight” practice proposed for most tropical crops (Harris

2006), is enough to obtain significant germinative benefits from planting hydrated seeds. However, if primed seeds are sown in soil at field capacity, this rapid hydration effect compared to dry seeds may be limited, although the benefits of being developmentally advanced still remain. In an agricultural context yield benefits associated with sowing hydrated seeds will vary depending on local soil moisture, with the most beneficial associated with sowing 'on-farm' primed seeds in water-stressed soils (Carrillo-Reche et al. 2018). Imbibition is primarily a passive process and is a driver for the resumption of metabolic activity (reflected by the increase in respiration) and so the priming duration must be long enough to ensure that the germination process is sufficiently advanced to enable pre-germinative benefits once the seed is sown. Since the actual timing for these events will vary depending on cultivar, seed quality and priming conditions (e.g. temperature), focusing on the germination advancement stages rather than a particular soaking time seems to be the best strategy for the optimisation and standardisation of 'on-farm' seed priming in order to maximise seed vigour.

Seedling vigour is the most important seed quality trait as the post-germination pre-emergence seedling growth phase is considered the most vulnerable stage and, thereby, the usefulness of seed priming (Finch-Savage and Bassel 2016). When the advantage of partial hydration is kept out of the equation, enhanced seedling vigour is evident when the priming process is stopped just before the beginning of the differentiation of embryo tissues into coleoptile and coleorhiza, but not before or after, highlighting the specificity of optimal priming protocols. At this stage, most of the pre-germinative metabolism has already taken place, i.e. mitochondrial multiplication, gene transcription, synthesis of amino acids and new proteins, but is still prior to the induction of post-germinative metabolism, i.e. cell division and expansion, which ensures that root emergence only occurs after sowing (He et al. 2015; Wojtyla et al. 2016; Ma et al. 2017). Furthermore, there is increasing evidence that the activation of cellular repair is the key process enhancing seed vigour following seed priming, so that it is likely that this optimal soaking time corresponds with the maximum DNA repair and antioxidant response to recover from prior oxidative damage (Sharma and Maheshwari 2015; Wojtyla et al. 2016; Forti et al. 2020). However, these invigorating effects are not arrested when seeds are dehydrated to their original moisture content and then allowed to 're-germinate'.

Dehydration, unfavourable storage conditions, and re-hydration lead to extensive oxidative damage that may revoke the seed repair attained during the priming process (El-Maarouf-Bouteau et al. 2013; Waterworth et al. 2019).

The onset of embryonic axes elongation can be understood as the milestone marking the transition from seed to seedling and, although technically falls within the 'safe limits' (as no germination is externally visible even when let air-dry), must be prevented. The declines in seed/seedling performance in both desiccation and vigour tests at and after this milestone are clear signs of excessively long priming duration ('over-priming'). The probable reason for this phenomenon is the loss of desiccation capacity, which is controlled by Type I and Type II proteins that accumulate/deplete as an adaptative mechanism for preparing the seed for germination or to extend the lifespan of the dry seed (Chen and Arora 2013). Type II proteins such as aquaporins, which are essential for water transport between cells and cell expansion for radicle protrusion, accumulate gradually over the course of germination (Chen and Arora 2013; Lutts et al. 2016). Whereas, Type I proteins such as late embryogenesis abundant proteins (which are involved in preventing membrane disintegration and protecting mitochondrial enzymes under dehydration, and are common in dry seeds), are progressively depleted after imbibition, and thus compromise desiccation tolerance (Grelet et al. 2005; Yang et al. 2007; An and Lin 2011).

In addition to the Type I/II protein balance, it is possible that the excessive accumulation of toxic fermentation products, primarily ethanol and lactic acid, in response to the prolonged hypoxic conditions during 'on-farm' seed priming conditions could also play a role in the gradual loss of vigour (Benvenuti and Macchia 1995). These fermentative products are effectively removed by lactate dehydrogenase and alcohol dehydrogenase, but at high concentrations they may become more difficult to eliminate (Benvenuti and Macchia 1995; Bewley et al. 2013).

3.5.3. Implications and practical considerations of 'on-farm' seed priming

In practice, farmers using 'on-farm' seed priming need to be able to distinguish between 'optimal' and 'safe' soaking times. When conditions allow seeds to be sown

within a few hours after priming, optimising soaking times to produce maximal moisture content and advancement benefits would be the best strategy. Air humidity and a long drying period after priming may impair the optimal soaking times by, for example, promoting the proliferation of fungal damage. Thus, when there is a risk of delayed sowing (e.g. due to heavy rain, or having to passively dry seeds overnight after priming), shorter soaking times can ensure that germination does not occur before planting. Current safe recommendations for 'on-farm' seed priming of barley is for "overnight" priming (~8 h) (Harris 2006).

It is important that farmers can obtain information on optimal soaking times for their own seeds and specific 'on-farm' priming conditions. From the methods proposed in this study for determining optimal soaking times, sectioning for microscopic observation of seed morphological changes is the simplest option. Having identified embryo axis differentiation as the marker for "over-priming", this method could be performed by farmers with a razor blade and a magnifying glass. However, the reproducibility of this within the farm context would be a challenge, and specific training for the identification of these subtle embryo differences would be required. The second method of monitoring seed respiration as a marker is a non-invasive technique and allows the accurate identification of both the initiation of phase II (which can be used for recommendation of safe limits) and the initiation of phase III (for recommendation of optimal soaking time). Although this method is not designed to be carried out by farmers, it could be performed by agricultural institutions for providing recommendations of general practices for common varieties within their region produced under comparable growing conditions. Both methods represent a much more rapid and cost-effective alternative to the current optimisation approach through a series of germination assays and mini-plot trials and, therefore, could facilitate the widescale adoption of 'on-farm' seed priming.

3.4. Conclusions

This study emphasises the importance of the two drivers of 'on-farm' seed "priming" benefits: moisture content and advanced germination at the moment of sowing. In an agricultural context, the former largely determines the time to germination but the magnitude will vary depending on soil moisture. However, the extent of the benefits from germination advancement will depend on the moment of

stopping the priming process and, thereby, the importance of optimising the soaking times in order to exploit the full benefits from this technology. Therefore, it is proposed that to achieve maximum seedling performance priming is stopped prior to the differentiation of the embryonic axis and/or the second burst of respiration. This optimal timing can be deduced from morphological observation of the embryonic axis or CO_2 flux patterns for each cultivar and priming conditions. These methods could easily be implemented for determining the optimal soaking times of other cultivars of barley. Extrapolation of these methods to other crops seems feasible although further testing would be required as seed respiration and germination rates can vary greatly depending on crop-specific characteristics of the seed, e.g. starch seeds versus oil seeds.

Chapter 4: Field performance and trans-generational effects of 'on-farm' seed priming and chitosan seed treatments on spring barley (*Hordeum vulgare* L.)

4.1 Abstract

Industrial seed priming is an effective strategy to enhance establishment and stress tolerance, however, it has not been adopted for arable crops in temperate agriculture because it is not commercially viable. Low-cost farmer-managed 'on-farm' seed priming and/or chitosan-based seed elicitor treatments may offer economic alternatives. Increased adaptation to stresses triggered by the application of elicitors can be passed to the next generation, but this has not been scaled-up to a field-based agricultural context. Therefore, a field experiment was conducted in 2018 to determine whether chitosan-based seed treatments (applied at 0.5 to 5 g l^{-1}), and 'on-farm' seed priming treatments (20 h and 24 h priming), either alone or in combination, could improve spring barley production in a European context. Seed collected from 2018 were sown in 2019 to assess whether the increased adaptation to stress acquired by the application of seed treatments can be passed to the next generation. Results from 2018 showed positive grain yield in response to all seed treatments, although yield increases were only significant when chitosan was applied to seeds at the lowest concentrations (14.9 % improvement relative to the control). For grain number, the effect of seed treatments was more evident, showing significant responses for chitosan at the lowest concentrations, 20 h priming and their combination (16, 12.5 and 13.2 % increase respectively). Mechanistically, crops from primed seeds showed improved emergence and seedling vigour that led to a greater number and size of tillers being retained for grain filling. However, these effects were not carried through to their progeny for the same traits measured in 2019. Similarities to the 2018 results were only found for yield, but it could not be determined whether this was simply by chance or if there was an underlying trend. Overall, these findings suggest that 'on-farm' seed priming, and chitosan-based treatments can be effective to ensure that yield potential is not restricted at an early stage in the crop season. However, it seems unlikely that these seed treatments can impart transgenerational legacies. If any effects, these are likely to be mild and the high climatic variability from season to season will hamper the linkage of inherited traits with their parent crop under field conditions.

4.2 Introduction

Cereal crops integrate two characteristic phases, (1) emergence to anthesis, which sets the structures for resource capture and grain formation (potential grain-bearing tillers), and (2) anthesis to ripening, which centres on the production of photoassimilates and the mobilisation of reserves to the grain. The former phase determines the grain number per m^2 (G no.) whereas the second phase determines the grain weight; both parameters together form the grain yield (GY). It is increasingly thought that barley yield is sink-limited, i.e., it produces more assimilate than can be stored by the grain (Bingham et al., 2007; Serrago et al., 2013; Kennedy et al., 2017), thus, producing more grains per ear, or more grain-bearing tillers per unit of area, would increase yield at no physiological cost since it would close this imbalance. However, producing more grains per ear offers little room for improvement as, unlike other cereals (e.g., wheat), barley only produces one fertile grain per spikelet. Therefore, increasing G no. by securing enough grain-bearing tillers early in the season could be a viable strategy to increase barley yields.

'On-farm' seed priming ensures good establishment in cereal crops (Rashid et al., 2006; Harris et al., 2008; Sime & Aune, 2019; Murungu et al., 2004). The simplicity and low cost of 'on-farm' seed priming allows cereal farmers of developing countries to benefit from increases in yield under the varied environmental conditions of low-input agriculture (Harris, 2006); however, 'on-farm' seed priming has not yet been tested in conventional agricultural systems of temperate climatic zones. Industrial seed priming is mostly performed commercially by seed companies in developed countries and limited to high value vegetable seeds (e.g., tomato, lettuce and pepper) due to the need for advanced technology and high energy costs (Paparella et al., 2015).

Chitosan is an active molecule that has attracted attention for its capacity to induce plant growth under both abiotic and biotic stresses (Xing et al., 2015; Hidangmayum et al., 2019). Chitosan is a naturally abundant biodegradable polysaccharide, mainly obtained from the exoskeletons of crustaceans and insects, and its application in agriculture is environmentally sustainable and inexpensive compared to common agrichemicals (Kashyap et al., 2015). Applying chitosan to seeds can enhance germination and seedling vigour, and elicit a range of defence

responses in young seedlings (Sharathchandra et al., 2004; Reddy et al., 1999; Lan et al., 2016; Guan et al., 2009). However, field-scale data quantifying the effects of chitosan seed treatments on yield are limited (Wang et al., 2015).

Studies have shown that the increased adaptation to stresses triggered by the application of elicitors can be passed to the next generation. For example, Walters and Paterson (2012) demonstrated that the progeny from of barley plants that had been treated with the elicitor saccharin, had enhanced resistance to rhynchosporium (causal agent *Rhynchosporium commune*) compared to the progeny from mock treated barley plants. This phenomenon where the progeny acquires the 'primed state' of defence from the maternal plant is known as transgenerational defence priming (Martinez-Medina et al., 2016). A wide variety of abiotic stresses can also imprint transgenerational responses, for example, plants grown from the seeds of parental plants subjected to N-deficiency or drought can demonstrate a 'stress memory' and respond more rapidly to similar environmental challenges (Kou et al., 2011; Walter et al., 2013). Currently, the study of transgenerational effects has been confined to lab experimentation although there is an urgent need to understand the application under field conditions. If effective adaptation occurs following chitosan and/or 'on-farm' seed priming treatments, and this results in greater yields, it is important to understand whether this capacity could also be inherited by the progeny of those plants.

Therefore, the aims of this study were to determine, a) whether 'on-farm' seed priming and chitosan seed treatments can increase crop yields in a temperate field-scale agricultural context; b) whether these treatments affect source:sink ratios and thus create trade-offs in grain quality; and c) whether crop trait effects conferred by elicitor treatments can be carried over to the next generation for spring barley.

4.3 Material and methods

4.3.1 Trial 2018

Plant material and preparation of seed treatments

A spring barley trial was prepared (spring, 2018) at the Balruddery farm (56°28'52.0"N, 3°07'52.6"W) on a sandy loam. Two cultivars were used: Concerto, which is considered a benchmark variety for spring barley in the UK, and RGT

Planet, a modern elite cultivar. Based on previous findings (Chapter 3), seeds were 'on-farm' seed primed during 20 h and 24 h which, morphologically, corresponded to stopping priming prior to differentiation of the embryonic axis or at the beginning of differentiation and expansion of embryonic axis respectively. Chitosan was applied as ChitoPlant® (ChiPro GmbH, Bremen, Germany) to the seeds either alone or in combination with 'on-farm' seed priming based on the manufacturer recommended doses. In total there were nine seed treatments and an untreated control (Table 4.1). Approximately 4,500 seeds (calculated by weight from the thousand grain weight of each cultivar) for each individual treatment were added to 2 l plastic buckets containing distilled water (1:5 (w/v) ratio). For chitosan treatments, powdered chitosan was added at a concentration of either 0.5, 2.5 or 5 g l^{-1}. Seeds were then incubated at 20 °C for either 20 or 24 h (seed priming treatments). The seeds that were treated with chitosan only (i.e. no 'on-farm seed priming treatment) were soaked in a chitosan solution for 15 min. After soaking, seed priming treatments were oven dried at 50 °C until moisture content reached 27-30 % (sufficiently dry to avoid clumping within the seed pipe during drilling). The moisture content of untreated seeds and chitosan only treatments ranged from 12 to 16 %. Subsequently, seeds were reweighed and split into four equal weight portions, which provided the four replicates for each cultivar x seed treatment combination; and packed in envelops ready for sowing.

Site, experimental design and crop husbandry

The trial was laid out using a randomized complete block design, with four replicates at Balruddery Farm (56°28'52.0"N 3°07'52.6"W), which belongs to the James Hutton Institute, Dundee (UK). Plots were sown on the 19th April with an eight-row plot seeder in small plots (1.55 x 2 m) at 360 seed per m^2. A total of 240 kg ha^{-1} of 22-4-14 fertiliser (7.5 sulphate [SO_4]) was applied in two equal splits (at sowing and in mid-May). Adjoining guards (of barley plants) surrounding the experimental plots were also sown to minimize potential edge effects. Weeds were controlled with pre-emergence herbicide Stomp® Aqua (BASF, Cheadle, UK) at a rate of 2.9 l ha^{-1}.

Table 4.1. Factor levels and resulting seed treatments in trial 2018.

Cultivar	Priming duration (h)	Chitosan conc. (g l^{-1})	Seed treatment code
Concerto	0	0	NP
		0.5	NP+0.5
		5	NP+5
	20	0	P20
		0.5	P20+0.5
		5	P20+5
	24	0	P24
		0.5	P24+0.5
		2.5	P24+2.5
		5	P24+5
RGT Planet	0	0	NP
		0.5	NP+0.5
		5	NP+5
	20	0	P20
		0.5	P20+0.5
		5	P20+5
	24	0	P24
		0.5	P24+0.5
		2.5	P24+2.5
		5	P24+5

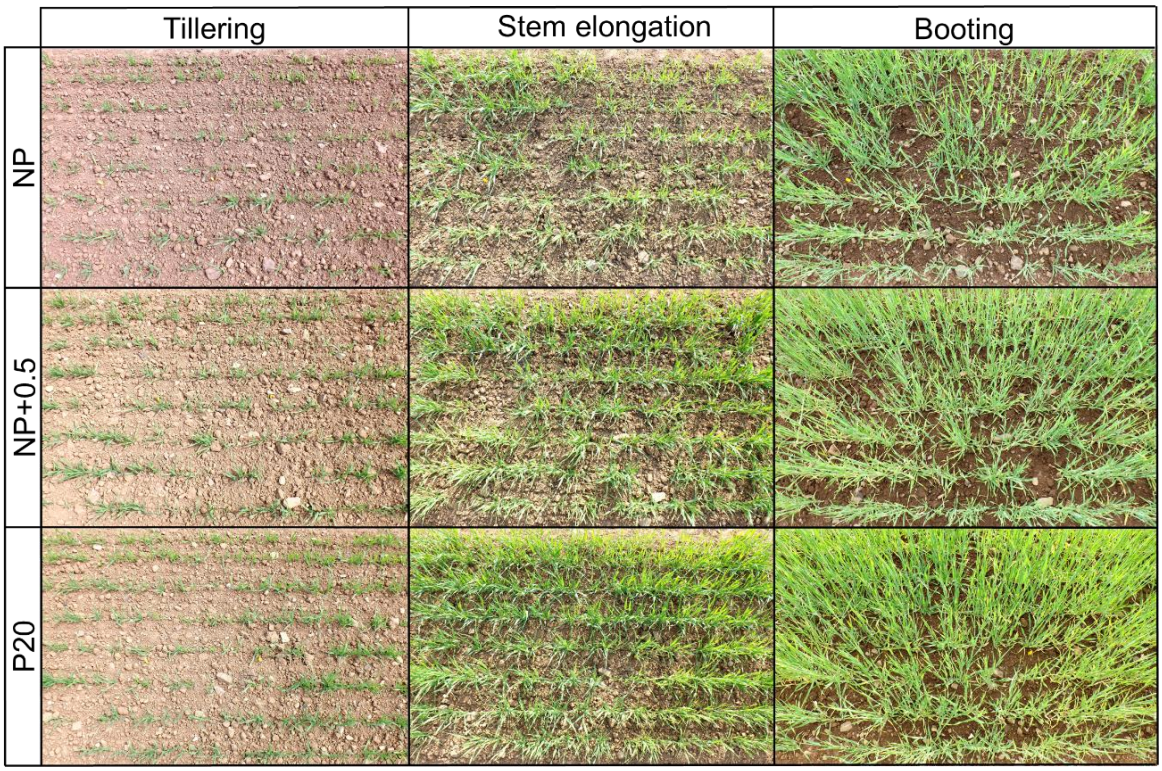
In-field measurements

Seedling emergence for each plot was estimated after the appearance of the first emerged seedlings (15 days after sowing (DAS)). In order to count the same section on each visit, a 0.5-meter section parallel to the row orientation was delimited by pinning two sticks on the soil between the central rows of each plot. Seedlings at both side rows of the marked section were counted 15, 17, 22 and 29 DAS until the counts from the latest visit coincided with the counts from the previous visit (from 22 to 29 DAS).

A single image of each plot was taken at tillering, stem elongation and booting with a digital camera (FinePix S4500, Fujifilm) with an objective 24–720 mm set at the minimum focal length. The camera was set to an automatic exposure time and an aperture with no flash; the images were stored as JPEG with native resolution of 4288 x 3216 pixels. The camera was hand-held pointing downwards from one border of the plot at approximately 1.5 m above the ground level and near the centre

of the plot but slightly angled to capture the whole plot in a single image. Pictures cropped to eliminate soil from the spaces in between the plots (Figure 4.1) prior to calculating the canopy area using CerealScanner plugin (Kefauver et al. 2018; https://integrativecropecophysiology.com/software-development/cerealscanner/), in FIJI (Schindelin et al., 2012), which is a specialist plugin for characterisation of early vigour in cereals (Fernandez-Gallego et al., 2019).

Figure 4.1. Example of images taken for calculating the canopy cover.



Crop height (H), taken from four representative plants per plot, was measured from ground level to the base of the highest fully expanded leaf ligule or, after ear emergence, to the base of the highest ear. These measurements were taken at stem elongation, booting and grain filling. The number of days from sowing to GS55 (when approximately 50% of the stems showed half-emerged spikes) was recorded for each plot. To evaluate potential differences in photosynthetic potential, leaf chlorophyll content (LC) was estimated with a SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan). Five readings were taken from each of four representative flag leaves per plot at booting, anthesis and grain filling.

Yield and grain quality

At ripening, grain was collected with a combine harvester and dried at constant moisture. Grain was passed through a 2.5 mm sieve to eliminate the ‘screenings’ (i.e. the small/broken grain and awns), and subsequently weighed. The percentage of grain retention (Retention %) was calculated from the difference in weight before and after sieving. A subsample of cleaned grain was used to determine grain N concentration (GN) and moisture content determined by using a calibrated near-infra red grain analyser (Infratec 1241, FOSS, Sweden). Thousand grain weight (TGW) was calculated using a MARVIN Seed Analyser (GTA Sensorik, Neubrandenburg, Germany). The grain weight of each plot was then adjusted to 85% dry matter to obtain grain yield (GY) and grain number (G no.) calculated from the GY and TGW.

4.3.2 Trial 2019: Transgenerational effects of seed treatments

To assess the potential transgenerational effects of seed treatments harvested seeds from the previous season were sown, on 5 April 2019, at equal grain number proportion of seeds m^{-2} at a close location to the first trial (56°29'05.0"N, 3°06'35.4"W). Due to limiting space, only the seven most promising treatments in terms of yield and control were put forward for this trial and so P24+0.5 and P24+2.5 were discarded.

The experimental design and crop husbandry were the same as in 2018 except that the second split of fertilisers was omitted. Measurements of canopy cover, yield components and grain quality were recorded as described above. At late anthesis (GS69 approximately), characteristic disease lesions of yellow rust (causal agent *Puccinia striiformis f.sp. hordei*) and rhynchosporium were observed. Disease severity of yellow rust (DSY) and rhynchosporium (DSR) were scored on a continuous scale (0 – 100 %) at plot level following the Agriculture and Horticulture Development Board (AHDB) Cereal trials protocol (HGCA, 2019).

4.3.3 Weather conditions

Mean temperature, accumulated precipitation and relative humidity data were collected by an automated meteorological station situated at a maximum distance of 1.2 km. from the experimental area. Meteorological data for the growing seasons

of 2015-2017 (earliest data available since implementation of the station at the site) were averaged for estimation of typical climate conditions at the site (Stanley et al., 2019). All weather data was supplied by the Natural Environment Research Council through the COSMOS-UK project (https://cosmos.ceh.ac.uk/).

4.3.4 Data organisation and analysis

All analyses were performed using R version 3.3.0 (R Development Core Team, 2016). To investigate associations between yield and grain quality and measured phenotypical traits, Pearson correlations were calculated. Interpretation of associations was assisted with dendrograms generated by hierarchical clustering algorithm, which groups most similar variables together, using Euclidean distances and Complete linkage method.

The data collected during the farm visits was organised according to Table 4.2, where the first sets of variables were aimed at describing establishment, the second set of variables described canopy cover at different stages and so on, for each individual seed treatment. These individual variables and groups of variables were used to perform a multiple factor analysis (MFA). MFA allows analysis of the relationship between individual variables and the groups of variables and a global characterization of the individual treatments by integrating these multiple groups of variables simultaneously. In brief, a principal component analysis (PCA) is performed for each group of variables, whilst within-group variable influences are balanced by dividing each variable by the square root of the 1st eigenvalue of the group to which it belongs (partial analyses). Subsequently, these normalised data are concatenated into a matrix to compute a global PCA where the influence of each variable group is the same (global analysis) (Abdi & Williams, 2010; Pagès, 2004). Only the two main dimensions were kept for analysis.

Table 4.2. Individual variables and variable grouping.

Phenotypic traits / Groups of variables[a]	Measurement	DAS[b]	Phenological stage	Growth stage[c]	Individual variable code
Establishment	Plant counts	15	Seedling Growth	11	E_{15}
	Plant counts	17	Seedling Growth	11	E_{17}
	Plant counts	22	Seedling Growth	11-12	E_{22}
Canopy Cover	Green area	29	Tillering	21	GA_{Ti}
	Green area	43	Stem Elongation	32-33	GA_{SE}
	Green area	57	Booting	41-49	GA_{Bo}
Height	Height	43	Stem Elongation	32-33	H_{SE}
	Height	64	Booting	45-51	H_{Bo}
	Height	110	Grain Filling	87	H_{GF}
Photosynthetic potential	Leaf chlorophyll	57	Booting	41-49	LC_{Bo}
	Leaf chlorophyll	82	Anthesis	69-71	LC_{At}
	Leaf chlorophyll	99	Grain Filling	77-83	LC_{GF}
Yield	Grain weight		Harvest	91-93	GY
	Grain no.		Harvest		G no.
Grain Quality	TGW		Post-harvest		TGW
	Grain N		Post-harvest		GN

[a]Each variable group is used in the MFA.
[b]DAS: days after sowing
[c]Growth stages according to Zadock scale

Effect of cultivar (Cv), seed treatment (Tr) and their interactions on yield components and grain quality were analysed using mixed effects models with replicate plots as a random effect. Assumption of normality and homoscedasticity of variances were checked by QQ-plots and residuals against fitted value plots respectively. Arcsine transformation was applied to DSY and DSR to meet normal distribution. Post hoc Fisher's LSD tests were performed to separate significant differences at *P* values < 0.05 with *predictmeans* package (Luo et al., 2014). *P* values were adjusted to avoid Type I errors (false positives) using the Benjamini–Hochberg correction (Waite & Campbell, 2006).

4.4 Results

4.4.1 Trial 2018

Yield components and grain quality

All seed treatments showed improved GY relative to the control (NP) although only NP+0.5 was significantly greater (14.9 % improvement) (Table 4.3). For G no., the treatment effect was more evident with treatments NP+0.5, P20 and P20+0.5 showing significantly greater grain per m^2, i.e. 16, 12.5 and 13.2 % increase relative to the control. There was a significant impact of cultivar ($P < 0.001$) and treatment ($P < 0.01$) in GY and G no. Whilst P20+0.5 attained the greatest GY and G no. for cultivar RGT Planet, cv Concerto did better with either 20 h soaking or with 0.5 g l^{-1} chitosan alone (Table S4.1), however, this variation did not result in a significant cultivar × treatment interaction. RGT Planet (the most recent elite cultivar of the two) had greater yield performance relative to Concerto.

The effect of treatment on grain quality had a significant effect but, after post-hoc analysis, this difference was not enough for discrimination of the treatments from the control for any of the grain quality parameters. The effect of P24 with chitosan tended to have greater GN, although this was not statistically different from the control. There was a significant effect of cultivar ($P < 0.01$) in Retention %, RGT Planet retained a greater percentage than Concerto; but not of treatment or cultivar × treatment interaction (Table S4.1).

Table 4.3. Mean cultivar and treatment effects for yield and grain quality traits: grain yield (GY), grain number (G no.), thousand grain weight (TGW), and grain nitrogen (GN) on 2018 trial. Values followed by different letters, within a column, differ significantly from each other: LSD test ($P > 0.05$). Significance levels of main effects Cultivar (Cv) and Treatment (Tr); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns non-significant.

		Yield		Grain Quality	
Main effects	Levels	GY (t ha^{-1})	G no. (m^{-2})	TGW (g)	GN (%)
Cultivar (Cv)	Concerto	4.02^{b}	7,914^{b}	49.7^{a}	1.57^{a}
	RGT Planet	4.49^{a}	8,733^{a}	50.1^{a}	1.56^{a}
Treatment (Tr)	NP	3.96^{y}	7,627^{x}	50.7^{z}	1.56^{zy}
	NP+0.5	**4.55^{z}**	**8,846^{z}**	50.2^{z}	1.54^{zy}
	NP+5	4.34^{zy}	8,475^{zyx}	49.9^{z}	1.56^{zy}
	P20	4.41^{zy}	**8,578^{zy}**	50.2^{z}	1.55^{zy}
	P20+0.5	4.35^{zy}	**8,630^{zy}**	49.2^{z}	1.55^{zy}
	P20+5	4.34^{zy}	8,439^{zyx}	50.2^{z}	1.51^{y}
	P24	4.09^{zy}	8,097^{zyx}	49.3^{z}	1.57^{zy}
	P24+0.5	4.01^{y}	7,930^{xy}	49.3^{z}	1.59^{z}
	P24+2.5	4.19^{zy}	8,332^{zyx}	49.2^{z}	1.59^{z}
	P24+5	4.31^{zy}	8,282^{yx}	50.9^{z}	1.60^{z}
Cv		***	***	ns	ns
Tr		**	**	*	**
Cv x Tr		ns	ns	ns	ns

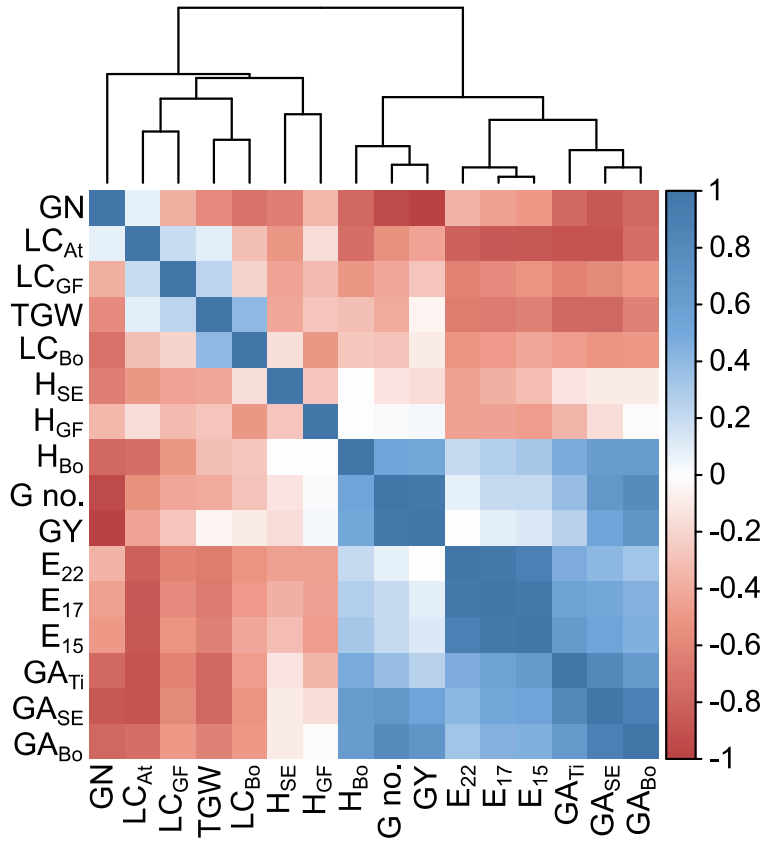
Seed treatment codes are in Table 4.1. Seed treatments significantly different from NP are shown in bold.

Pearson correlations and hierarchical clustering

Pearson correlations were calculated to explore the relationships between yield components and grain quality, as well as their links to the phenotypical traits during crop growth across individual seed treatments (Figure 4.2). GY was strongly correlated to G no. (R = 0.97), although TGW was not correlated to either of the two yield components (R = 0.20 and -0.06 respectively). GN had a moderate negative relationship with G no. (R = -0.47) and GY (R = -0.51). Relationships between consecutive GA measurements changed from moderately to strongly correlated to GY as the crop advanced (R = 0.44, 0.66 and 0.76 at tillering, stem elongation and booting respectively) but particularly to G no. (R = 0.53, 0.75, 0.84 respectively) showing a strong correlation at the booting stage. Height measurements at booting

were also highly associated with GY and G no. (0.65, 0.66 respectively). TGW was moderately correlated to LC at booting and at grain feeling (R = 0.55 and 0.42 respectively) and weakly at anthesis (R = 0.32). Only weak or very weak associations were found for GN and phenotypical traits.

Figure 4.2. Correlation matrix and dendrogram representing associations among phenotypical traits, yield and grain quality parameters. Darker blue shows greater positive correlation whilst darker red shows greater negative. The length of the dendrogram branches represents the distance between variables or clusters of variables calculated from Pearson correlations. Traits abbreviations are as in Table 4.2.



Hierarchical clustering (represented by a dendrogram in Figure 4.2) provided an overview of these relationships at a higher level. This method split data into two main blocks. In the right-hand block, emergence counts and GA were closely connected and, in turn, linked to yield parameters and height at booting. In the left-hand block, branches among variables were generally longer, illustrating a lower degree of association between these variables. TGW appeared to be linked to LC measurements whilst height measurements at stem elongation and grain filling, and GN seemed fused arbitrarily at higher distances.

Multiple Factor Analysis (MFA) dimensions and individual treatments

Only groups of variables with clear association, i.e. Establishment, Canopy Cover and Yield, were further used to characterise treatments phenotypical differences in an MFA. The analysis returned two main dimensions, which encompassed 86 % of the total phenotypical trait variance. All variables were strongly positively correlated to the first MFA dimension. The three groups of variables Canopy Cover, Yield and Establishment groups contributed similarly (37, 34 and 30 % respectively) to the construction of this dimension (Figure 4.3). Treatments towards the right side in Figure 4.4, e.g. RGT Planet P20+0.5 and NP+0.5 were considered the highest rating for these variables and Concerto P24+5 and NP the lowest. In the second dimension, 47 % of loadings belonged to Establishment variables (towards upper side) and 38 % to Yield variables (towards down side). Thus, for example, Establishment rating was proportionally greater than its Yield for Concerto NP, whilst the opposite was the case for RGT Planet P20.

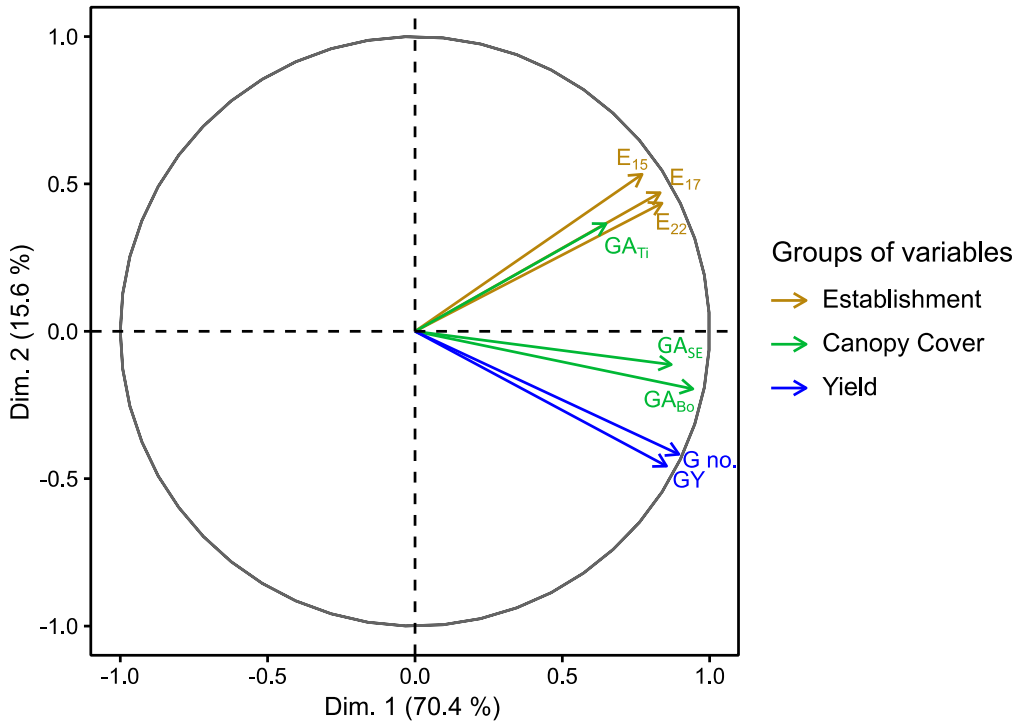


Figure 4.3. Relationships between individual variables and groups of variables (variable codes are in Table 4.2). Variables with arrows closer to the circle are more represented in the global analysis.

It is evident that the two cultivars had distinct growth responses to the different treatments (Figure 4.4). Individual treatment effects of RGT Planet tended to appear

in, or close to, the right quadrats whilst treatment effects for Concerto were mostly in, or close to, the left quadrats. Both cultivars showed positive responsiveness to the seed treatments (with the exception of P24+0.5 of Concerto) in terms of canopy cover, yield and establishment. This was represented by the cv Concerto treatments being projected to the right hand-side of their cultivar control (NP). However, the second dimension showed that treatment effects on Concerto were more evident in yield than in establishment, whilst the opposite seemed to be norm for RGT Planet. In addition, specific seed treatments exerted varying responses on the cultivars. For example, whilst both NP+0.5 treatments were well separated from their respective controls for both cultivars; P20 treatment had a divergent effect as Concerto P20 was well separated from Concerto NP but RGT Planet P20 was scarcely separated from RGT Planet NP. Thus, treatment effects were cultivar-dependent, i.e., treatments did not necessarily produce similar effects in both cultivars.

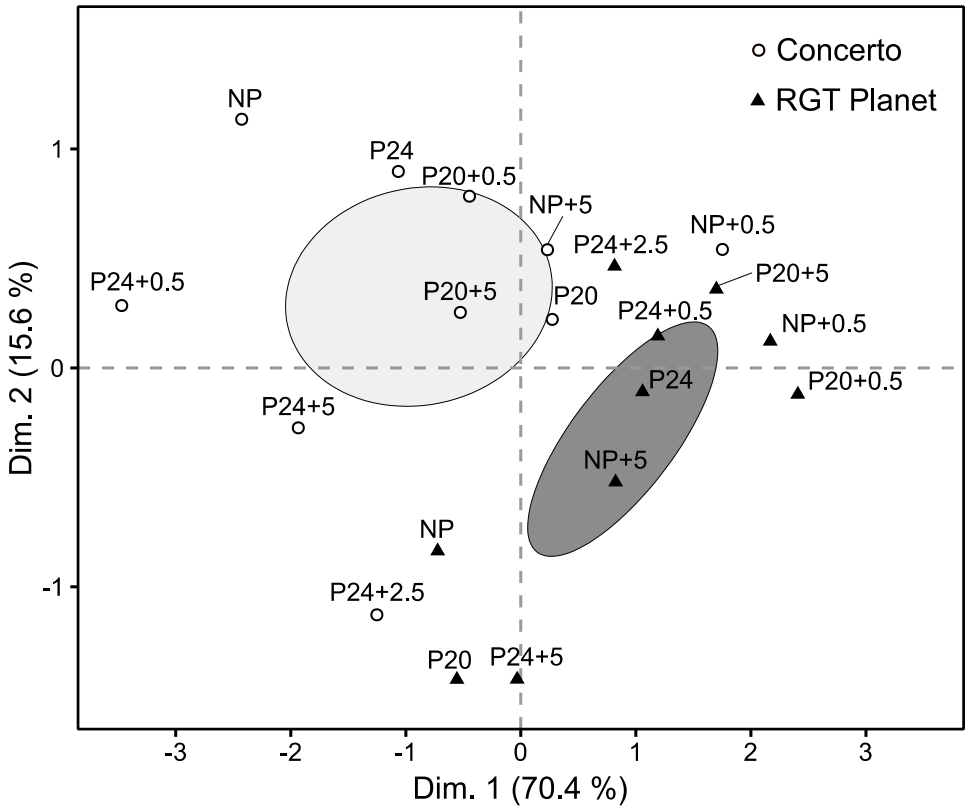


Figure 4.4. Representation of individual treatments on the basis of the first two dimensions by cultivar. Ellipses represent 95 % CIs for Concerto (light grey) and RGT Planet (dark grey).

Factor level decomposition

In order to resolve overall effects of ‘on-farm’ seed priming soaking times and chitosan concentration on the phenotypic traits, data was averaged for each factor level (centroid) and broken down into each phenotypical trait (partial points) (Figure 4.5). MFA analysis of ‘on-farm’ seed priming depicted a clear differentiation between P20 treatments and controls in terms of yield performance (Figure 4.5a). P24 treatments performed halfway between the control and P20 treatments for all traits, although not clearly separated from the control in terms of establishment. All chitosan centroids were on the right, distant from the control, revealing overall positive effects on the *x* axis correlated variables (Figure 4.5b). However, differentiation among concentration levels was less evident, with 0.5 g l^{-1} chitosan (+0.5) slightly separated to the up-right side due to greater effects on Establishment.

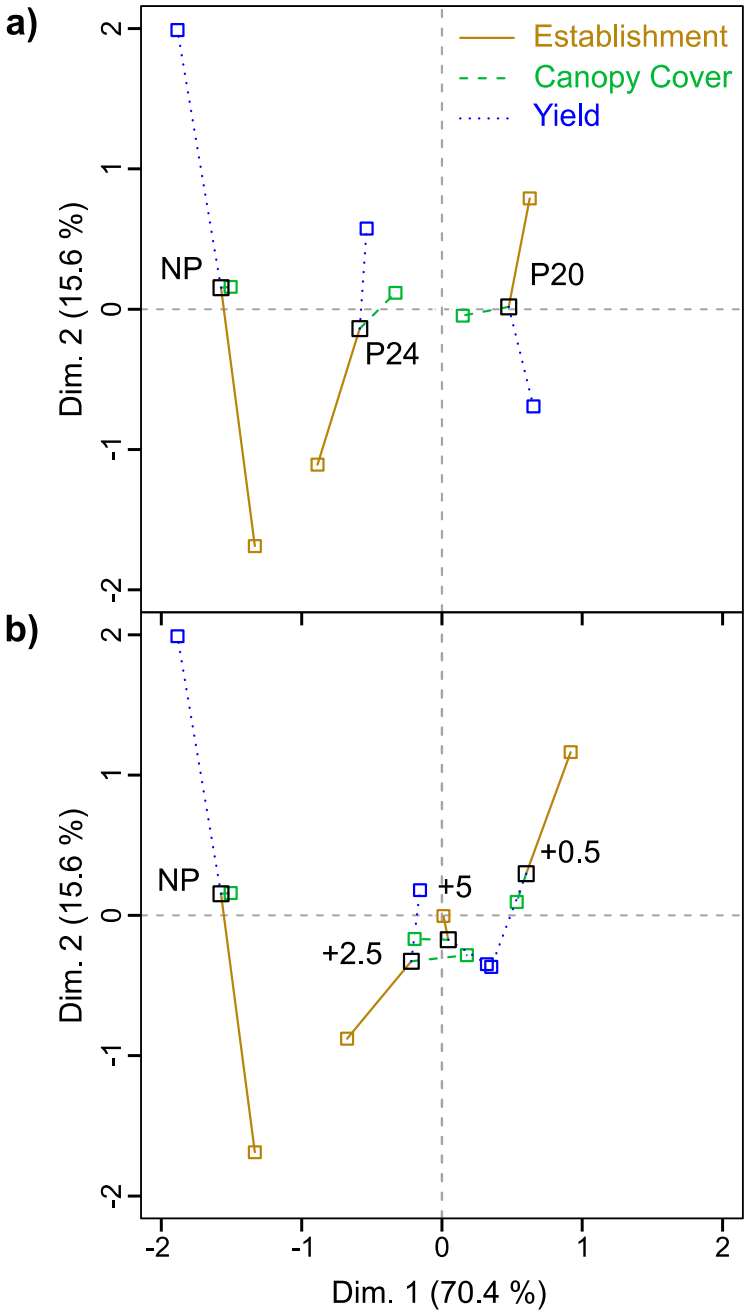


Figure 4.5. Projection of the groups of variables (coloured squares) onto the global analysis according to (a) 'on-farm' seed priming levels, 20 h (P20) and 24 h priming (P24); and (b) chitosan concentrations levels, 0.5 (+0.5), 2.5 (+2.5) and 5 g l^{-1} (+5) against untreated (NP) in 2018. Each dark square of a given factor level is the centroid of the treatments belonging to this level.

4.4.2 Meteorological conditions

There were contrasting weather conditions for the two experimental seasons especially in terms of precipitation (Figure 4.6). Compared with the average of the three previous seasons (318.4 mm precipitation and 79.1 % relative humidity), the

2018 season was considered very dry with 189.8 mm of accumulated precipitation and an average relative humidity of 78 % during the period from sowing to harvest. Conversely, the 2019 season was considered humid with an average of 395.3 mm rainfall and 86 % relative humidity. Average mean daily air temperature was 13.3°C for 2018 and 12.2°C for 2019, which was slightly warmer than the average of the three prior seasons (11.7°C).

4.4.3 Trial 2019: evaluation of transgenerational effects

Yield components, grain quality and disease

The mild weather conditions of 2019 were reflected in greater GY (23 % more on average) than 2018; however, yield components were not significantly affected by cultivar or seed treatment (Table 4.4). Although P20 and NP+0.5 had the greatest G no. as occurred in the 2018 trial, this was not significant relative to the control. There was a slightly significant interaction between cultivar and seed treatment for TGW ($P < 0.05$) due to the control having significantly greater TWG than P20+5 in Concerto, and P24+5 than P24 in RGT Planet. GN was unaffected by cultivar or seed treatments.

Low and very low levels of yellow rust (DSY) and rhynchosporium (DSR) respectively developed at late anthesis (Table S4.2). No differences between cultivars or treatments were found.

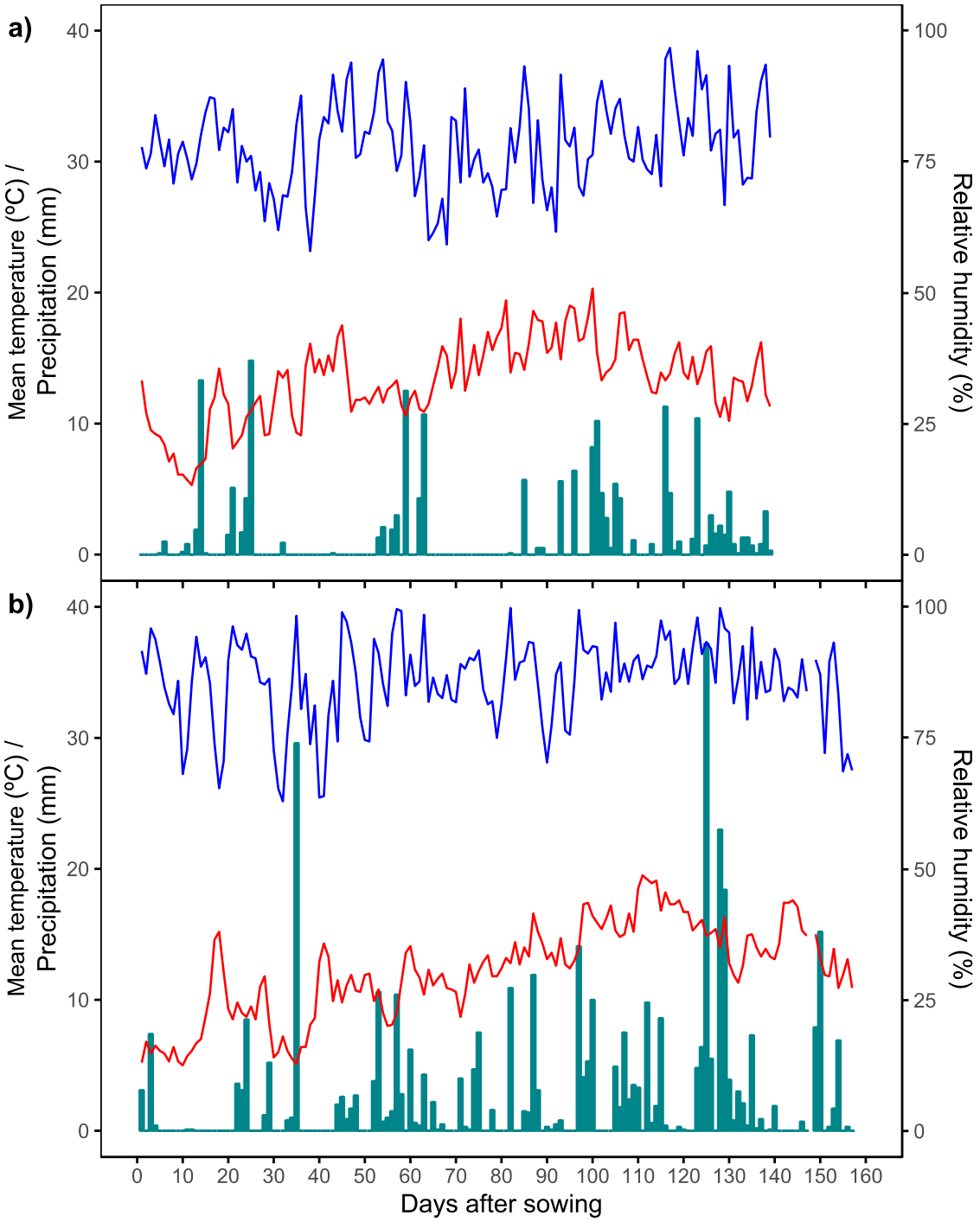


Figure 4.6. Climatic conditions during, (a) season 2018 and (b) season 2019. Daily mean temperature represented by red lines, daily precipitation by turquoise bars and daily mean relative humidity and by blue lines. Data provided by COSMOS-UK.

Table 4.4. Mean cultivar and treatment effects for yield and grain quality traits: grain yield (GY), grain number (G no.), thousand grain weight (TGW), and grain nitrogen (GN) on 2019 trial. Values followed by different letters, within a column, differ significantly from each other: LSD test ($P > 0.05$). Significance levels of main effects Cultivar (Cv) and Treatment (Tr); ns non-significant, * $P < 0.05$.

		Yield		Grain Quality	
Main effects	Levels	GY (t ha^{-1})	G no. (m^{-2})	TGW (g)	GN (%)
Cv	Concerto	5.26[a]	11,030[a]	44.0[a]	1.57[a]
	RGT Planet	5.27[a]	11,170[a]	43.6[a]	1.57[a]
Tr	NP	5.32[z]	11,143[z]	44.1[z]	1.56[z]
	NP+0.5	5.32[z]	11,340[z]	43.3[z]	1.57[z]
	NP+5	5.38[z]	11,268[z]	44.0[z]	1.57[z]
	P20	5.41[z]	11,344[z]	44.1[z]	1.55[z]
	P20+0.5	5.23[z]	10,939[z]	43.9[z]	1.60[z]
	P20+5	5.13[z]	11,006[z]	42.9[z]	1.58[z]
	P24	5.08[z]	10,761[z]	43.4[z]	1.57[z]
	P24+5	5.29[z]	10,997[z]	44.3[z]	1.57[z]
Cv		ns	ns	ns	ns
Tr		ns	ns	ns	ns
Cv x Tr		ns	ns	*	ns

Seed treatment codes are in Table 4.1. Seed treatments significantly different from NP are shown in bold.

Multiple Factor Analysis (MFA) dimensions and individual treatments

The main two dimensions encompassed 79.7 % of the total variance of the traits measured in this trial with most of the groups of variables having high loadings in both dimensions (Figure 4.7). The top right quadrat was dominated by Canopy Cover and Yield variables indicating a positive correlation between them. However, Establishment variables (on the top left quadrat) showed low positive relationship to green area at tillering and no relationship with yield variables. These trait by trait correlations contrasted with the ones in 2018, where the set of Establishment, Canopy Cover and Yield variables were positively and closely related.

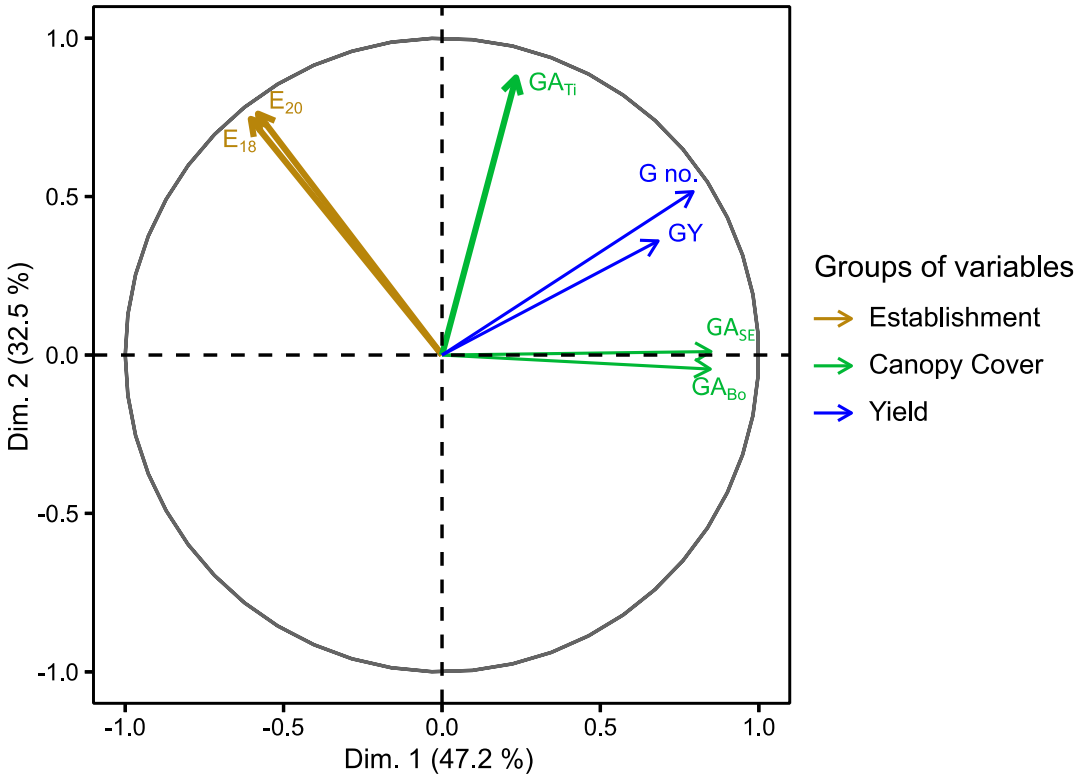


Figure 4.7. Relationships between individual variables and groups of variables (variable codes are in Table 4.2). Variables with arrows closer to the circle are more represented in the global analysis. Thin arrows are more strongly correlated to dimension 1, while thick arrows are more strongly correlated to dimension 2.

Unlike 2018 trial, there was no clear differentiation between cultivars for the measured phenotypic traits (Figure S4.1). This is in line with the low differences observed in yield and grain quality parameters between cultivars. Similarly, effects of 'on-farm' seed priming or chitosan concentrations were minimal relative to the control (Figure S4.2).

4.5 Discussion

4.5.1 Effect of 'on-farm' seed priming and chitosan on yield components

This is the first study on a field-scale of 'on-farm' seed priming of cereals in a European conventional agricultural system. Barley responded positively to all combinations of 'on-farm' seed priming and chitosan treatments, with substantial increases in GY and G no. The priming duration of 20 h gave consistently greater yield components than the 24 h treatments and, thus, confirms that the optimal soaking time of 20 h for these two cultivars (as hypothesised in Chapter 3) can be

successfully translated to field-scale conditions. Although P24 brought seeds closer to germination (up to the state of embryo differentiation), it probably also entailed a loss of desiccation tolerance and a greater accumulation of toxic fermentative products from the prolonged hypoxic conditions that would compromise the vigour of the future seedling. Seed priming for 20 h should not be taken as the optimal soaking times for all barley cultivars however, as the precise duration will vary depending on cultivar and priming conditions (e.g. temperature) (Paparella et al., 2015). For example, Rashid et al. (2006) found in a series of trials with local Pakistani cultivars and an old US cultivar that the optimal soaking time varied between 12-16 h.

Although there is little available data linking chitosan seed application and effect on yield (Wang et al., 2015), a number of studies have demonstrated enhanced germination, seedling growth and protective effects for cereal crops when chitosan is applied as a seed treatment (Sharathchandra et al., 2004; Reddy et al., 1999; Guan et al., 2009; Siddaiah et al., 2018). However, the implications for barley, beyond the early growth stages and subsequent yield performance have not yet been determined. In this trial, greater yield components were generally obtained at the lowest chitosan dose tested (0.5 g l^{-1}). However, this trend was reversed when chitosan treatments were combined with P24 as higher concentrations of chitosan tended to produce higher yields, validating that the activity of chitosan as a plant regulator is concentration-dependent (Yang et al. 2019). Solutions containing high concentrations of chitosan may create a film around the seed that hamper water absorption (Jabeen & Ahmad, 2013) or induce cell apoptosis (Li et al., 2019); although it is not clear why the concentration of the chitosan would interact with a longer seed priming duration. Perhaps prolonged hypoxia during P24 priming may generate excessive nitric oxide and fermentative products and the antioxidant properties of chitosan can counteract these effects to some extent.

Genetic background of the cultivar also determined which treatments were more beneficial and the magnitude of response to them. For example, RGT Planet was less sensitive to the duration of ‘on-farm’ seed priming, whereas 24 h priming seemed excessively long for Concerto. The combination of the highest performing priming duration and chitosan concentration (i.e. P20+0.5) did not always result in an additive value effect. Whilst P20+0.5 was indeed the best treatment for RGT

Planet, Concerto achieved greater yields with either 20 h soaking or with 0.5 g l^{-1} chitosan alone illustrating a genetic response to specific treatments. In general, combined priming/chitosan treatments did not significantly differ from their corresponding single priming or chitosan treatments, suggesting that the treatment combinations were not necessarily complementary. It is possible that 'on-farm' seed priming and chitosan have overlapping mechanisms of action, at least, on their direct metabolic effects on seed development. The former promotes α-amylase production during germination, which plays a crucial role in starch mobilization, and provides the embryo and the subsequent young seedling with carbohydrates for respiration (Ashraf & Foolad, 2005); and the accumulation of antioxidants and soluble phenolics in the seedlings (Farooq et al., 2017). Likewise, chitosan significantly stimulates amylases and the production of antioxidants in germinating seeds (Lan et al., 2016) as well as upregulating metabolites involved in photosynthetic C fixation and N assimilation of seedlings (Zhang et al., 2017). Therefore, both have similar mechanisms resulting in rapid germination and seedling growth which, in turn, may be translated into improved crop stands.

4.5.2 Understand the mechanism for yield variation

The concatenated positive association of establishment, vegetative growth and yield in this trial suggested that seed treatments provide a head start at emergence that is upheld until harvest resulting in greater yields. Likewise, the fact that GY was very strongly correlated to G no. rather than to TGW, also pointed towards tillering-stem elongation, which is when G no. are largely defined in barley (Ugarte et al., 2007; Arisnabarreta & Miralles, 2008; Křen et al., 2014); as the key stages for the GY variation in this study.

The tillering and stem elongation stages are sensitive to mean temperature (Ugarte et al., 2007). Cold temperatures allow appropriate tiller formation whilst warm temperatures can hasten stem elongation causing yield losses up to 46 % that cannot be recovered even if there is a good flush of re-tillering in later stages of the crop cycle (Ugarte et al., 2007; García del Moral & García del Moral, 1995). Thus, in the 2018 season it is likely that the relatively warm mean temperature during tillering-stem elongation, together with the lack of rain, constrained tiller production in this study. Consequently, plots with greater plant populations and fully emerged

seedlings at the beginning of the tillering stage, e.g. 20 h primed and/or chitosan alone treated plots in this study, had a greater chance of retaining more shoots to booting.

The number of shoots together with vigorous growth was involved in tiller survival as indicated by the increasingly stronger association of canopy cover (estimated as green area) throughout tillering, stem elongation and the booting stages, to grain number. Although greater canopy cover would not necessarily mean greater vigour (as it could simply derive from the increased plant numbers at seedling growth stage), the fact that height at booting was also strongly positively correlated to G no. suggests enhanced vigour per shoot as a mechanism. A number of vigour-related traits have been linked to survival of tillers with anteriority. Tillers with greater rate of leaf emergence, with at least one-third of the height of their main stem at stem elongation or with greater leaf area and canopy size before stem elongation have been found to be more fertile in barley (Kirby & Jones, 1977; Kennedy et al., 2017; García del Moral & García del Moral, 1995). Thus, these results highlight the importance of ensuring good sized tillers that are able to intercept more light prior to GS31 and minimise pre-anthesis tiller mortality (Kennedy et al. 2017). Although re-tillering may take place during heading and anthesis if environmental conditions are favourable, its contribution to yield is commonly negligible (Kennedy et al., 2017).

4.5.3 Effects on source-sink ratios and grain quality

It is conceivable that if G no. is significantly increased, inter-plant resource competition may also increase, unbalancing source-sink ratio and compromising attainment of full TGW potential. Nevertheless, there was no association between TGW and G no., so it is unlikely that source capture was compromised in this trial. However, given that plant densities were relatively low in this trial, it cannot be ruled out that under more restricted conditions in terms of available assimilates per shoot it may have a negative impact in TGW.

There is a general view that crops that stay greener for longer can maximise TGW resulting in a higher percentage of grain retention. In an effort to non-invasively monitor this process, photosynthetic potential was estimated (expressed as chlorophyll content of the flag leaf) from pre-anthesis to ripening, and showed a

moderate association with TGW. Seeds soaked for 20 h showed low photosynthetic capacity when compared to the control average, however, this did not affect either TGW or percentage of grain retention of these plants indicating that they were not constrained by these effects in this trial. Assuming that post-anthesis spike photosynthesis (which is another important source of photoassimilates for grain filling) followed a similar pattern as the measure of leaf photosynthesis, this can be explained by the fact that mobilisation of stem soluble carbohydrate to the grain can be more efficient than maintaining photosynthetic activity in latter stages of grain filling (Serrago et al., 2013; Bingham et al., 2007). Thus, taken together, the results of this trial reinforce the view that barley is not commonly source-limited during grain filling (Serrago et al., 2013; Bingham et al., 2007; Kennedy et al., 2017).

4.5.4 Transgenerational effects of elicitor treatments

The contrasting weather conditions between the 2018 and the 2019 season resulted in very distinct crop development during each season. Whilst establishment played a crucial role in 2018, no link was found between establishment and subsequent canopy development in 2019 indicating that establishment was not a limiting factor during 2019. This was also evident at cultivar level. Whilst RGT outperformed Concerto in 2018, especially in terms of yield (which was expected given that RGT Planet is known to be a higher yielding cultivar than Concerto (HGCA, 2019)); both cultivars performed similarly during 2019.

This study has provided no evidence of transgenerational effects following either chitosan or ‘on-farm’ seed priming in spring barley. Although seed treatments did exert changes in 2018 that resulted in better overall performance, those effects were not carried through to their progeny for the traits measured in 2019. Resemblance to 2018 results can only be found for yield components, but not for other traits, as NP+0.5 and P20 repeated among the highest values for yield components, but it cannot be determined whether this was simply by chance or there was an underlying trend. In this respect, these results depict a similar picture to the only data available of in-field elicitor-induced transgenerational effects (Adrian Newton (James Hutton Institute), unpublished data). Therefore, it can be hypothesised that either (1) there is no transgenerational effect associated with these treatments, or (2) there is an

underlying transgenerational effect, but their effects are too mild to be detected under field conditions.

Chitosan and ‘on-farm’ seed priming may not exert the type of stimulus needed to produce adaptative changes onto the next generation. Transgenerational effects are caused by elicitors or stress events in the maternal plants that produce long-lasting epigenetic changes, e.g., DNA methylation and histone modifications; or accumulation of transcriptional factors that are passed on to their progeny (Ramírez-Carrasco et al., 2017; Walter et al., 2013). In this respect, there is increasing evidence of elicitors, such as β-Aminobutyric acid (BABA) and salicylic acid derivates, and abiotic stresses, such as heat stress, drought or N-deficiency that can facilitate transgenerational changes (Ramírez-Carrasco et al., 2017; Walter et al., 2013; Kou et al., 2011). Similarly, both ‘on-farm’ seed priming and chitosan seed treatment can be perceived as a first stress/elicitation event to the maternal plant. The former represents an abiotic stress due to the hypoxic conditions of the treatment and/or membrane damage caused by rapid uncontrolled imbibition (Chen & Arora, 2013). This rapid imbibition of seeds, during ‘on-farm’ seed priming, is known to disrupt cell membranes and cause localised cell death in cotyledons and the embryonic axis of seeds producing reactive oxygen species (ROS) (Powell & Matthews, 1978; Bailly, 2004). In contrast, chitosan may be perceived as a biotic stress that mimics pathogen-associated molecular pattern molecules (PAMPs), widely known to induce systemic acquire resistance (SAR) (Iriti & Varoni, 2017; Alexandersson et al., 2016). However, there is currently no evidence of whether seed priming related stresses or chitosan can induce changes at an epigenetic level.

Apart from the nature of the elicitor/stress, the timing might be also an important factor. Although epigenetic effects through seed treatments have been suggested as a plausible strategy to imprint transgenerational benefits, the experimental evidence is lacking (Chen & Arora, 2013; Worrall et al., 2012). It is conceivable that transgenerational benefits will be more likely to pass onto the progeny when the triggering stimulus (either stress or elicitor) takes place during the period of maternal seed development so that the progeny seeds are directly bestowed with the priming state. For example, progeny from oilseed rape and wheat that were drought-stressed during flowering of the maternal plants have demonstrated higher vigour and adaptability to subsequent stresses than the progeny seeds from non-stressed

maternal plants (Hatzig et al., 2018; Tabassum et al., 2017). Therefore, chitosan could be a potential candidate for transgenerational legacy if applied, for example, as a leaf spray.

The other possible explanation is that there were transgenerational effects but they were mild, and the optimal conditions experienced during 2019 did not provide a sufficiently challenging environment that would have shown expression of these effects. Some physiological legacies, not necessarily involving epigenetic changes, such as seedling vigour can be inherited from the maternal plant through alterations in seed composition such as a greater accumulation of protein content and storage metabolites (Hatzig et al., 2018; Richards, 2000). However, warm temperatures and well distributed rainfall throughout the season would have hampered detection of these potential effects. Moreover, disease severity (yellow rust and rhynchosporium) was at a low level and too late in the season to constitute a challenge for enabling differentiation of potential effects on defence priming.

The high variability of the experimental field conditions significantly influenced both crop development and the challenge stimuli (disease and/or drought and the timing of occurrence), which hampered any observation of induced heritable traits. Moreover, a greater understanding of the elicitor/stress stimulus, timing and mode of applications need to be gathered first at lab level before moving on to field experimentation. Molecular and epigenetic markers would allow a more robust method for associating phenotypes with transgenerational memory. This can be done, for example, by quantifying the transcript levels of marker defence genes such as PATHOGENESIS RELATED GENE-1 (PR-1) or methylation patterns such as Cytosine methylation, as has been done for elucidation of transgenerational defence priming and N-deficiency memory respectively (Ramírez-Carrasco et al., 2017; Kou et al., 2011).

4.6 Conclusions

Successful establishment before GS31 is a bottleneck for attaining enough yield bearing structures in spring barley, therefore, seed treatments and elicitors could be a timely and convenient approach to ensure that yield potential is not restricted early in the crop season. This study suggests that chitosan-based and ‘on-farm’ seed priming treatments (separately or in combination) can improve shoot vigour which,

ultimately, results in greater yields without causing side effects. It is foreseeable that these seed treatments are especially beneficial under stress conditions such as rapidly drying seed beds or when competing with weeds for light where enhanced growth will reduce tiller mortality. However, data on the effects of contrasting climates, soil types and genetic background are now needed to more clearly define the potential benefits that these seed treatments can deliver.

Whether chitosan-based and ‘on-farm’ seed priming treatments can impart transgenerational legacies remains unknown. The high variability of climatic conditions from season to season is likely to be a major burden assessing transgenerational effects in the field as it hampers the linkage of potentially inherited traits with their parent crop. Additionally, practical questions such as elicitor/stress stimulus and timing of the application in crop species need to be further tested under controlled conditions before moving on to field experimentation. Currently, the molecular and epigenetic basis of transgenerational memory are still largely unknown and, thus, will require further research before they can be exploited in sustainable agriculture.

Chapter 5: In-field evaluation of host defences induced by seed treatments on winter barley (*Hordeum vulgare* L.)

5.1. Abstract

Control of cereal foliar diseases depends largely on the application of non-sustainable chemical fungicides. Enhancing host defences, i.e. induced resistance, disease tolerance and/or escape, in combination with current disease management regimes may be a valuable strategy to reduce pesticide use and provide durable disease control as part of integrated pest management (IPM) programmes. Since both 'on-farm' seed priming (OSP) and chitosan priming (CHP) have been reported to confer varying levels of host defence, this study sought to investigate their potential to deliver disease control as a strategy for the sustainable management of crop pathogens in winter barley. Field experiments were conducted at two different field sites, and included fungicide and non-fungicide treatments, three cultivars, and OSP, CHP and a control treatment (NP) as seed treatments at each site. Results showed no significant effects of seed treatments on disease severities of powdery mildew (*Blumeria graminis*) or rhynchosporium (*Rhynchosporium commune*), except for powdery mildew at one of the sites. Further analysis revealed that these differences were due to a negative association between post-stem elongation powdery mildew levels and rate of stem elongation in the non-fungicide treatments, where OSP showed the highest rate and lower disease severity. Estimated tolerance varied by cultivar but not by treatment. At both sites, strong negative correlations were found between canopy size and senescent tissue (mostly attributable to powdery mildew infection) at advanced tillering. Overall, no evidence was found to suggest that chitosan or 'on-farm' seed priming can induce resistance. It is likely that the continuous interactions with biotic and abiotic elements hinder the expression of potential induced resistance in field crops. These field trials, however, enabled the identification of candidate traits to deliver disease tolerance (and escape) for primary and secondary spreads of powdery mildew, such as large canopies and rapid stem elongation respectively. The greater remaining healthy tissue of large canopies may allow them to compensate for a loss of radiation interception in primary infections. Rapid stem elongation can limit the upward spread of powdery mildew by developing upper leaves away from the optimal microclimate for the fungus lower down the stem. Thus, seed treatments may deliver disease tolerance and escape traits, but these benefits will be conditional upon conferring successful establishment and vigour first.

5.2. Introduction

Plant host defence against pathogens and parasites can involve three elements, i.e. ‘resistance’, which is the capacity of a crop to eliminate or limit pests and pathogens by genetic and molecular mechanisms, ‘tolerance’, which is the ability to maintain performance in the presence of expressed disease and ‘escape’, which is the ability to restrict the dispersal of spores within the canopy and hence the spread of the disease (Walters et al. 2012; Ney et al. 2013).

A number of natural and synthetic substances that have the potential to induce host resistance have been identified. These so-called plant defence elicitors include chitosan, which acts as a priming stimulus for systemic resistance by mimicking pathogen-associated molecular pattern molecules (PAMPs) (Alexandersson et al. 2016; Iriti and Varoni 2017). Chitosan can induce resistance in crop plants against a wide range of pathogens including via direct application to seeds. For example, seeds from tomato, pearl millet and wheat immersed in a chitosan solution had subsequent protection against *Fusarium oxysporum*, *Sclerospora graminicola* and *Fusarium graminearum* respectively, through the accumulation of defence-related secondary metabolites, e.g. beta-1,3 glucanase and ferulic acid (Benhamou 1994; Reddy et al. 1999; Sharathchandra et al. 2004). Similarly, induced resistance responses have been associated with crops following ‘on-farm’ priming of seeds (Rashid et al. 2004a; Harris et al. 2005), with reports of a decrease of 20 % in downy mildew (causal agent *Sclerospora graminicola*) infection of pearl millet after ‘on-farm’ seed priming (Harris et al. 2005). It was hypothesised that the anaerobic conditions of the treatment may trigger an accumulation of phytohormones involved in induced resistance that, upon pathogen attack, could accelerate and strengthen defence responses (Harris et al. 2005).

In addition to induced resistance, seed priming with water or chitosan may have other physiological effects that result in traits that can confer a varying degree of disease tolerance and/or escape. For example, chitosan application can result in a larger canopy size (Chapter 4) and therefore an increased net photosynthetic rate (Khan et al. 2002), both of which are candidate traits that can lead to tolerance of foliar diseases in cereal crops (Bingham et al. 2009). Enhanced crop vigour following ‘on-farm’ seed priming, can lead to considerably decreased severity of the

symptoms caused by mungbean yellow mosaic virus (MYMV) due to the improved state of readiness of the plant to defend itself (i.e. plant 'tolerance') (Rashid et al. 2004a). The rapid emergence of crops following 'on-farm' seed priming can reduce the size of the 'infection window' available to soil-borne diseases such as collar rot (*Sclerotium rolfsii*) and *Fusarium* wilt (Musa et al. 2001), whilst the decreased time to maturity can reduce exposure to late-season pests (i.e. 'escape') (Harris et al. 1999). Increased height or rapid stem extension may also be traits that confer disease escape, for example by hampering the spread of disease to upper leaves by splash-spread diseases such as rhynchosporium (*Rhynchosporium commune*), (Walters et al. 2012).

Effective control of diseases solely through induced resistance, tolerance and/or escape mechanisms is unlikely; however, unlike fungicides or genetic-mediated resistance, these strategies are broad-spectrum and so do not generate pathogen selective pressure. Enhancing host defences, in combination with current disease management regimes, may be a valuable strategy to reduce pesticide use and provide durable disease control in integrated pest management (IPM) programmes of cereal grains (Walters et al. 2012). In barley, it is especially important to protect crops from early epidemics during the vegetative growth as yield largely relies on maximising tiller production and survival (Walters et al. 2012). Thus, IPM findings in winter barley, with more overwintering disease and more routinely exposed to pathogens than the spring crop, may be particularly valuable as strategy to retain side tillers that might otherwise be lost to disease (Zhan et al. 2008).

Therefore, the overall aim of this chapter is to investigate the potential of 'on-farm' seed priming and chitosan-based seed treatments to deliver disease control as a strategy for the sustainable management of winter barley pathogens. The specific objectives of this work were to test the hypotheses that 'on-farm' seed priming and chitosan seed dressing can: (a) induce disease resistance; b) confer disease tolerance; c) confer disease escape; and d) increase crop yields in a temperate field-scale agricultural context.

5.3. Material and methods

5.3.1. Plant material and preparation of seed treatments

Three winter barley genotypes with differential disease ratings to common foliar diseases, according to Agriculture and Horticulture Development Board (AHDB), were selected (Table 5.1). Seed treatments consisted of an 'on-farm' seed priming treatment (OSP), 0.5 g l^{-1} chitosan (CHP) applied as ChitoPlant® (ChiPro GmbH, Bremen, Germany) at a concentration based on findings from Chapter 4, and a non-primed control (NP), which consisted of dry seeds. Preliminary tests were carried out to determine the optimal 'on-farm' seed priming duration for each cultivar as described in Chapter 3 *Respiration measurements*. The optimal priming durations were 20, 24 and 28 h for SY Venture, KWS Tower and KWS Cassia respectively (see Figure S5.1).

Approximately 13,400 seeds (calculated by weight from the thousand grain weight of each cultivar) of each cultivar were poured into labelled 5 l plastic buckets containing either distilled water or 0.5 g l^{-1} chitosan solution (1:5 (w/v) ratio). These buckets were then incubated at 20 °C for the corresponding optimal priming durations for each cultivar, or for 15 min for CHP treatments. After soaking, OSP seeds were oven dried at 50 °C until moisture content was reduced to 27-31 % (sufficiently dry to avoid clumping within the seeder drill piping). The moisture content of the NP and CHP treatments ranged from 12 to 16 %. Subsequently, seed were reweighed and split into twelve equal weight portions, which provided the twelve replicates for each cultivar x seed treatment combination; and packed in envelops for sowing.

Table 5.1. Details of cultivars used in both growth trials.

Cultivar	Date listed	Type	Resistance mildew[a]	Resitance rynchosporium[a]
SY Venture	2012	Two-row malting	6	5
KWS Cassia	2010	Two-row feed	4	5
KWS Tower	2014	Two-row feed	5	6

[a]Resistance ratings according to AHDB Recommended list 2018 on a scale of 1–9, with higher values indicating higher resistance of the variety.

5.3.2. Field sites, experimental design and crop husbandry

Winter barley trials were conducted at two sites near Dundee (UK) (Table 5.2). The first site, Balruddery, was selected as a representative site for growing barley within a rotation. The second site, Mylnefield, has had barley repeatedly cultivated as a monoculture and has been used as a disease nursery for cultivar testing for over 30 years.

Table 5.2. Details of both growth trials.

Site	Sowing date	Latitude, longitude	Elevation (m)	Soil texture	Previous crops	Harvest date
Balruddery	17 Oct.	56°29'03.5"N 3°06'34.4"W	118	Sandy loam	Barley (2017), Peas (2018)	31 Jul.
Mylnefield	29 Oct.	56°27'21.4"N 3°04'25.2"W	13	Sandy loam	Barley from 1986	2 Aug.

In both sites, the experimental design consisted of two fungicide treatments, either no fungicide (F0) or fungicide (F1) applied alternately per column; and three replicates (Figure 5.1). Fungicides were applied with a hand pump rucksack (Table 5.3). Weeds were controlled with pre-emergence herbicides Pincer® (Agform, Wickham, UK) and PicoMax® (BASF, Cheadle, UK) at 0.6 and 3 l ha^{-1} respectively. Adjoining guards of barley surrounding each column were sown to act as a buffer for the fungicide applications and to reduce potential edge effects. Each column contained 18 plots and was split in two sub-reps with the nine cultivar × seed treatment combinations randomized within each sub-rep. Thus, each fungicide × cultivar × seed treatment combination comprised six replicates.

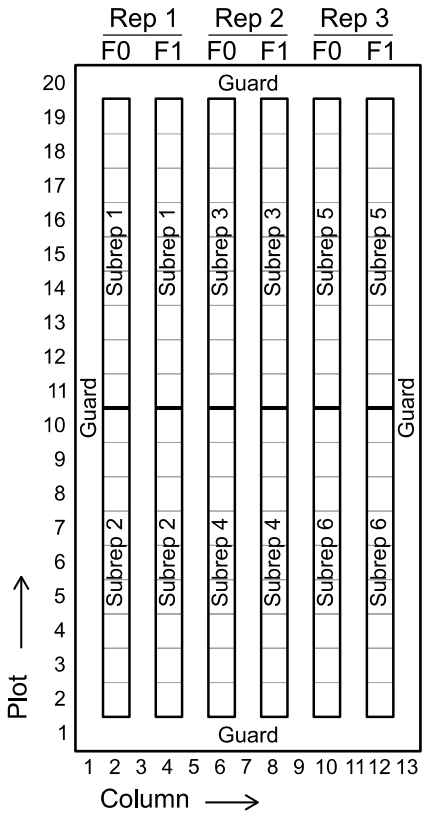


Figure 5.1. Experimental design at both sites. Whole plots were arranged along columns and sub-plots by rows, with guards in the middle of the whole plots and sub-plots. Fungicide was applied alternately per column (either none (F0) or full treatment (F1)) and sub-replicated in the same column. Each sub-replicate contained nine plots where cultivars x seed treatments combinations were randomised.

Plots were sown with an eight-row plot seeder (1.55 x 2.00 m) at 360 seed m^{-2} together with a seedbed application of 350 kg ha^{-1} of 0:20:30 nitrogen:phosphorus:potassium (N:P:K). Approximately, a total of 340 kg ha^{-1} of 29:0:0 (7 sulphate [SO_4]) was applied at each site. At Balruddery, a half dose was applied in March and the other half in April, whereas a full dose was applied in March at Mylnefield. This was done because an irrigation system was installed in Mylnefield at the beginning of April, which would have interfered with the second fertiliser application. The objective of this irrigation system was to promote rhynchosporium spore dispersal by simulating rain via the overhead sprinklers (Rightrain, Ringwood, UK) distributed across the experimental field. Irrigation was provided from developmental stage GS31 to 71 and consisted of applications of approximately 15 mm of water three times a week.

Table 5.3. Fungicide programme and active substances.

Treatment	Commercial product	Active substance	Rate (l/ha)	GS applied[a]
T0	Proline	Prothioconazole	0.5	GS 30
	Corbel	Fenpropimorph	0.5	
T1	Siltra Xpro	Bixafen & prothioconazole	0.6	GS 31-32
	Rover 500	Chlorothalonil	1	
	Vegas	Cyflufenamid	0.3	
T2	Tucana	Pyraclostrobin	1	GS 49
	Imprex	Fluxapyroxad	2	
	Joules	Chlorothalonil	1	
	Proline	Prothioconazole	0.3	

[a]There were 19 days between T0 and T1 application and 29 days between T1 and T2 application at both sites. Specific timing of applications can be found in Figure 5.3.

5.3.3. In-field imaging

Image collection

Images of each plot were collected from the stage of emergence of the first seedlings to approximately stage GS71-75 (specific timing of image acquisition are shown in figure 5.2). Where possible, images were taken between the hours of 10:00 – 14:00, particularly on overcast days. Images were taken 80 cm above the canopy with a Canon EOS 1200D digital camera (Canon, Japan). The camera was held parallel to the ground with a monopod and focused near the central area of the plot. The camera was set at 18 mm focal length, automatic aperture with no flash and 1/250 shutter speed. The images were stored as JPEG with native resolution of 3456 x 5184 pixels. Prior to the first images being collected, a 1 m section, parallel to the row orientation, was delimited by placing two sticks on the soil between the central rows of each plot. This allowed posterior conversion of pixels to m^2 as the long side of the picture (5184 pixels) captured the two sticks at the extremes of the picture (approximately equivalent to 1 m).

Image processing for emergence counts

An image capturing the delimited area per plot was used for seedling counts (Figure S5.2a for illustration), and emergence counts in the same section of the plot in each visit. Seedlings at both side rows of the marked section were counted with a cell counter plugin and zoomed 50x in FIJI software (version 2.0.0-rc-49/1.52s) (Schindelin et al. 2012) (Figure S5.2b for illustration). Images for emergence counts were taken every 2-3 days from the appearance of the first emerged seedlings until

it was considered that emergence had reached its end, i.e., when count numbers from the latest visit coincided with the counts from the previous visit.

Image processing for total plant tissue and percentage of senescent tissue estimation at advanced tillering

A single image per plot capturing the delimited area was taken to evaluate early vigour and the severity of an early powdery mildew epidemic at the end of advanced tillering. The timing of image acquisition was at approximately three and two weeks after the first observation of disease symptoms at Balruddery and Mylnefield respectively, and 35 and 23 days before the T0 fungicide application respectively. To facilitate image segmentation, image acquisition was carried out on a cloudy day to avoid overly bright leaves, and several hours after a rain event whilst the soil was still humid, which improved the colour contrast between the green shoot and the soil. Segmentation of soil, green plant tissue and senescent tissue was performed using FIJI software (Figure S5.3 for illustration). In brief, pixels within each picture were automatically classified into two clusters depending on their distance to a cluster centroid generated by the k-means++ algorithm using the k-means Clustering plugin (https://github.com/ij-plugins/ijp-toolkit/wiki/k%E2%80%90means-Clustering) in FIJI. This roughly classifies pictures into two layers containing dark/brownish (attributable to soil), greenish and yellowish/light brown pixels (attributable to plant tissue). The layer corresponding to plant tissue was retained and most of the stones and small particles within the area eliminated setting a threshold for particles with high circularity. Subsequently, the resultant RGB image was converted to CIELab colour space (Commission Internationale de l'Eclairage, L* lightness, a* green–red component, b* blue–yellow component) to more finely classify pixels by colour thresholding. Pixels from 0 to 255, 0 to 105 and 120 to 255 degrees for the channels L*, a* and b* were considered greenish and from 0 to 255, 106 to 135 and 120 to 255 degrees for the channels L*, a* and b* were considered yellowish. At least ten randomly selected images per site were visually inspected to verify the quality of the segmentation before bulk processing. Total plant tissue (TPT) cover was calculated as the sum of greenish pixels and yellowish pixels and converted to m^2 being expressed as m^2 of TPT m^{-2} of soil. Percentage of senescent tissue (PST) was calculated from the proportion of yellowish pixels in the total plant tissue.

Image processing for canopy green cover

One or two images of each plot were taken (reliant on weather conditions) that targeted the central rows of the plot, but not necessarily from the delimited area, from plants at stages GS30 to GS71-75 approximately every fortnight. Canopy green area was calculated using CerealScanner plugin (Kefauver et al. 2018; https://integrativecropecophysiology.com/software-development/cerealscanner/), in FIJI, which is a specialist plugin for the characterisation of canopy growth in cereals (Fernandez-Gallego et al. 2019).

5.3.4. In-field measurements

Disease severity

Disease severity of powdery mildew (*Blumeria graminis*) and rhynchosporium were scored on a continuous scale (0 – 100 %) at plot level following the AHDB Cereal trials protocol (HGCA, 2019) from GS30 (approximately when T0 was applied) until the distinction between chlorotic and senescent tissue was no longer possible (approximately after GS69). Disease scorings and image acquisition for canopy cover were carried out as close as practically possible (maximum 3 d between the two measurements) and approximately every fortnight.

Height and maturity

Crop height was measured after visually determining the most representative part of the average plot height at stages GS31, GS33, GS49 and GS71. At Balruddery, only the GS71 measurement (final height) was performed. Height was taken from the ground level to the base of the highest fully expanded leaf ligule or, after ear emergence, to the base of the highest ear. The number of days from sowing to GS49 (when approximately 50% of the stems showed awns visible) was recorded for each plot as an estimate of time to crop maturity.

5.3.5. Yield and grain quality

At ripening, grain was collected with a combine harvester and dried at constant moisture. Grain was passed through a 2.5 mm sieve, for elimination of remaining awns and small/broken seeds, and weighed. A subsample of cleaned grain was used to determine grain N concentration (GN), and moisture content determined by

using a calibrated near-infra red grain analyser (Infratec 1241, FOSS, Sweden). Thousand grain weight (TGW) was calculated using a MARVIN Seed Analyser (GTA Sensorik, Neubrandenburg, Germany). The grain weight of each plot was then adjusted to 85 % dry matter to obtain grain yield (GY) and grain number (G no.) calculated from the GY and TGW.

5.3.6. Meteorological conditions

Mean temperature, accumulated precipitation and relative humidity data were collected by an automated meteorological station situated at a maximum distance of 300 m from the experimental area (Figure 5.2). Balruddery weather data was supplied by the Natural Environment Research Council through the COSMOS-UK project (https://cosmos.ceh.ac.uk/) and Mylnefield weather data by the James Hutton Institute.

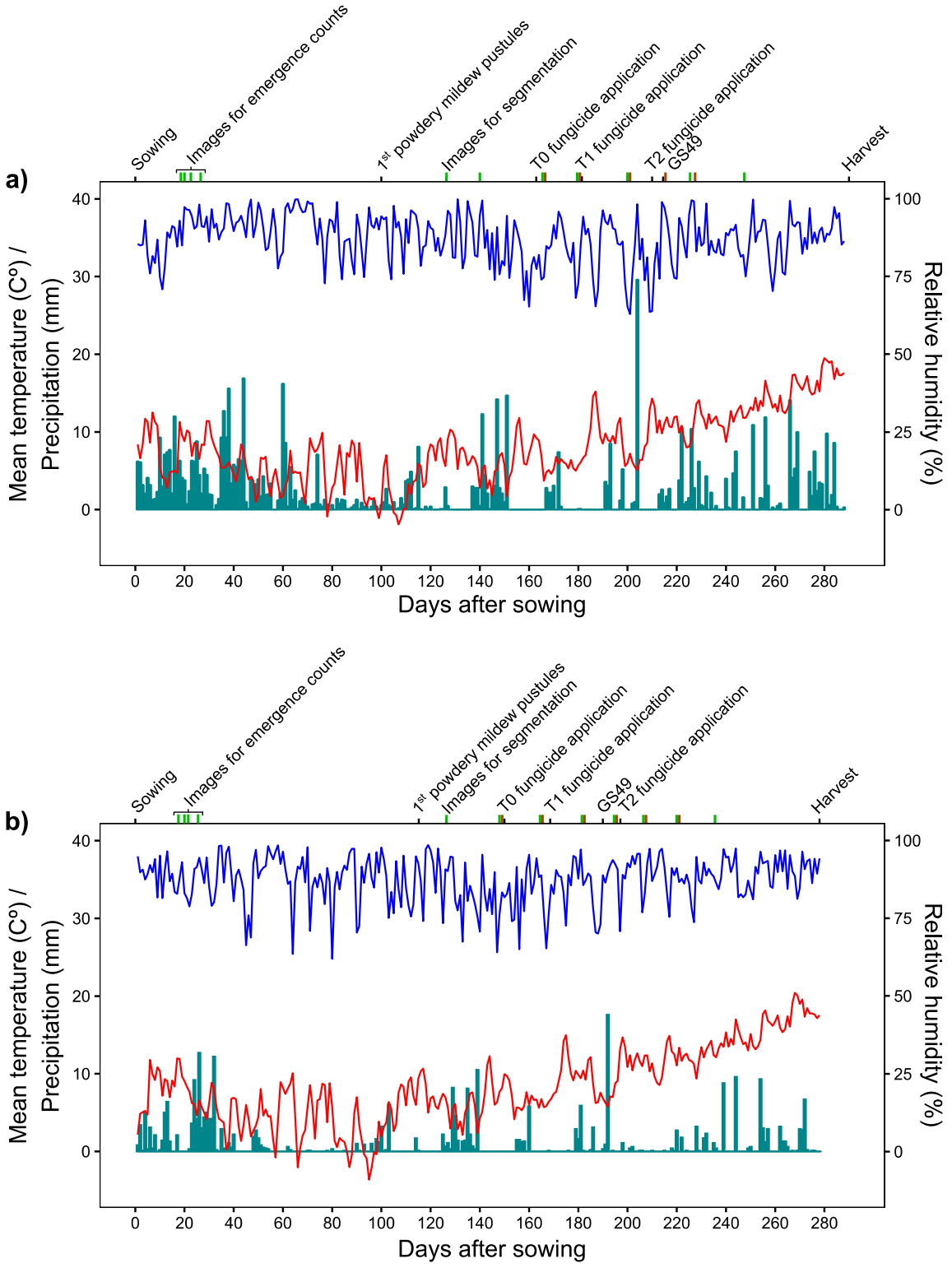


Figure 5.2. Climatic conditions and key activities during the growing season at (a) Balruddery and (b) Mylnefield. Daily mean temperature (red lines), daily precipitation (turquoise bars) and daily mean relative humidity (blue lines). Climatic data provided by COSMOS-UK and the James Hutton Institute. Green ticks over the upper box bar represent an image acquisition event and brown ticks represent a disease score event.

5.3.7. Data analysis

Disease scores and canopy green cover were integrated over time using the trapezoidal method (Waggoner and Berger 1987) and named area under disease progress curve (AUDPC), and healthy area duration (HAD). AUDPC measures the proportion of disease-induced green area loss over time, whilst HAD can be considered a measure of the size of the canopy and the remaining area of healthy photosynthetic tissue (Bingham et al. 2009; Walters et al. 2012).

All analyses were performed using R version 3.3.0 (R Development Core Team 2016). Effect of fungicide (Fun), cultivar (Cv), seed treatment (Tr) and their interactions in crop traits or disease (e.g., GY, AUDPC, TGW) were analysed using mixed effects models. Spatial effects of column and/or subrep were tested selecting the model with lower Bayesian information criterion (BIC) and accounted as random effects. Assumption of normality and homoscedasticity of variances were checked by QQ-plots and residuals against fitted value plots respectively. Percentage of senescent tissue (PST) data was $\log_{10}$ transformed to meet normal distribution. Post-hoc Fisher's LSD tests were performed to separate significant differences at *P* values < 0.05 with *predictmeans* package (Luo et al. 2014). *P* values were adjusted to avoid Type I errors (false positives) using the Benjamini–Hochberg correction (Waite and Campbell 2006).

Assessments of specific candidate traits that may confer tolerance or escape characteristics were performed using pairwise correlations for each cultivar. Pearson's correlation between early growth (expressed as TPT) and percentage disease symptoms (PST) was calculated to investigate whether a larger canopy can confer tolerance in pre-stem elongation epidemics. Spearman's correlation was calculated to investigate whether height can be involved in escape of secondary spread of disease to upper leaves. Specifically, AUDPC accumulated after anthesis in the top four leaves (i.e., flag leaf, leaf 2, leaf 3 and leaf 4 (see Figure 1.2 for illustration)) was correlated against height rate from GS33 (when leaf 3 and leaf 2 emerge) to GS49 coinciding with the rapid stem extension phase.

Disease tolerance was estimated according to Foulkes et al. (2006) with some modifications. The degree of 'tolerance' was modelled by linear regression as the slope of the relationship between GY and HAD including Cv and Tr as moderator

variables. Site and spatial effects of row x column within sites were controlled for by including them as random effects. In order to generate sufficient GY-HAD variation for estimation of slopes, data from both sites was pooled and the effect of fungicide treatments was accounted for as variation in HAD (Parker et al. 2004; Foulkes et al. 2006). To validate this approach, the regression slopes were visually checked, by specifically making sure that, (a) data was dispersed along the fitted line (i.e., did not show fungicide/untreated clusters), and (b) slopes did not excessively deviate from the fitted tolerance line (Figure S5.4). Failure to fulfil these conditions would have undermined the analysis by producing spurious results.

5.4. Results

5.4.1. Emergence and early growth

Chitosan priming had a positive effect on emergence compared to non-primed seeds, with 22 and 13 more seedlings m^{-2} at Balruddery and Mylnefield respectively at the end of the seedling growth stage, although this increase was only significant at the Balruddery site ($P < 0.01$) (Figure 5.3). The effect of 'on-farm' seed priming (OSP) was significantly related to earliness in emergence (first count event) at Balruddery ($P < 0.01$) but not at Mylnefield. However, this earliness in emergence was not translated into a significant number of seedlings at the end of the seedling growth stage in either of the sites.

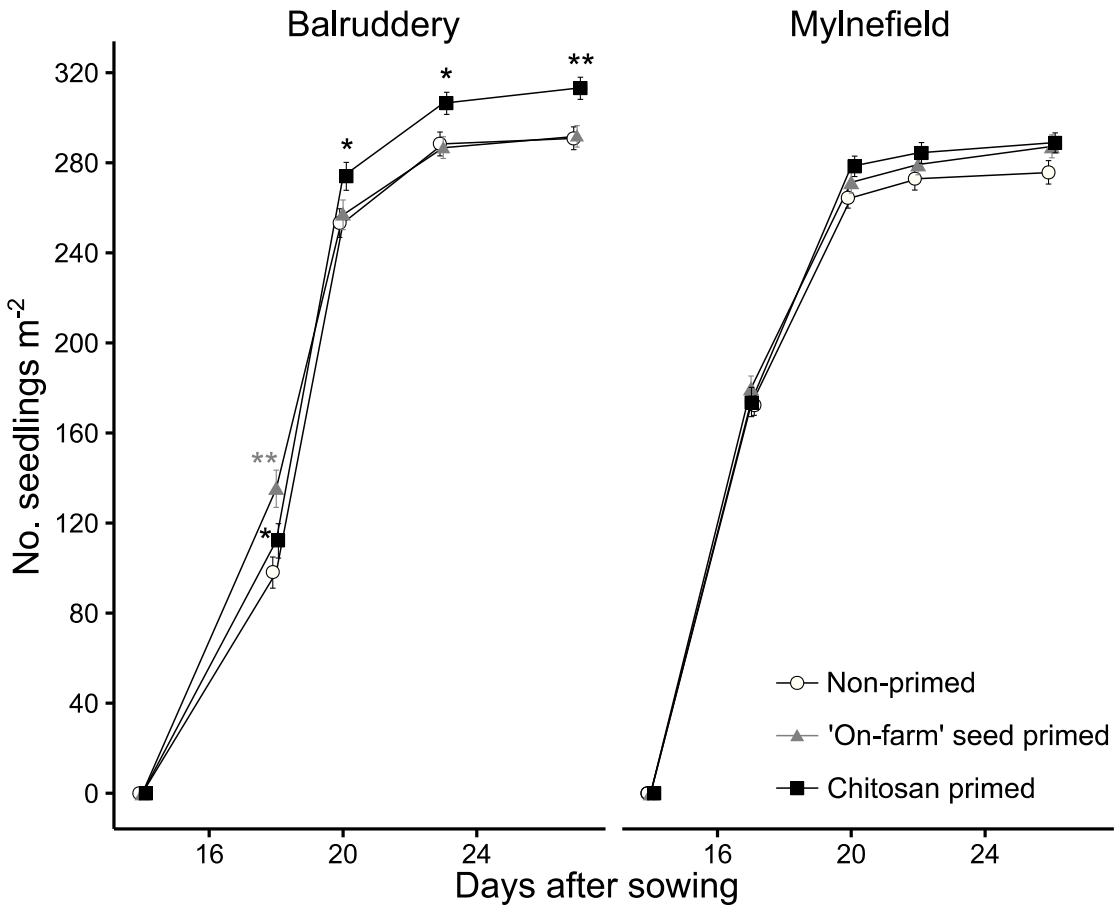


Figure 5.3. Emergence over time. Only seed treatment (Tr) effect is presented as the effect of cultivar (Cv) was not significant across time points. Asterisks denote significant differences (* $P < 0.05$, ** $P < 0.01$) against the non-primed control at each time point (LSD test). Error bars show ± SE.

Total plant tissue (TPT) produced by advanced tillering was estimated using image segmentation. Both sites yielded very similar results with TPT varying by cultivar and seed treatment, but with no interaction between them indicating that the seed treatment effect was similar between the cultivars (Figure 5.4). KWS Cassia and KWS Tower produced significantly more TPT than SY Venture ($P < 0.001$). Non-primed seeds had the greatest TPT overall, whilst plants grown from 'on-farm' primed seeds had significantly less TPT at both sites. These results contrasted with the significant CHP impact on final emergence, indicating that the effects on emergence did not continue during development up to advanced tillering.

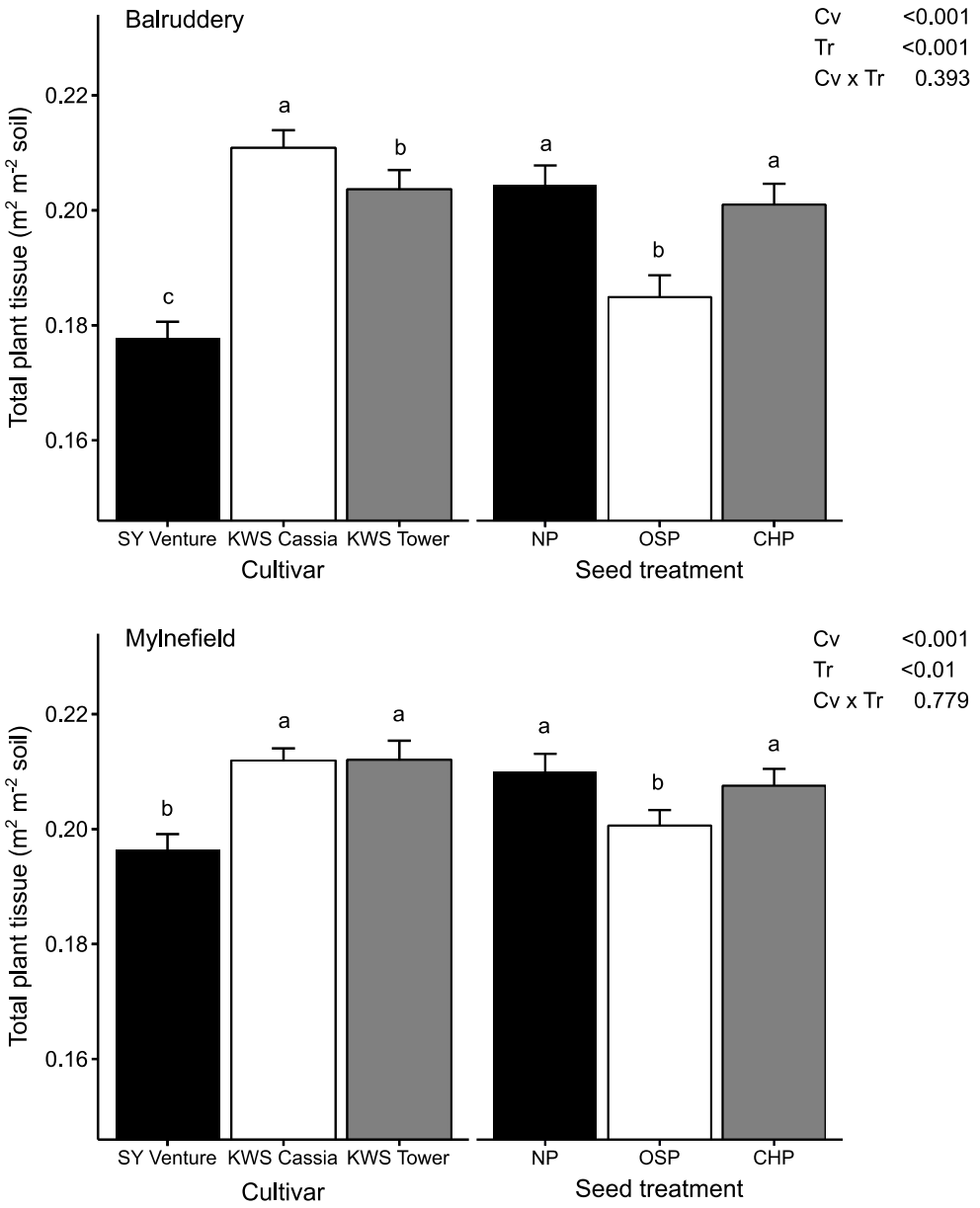


Figure 5.4. Total plant tissue estimated by image segmentation at advanced tillering at Balruddery (a) and Mylnefield (b). *P* values from analysis of deviance are for cultivar (Cv), and seed treatment (Tr) effects and the Cv x Tr interaction. Bars with different letters are significantly different from each other (LSD test). Error bars show the mean +SE.

5.4.2. Effect of vigour as candidate trait for tolerance

At the time of image acquisition for image segmentation, both sites were infected with powdery mildew (*Blumeria graminis* f.sp. *hordei*). Most plots at Mylnefield presented discoloured yellow leaves (indicative of the infection depleting the leaf of nutrients) with some grey/brown leaf tips; whilst, at Balruddery, damaged tissue was

predominantly grey/brown (indicative of an older infection) and also covered with whitish pustules expanding to healthy tissue. Consequently, there was a greater percentage of senescent tissue across cultivars and treatments at Balruddery than at Mylnefield (42 % compared with 29 %). As for TPT, there were no interactions between factors in any of the trails. The main effects, cultivar (Cv) and seed treatment (Tr) are shown in Figure 5.5, and post-hoc analyses ranked cultivars as SY Venture > KWS Cassia > KWS Tower. Seed treatments showed a similar pattern at both sites with OSP having significantly more senescent tissue than non-primed seeds.

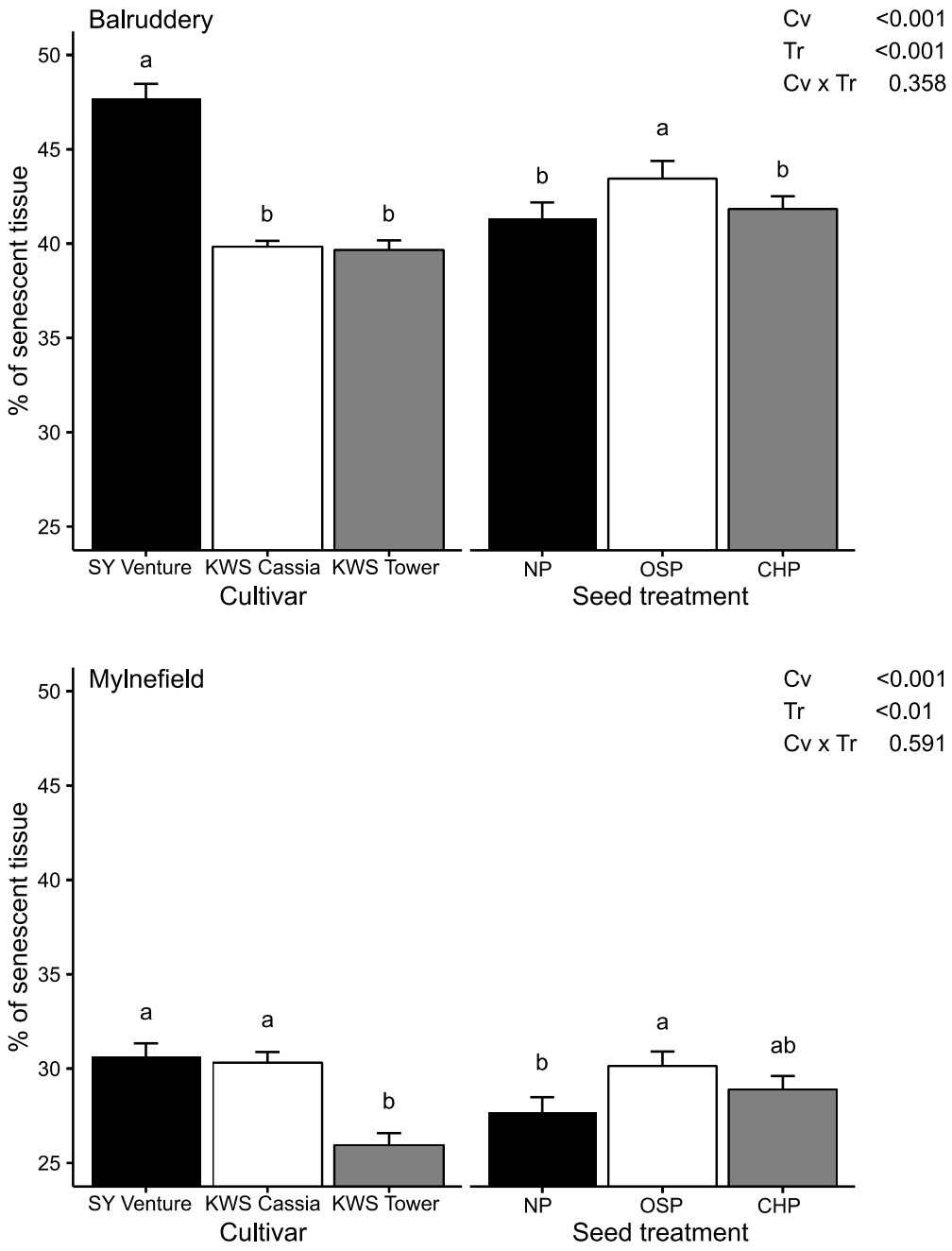


Figure 5.5. Percentage of senescent tissue estimated by image analysis at advanced tillering in Balruddery (a) and Mylnefield (b). *P* values from analysis of deviance are for cultivar (Cv), and seed treatment (Tr) effects and the Cv x Tr interaction. Bars with different letters are significantly different from each other (LSD test). Error bars show the mean +SE.

In order to investigate whether plants with larger canopies tend to be more infected during an early disease event, Pearson's correlations between total plant tissue and percentage of senescent tissue (PST) were plotted. A consistent negative

correlation at both sites for all three cultivars was evident ($P \leq 0.05$), with the relationship being stronger at Mylnefield (Figure 5.6).

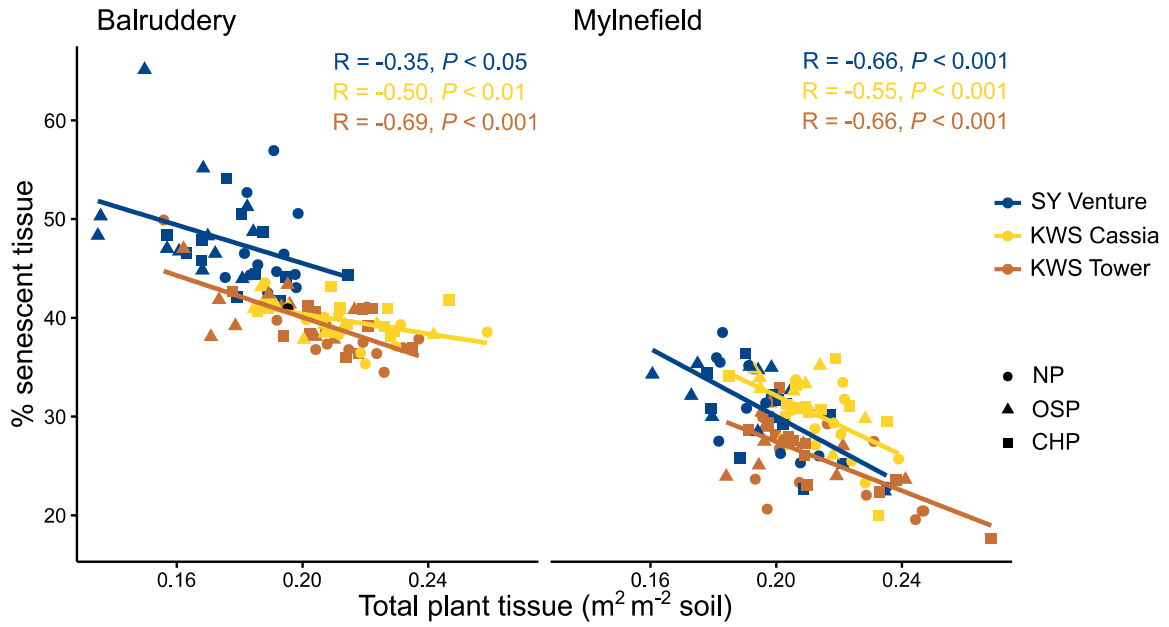


Figure 5.6. Relationship between total plant tissue and percentage of senescent tissue at Balruddery (a) and Mylnefield (b).

5.4.3. Disease severity and resistance

Powdery mildew and rhynchosporium were the dominant diseases, although with varying severity and timing between the two sites. Powdery mildew pustules appeared earlier at Balruddery (approximately two months before the start of stem elongation and before the first fungicide applications) covering up to 22 % of the leaf area (assessed by visual scoring), whilst at Mylnefield the first pustules appeared about a month later, covering up to 14 % of the leaf area. However, with the appearance of new leaves at the end of stem elongation, powdery mildew infection was reduced to very low levels (< 5 %) at Balruddery whilst, at Mylnefield, a new outbreak arose and affected parts of leaves 3 and 4 (up to 16 % of the total scored leaf area). At Mylnefield, rhynchosporium lesions at traceable levels appeared just before anthesis whilst, at Balruddery, there were no rhynchosporium lesions until mid-late anthesis; however, similar levels of severity were recorded at milk development at both sites. In terms of visible lesions, fungicide controlled the second rise of powdery mildew, which occurred after stem elongation, and completely prevented a rhynchosporium outbreak in both sites.

Area under disease progress curve (AUDPC) was used to integrate the periodic measurements of disease scores over time as an estimate of disease intensity. The main differences in AUDPC were due to the effect of genetic variation (cultivar effect) on both diseases (Table 5.4). At both sites, KWS Tower was the most resistant followed by SY Venture and, lastly, by KWS Cassia. At Balruddery, fungicide applications did not significantly reduce powdery mildew AUDPC, largely, because much of the mildew scored corresponded with lesions produced before the first fungicide application rather than connected to the effectiveness of the fungicide controlling the disease. The interaction between fungicide and cultivar for the powdery mildew AUDPC at Mylnefield was due to the fungicide being more effective at controlling powdery mildew in cultivar KWS Cassia compared to SY Venture. However, the interaction between fungicide and cultivar for the rhynchosporium AUDPCs was due to the complete prevention of rhynchosporium lesions in fungicide-treated plots at both sites. The effect of treatments on AUDPC was only perceptible at Mylnefield for powdery mildew where OSP showed the lowest AUDPC (Table 5.5). Similarly, the rhynchosporium AUDPC was also the lowest for OSP, although this was not significantly different from NP (P = 0.27).

Table 5.4. Analysis of deviance *P* values for fungicide, cultivar and treatment on HAD (calculated form GS30 to GS71-75) and AUDPCs (from GS30 to GS69).

Site	Term	AUDPC powdery mildew	AUDPC rhynchosporium	HAD
Balruddery	Fun	0.069	**< 0.001**	0.435
	Cv	**< 0.001**	**< 0.001**	**< 0.001**
	Tr	0.954	0.136	**0.007**
	Fun x Cv	0.212	**< 0.001**	0.365
	Fun x Tr	0.563	0.165	0.176
	Cv x Tr	0.870	0.243	0.669
	Fun x Cv x Tr	0.701	0.285	0.372
Mylnefield	Fun	**0.003**	**< 0.001**	0.056
	Cv	**< 0.001**	**< 0.001**	**< 0.001**
	Tr	**0.040**	0.189	0.053
	Fun x Cv	**< 0.001**	**< 0.001**	0.940
	Fun x Tr	0.079	0.190	0.232
	Cv x Tr	0.981	0.458	0.706
	Fun x Cv x Tr	0.943	0.457	0.118

Most of the variation in healthy area duration (HAD) was accounted for by the cultivar effect ($P < 0.001$). The effect of seed treatment was significant at Balruddery but not at Mylnefield (Table 5.4). Post-hoc analysis revealed that NP had significantly greater HAD than OSP ($P < 0.01$) and CHP ($P < 0.05$) at Balruddery and Mylnefield respectively (Table 5.5).

Table 5.5. Effect of seed treatments on healthy area duration (HAD) and AUDPCs. Values in each row followed by different letters differ significantly from each other: LSD test ($P > 0.05$).

	Tr		
	NP	OSP	CHP
AUDPC powdery mildew			
Balruddery	723[a]	724[a]	725[a]
Mylnefield	453[ab]	**427[b]**	462[a]
AUDPC rhynchosporium*			
Balruddery	156[a]	189[a]	176[a]
Mylnefield	135[a]	115[a]	141[a]
HAD			
Balruddery	6,440[a]	**6,243[b]**	6,395[a]
Mylnefield	5,989[a]	5,898[ab]	**5,809[b]**

*values correspond to F0 as there was no AUDPC for rhynchosporium under F1.
Seed treatments significantly different from NP are shown in bold.

5.4.4. Effect of stem elongation rate as a candidate trait for disease 'escape'

To further explore whether the AUDPC variance found at Mylnefield was to some extent due to involvement of disease escape mechanisms, a correlation analysis between rate of stem elongation and AUDPCs from anthesis to grain filling was performed for the plots with no fungicide application (Figure 5.7). For the case of powdery mildew, this correlation was significantly negative for all cultivars showing an average elongation rate above 2.4 cm d^{-1} ($P < 0.01$). However, the same was not applicable for rhynchosporium disease as no significant association was found. Stem elongation rate variation was strongly driven by cultivar ($P < 0.001$) and, to a lesser extent, by Tr ($P < 0.05$). OSP had significantly greater height rate ($P < 0.05$) (Table S5.1).

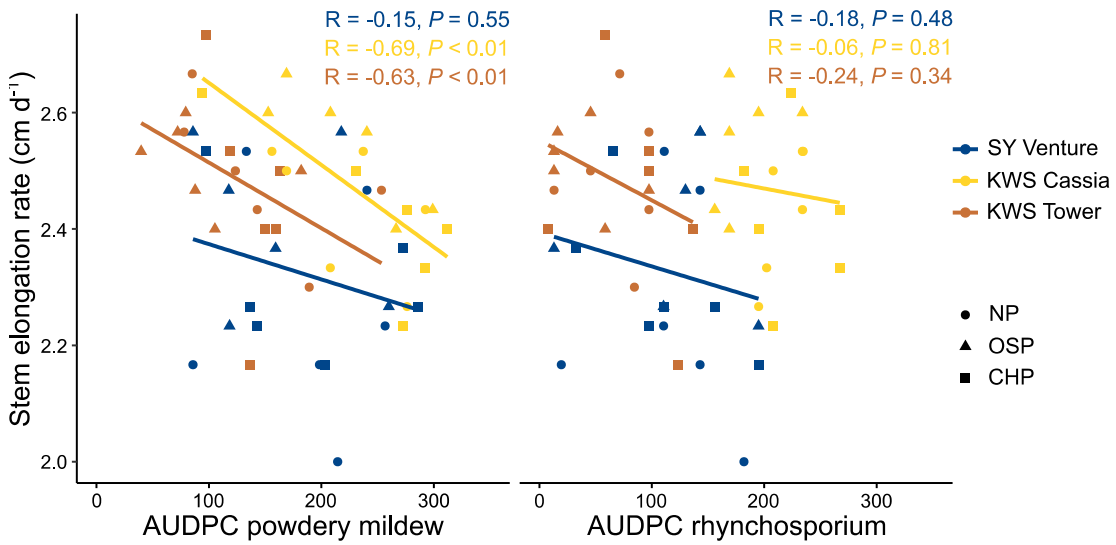


Figure 5.7. Relationship between stem elongation rate from GS33 to GS49 against (a) powdery mildew AUDPC and (b) rhynchosporium AUDPC from anthesis in Mylnefield. R: correlation coefficient.

5.4.5. Effects on yield and yield components

Yields were greater at Balruddery (7.73 t ha^{-1}) than at Mylnefield (6.68 t ha^{-1}), which was mainly attributed to differences in average grain number (13,600 and 11,500 respectively) rather than in TWG (56.68 g vs. 57.8 g respectively). There was a significant grain yield response to fungicide application (P < 0.05) with averaged increments across Cv and Tr of 2.02 t ha^{-1} at Balrudery and of 1.15 t ha^{-1} at Mylnefield relative to plots with no fungicide application (Table 5.6). This grain yield response was primarily due to increasing grain number (25 and 13 % relative to F0 at Balrudery and Mylnefield respectively) rather than through increments in TGW (4 and 5 % respectively). The effect on TGW was significant at Mylnefield (P < 0.001), although not at Balruddery (P = 0.06). The interaction between fungicide application and cultivar at Balruddery was significant (P < 0.05). KWS Tower showed a higher fungicide benefit (2.52 t ha^{-1}) compared with SY Venture (1.92 t ha^{-1}) or KWS Cassia (1.62 t ha^{-1}), despite KWS Cassia being the cultivar with less disease lesions. By contrast, there was no interaction between fungicide and cultivar at Mylnefield indicating that all cultivar genotypes responded to the same extent to fungicide application. Although seed treatments did not significantly alter yield at Mylnefield they did at Balruddery: post-hoc analysis showed that grain yield was

significantly lower for OSP compared to NP by having a negative impact on grain number, as TGW remained unaffected (Table 5.7).

Table 5.6. Analysis of deviance *P* values for fungicide, cultivar and treatment on agronomic variables.

Site	Term	GY (t ha^{-1})	G no. (m^{-2})	TGW (g)
Balruddery	Fun	**0.010**	**0.009**	0.060
	Cv	**< 0.001**	**< 0.001**	**< 0.001**
	Tr	**0.028**	**0.005**	0.264
	Fun x Cv	**0.003**	**0.041**	**0.022**
	Fun x Tr	0.193	0.281	0.237
	Cv x Tr	0.864	0.819	0.609
	Fun x Cv x Tr	0.762	0.829	0.436
Mylnefield	Fun	**0.015**	**0.045**	**< 0.001**
	Cv	**0.047**	**< 0.001**	**< 0.001**
	Tr	0.072	0.076	0.983
	Fun x Cv	0.630	0.738	**0.023**
	Fun x Tr	0.103	0.243	0.103
	Cv x Tr	0.793	0.817	0.969
	Fun x Cv x Tr	0.082	0.111	0.460

Table 5.7. Effect of seed treatment on grain yield (GY), grain number (G no.) and thousand grain weight (TGW). Values between the two farms for each parameter not sharing the same letter differ significantly from each other: LSD test (*P* > 0.05).

	Tr		
	NP	OSP	CHP
GY (t ha^{-1})			
Balruddery	7.89^{a}	**7.54^{b}**	7.77^{ab}
Mylnefield	6.75^{a}	6.77^{a}	6.51^{a}
G no. (m^{-2})			
Balruddery	13,929^{a}	**13,211^{b}**	13,736^{a}
Mylnefield	11,692^{a}	11,723^{a}	11,281^{a}
TGW (g)			
Balruddery	56.5^{a}	57.0^{a}	56.5^{a}
Mylnefield	57.8^{a}	57.8^{a}	57.8^{a}

Seed treatments significantly different from NP are shown in bold.

5.4.6. Effects on overall tolerance

Overall disease tolerance was represented as the slope of grain yield against HAD where the steepness of the slope shows the degree of tolerance (the steeper,

the more intolerant). There was a significant interaction between HAD and Cv (P < 0.001) indicating that the cultivars had different degrees of tolerance (Figure 5.8a). KWS Tower and Venture had similar degrees of tolerance whilst KWS Cassia was significantly less tolerant (Figure 5.9a). However, treatments did not have a significant effect on tolerance (P = 0.21) (Figure 5.8b). In general, crops from CHP treated seeds had a less steep slope than the non-primed control but these differences in slope were not significant (Figure 5.9b).

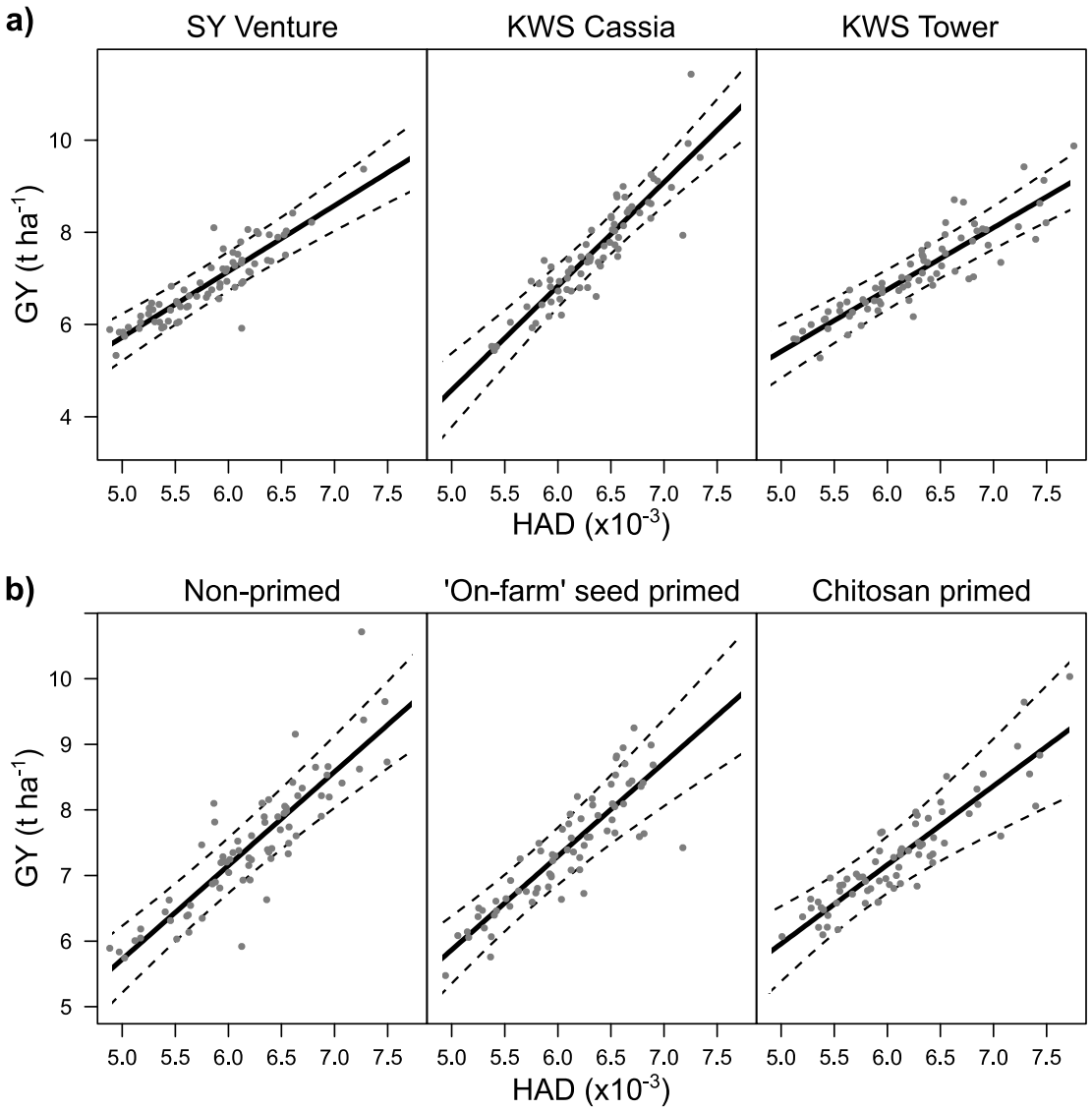


Figure 5.8. Disease tolerance estimated as the slope of GY on HAD across sites and fungicide treatments. a) Cultivar effect with all seed treatments pooled together, and b) seed treatment effect with all cultivars pooled together. Solid line represents regression line and dashed lines represent 95 % confidence intervals.

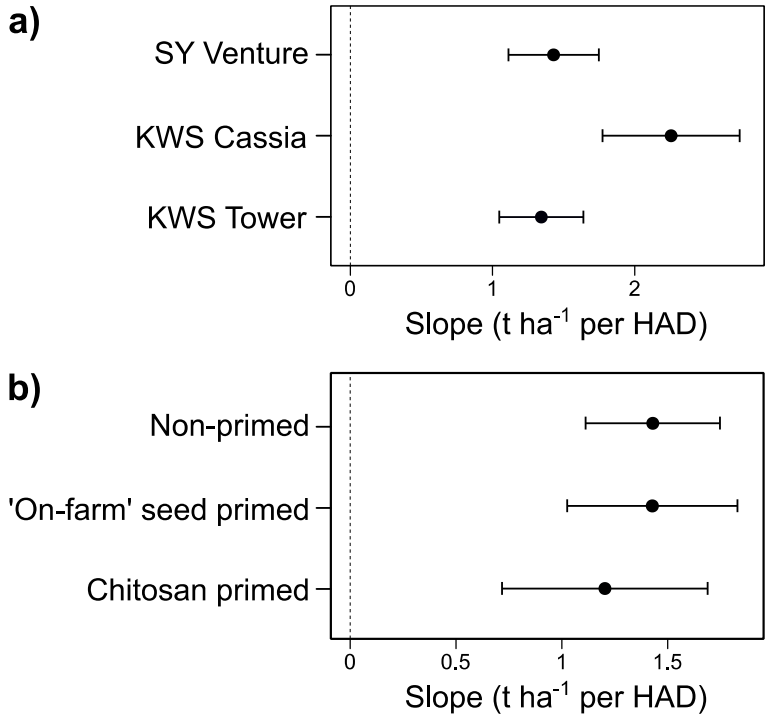


Figure 5.9. Effect sizes for estimated slopes within (a) cultivar and (b) treatment factor. Error bars represent 95% confidence intervals (CI). Effect sizes closer to zero represent more tolerant levels with each factor. Levels within factor are considered to be significantly different from one another when their CI do not overlap.

5.5. Discussion

‘On-farm’ seed priming and chitosan seed dressing offers limited scope to control disease in winter barley of temperate agriculture, either alone or as a complement to fungicides, regardless of the cultivar of choice. This study has illustrated the varied responses of diseases to conventional management, i.e., varietal resistance and fungicides; however, seed treatments do not seem to complement the control of disease.

5.5.1. Induced resistance

These trials indicate that disease symptoms are primarily controlled by genetic-mediated resistance, (i.e., the cultivar), and, secondarily, by fungicides that can further control the development of disease lesions on new leaves after GS32. However, in general, neither chitosan nor ‘on-farm’ seed priming further decreased the appearance of lesions, which would have been indicative of induced disease resistance. These results are consistent with those of Wang et al. (2015) who found

no effect on disease in a series of winter wheat field trials following chitosan seed dressing.

The continual interactions between multiple abiotic and biotic agents can compromise the ability of elicitors to further promote host resistance in the field (Walters et al. 2013; Alexandersson et al. 2016; Iriti and Varoni 2017). For example, Walters et al. (2011) ascribed the poor response to elicitors applied to spring barley against powdery mildew and rhynchosporium to the potential for crops already being in an induced state before the application of the elicitors. Stresses such as overwinter cold acclimation, which induces transcription of a wide array of pathogenesis-related (PR) genes (Kuwabara and Imai 2009), could also mask elicitor-induced disease resistance. Another possibility is that resistance could also be induced by soil microbial communities as demonstrated by Wiese et al. (2003) where high organic matter soils showed lower mildew infection, whilst the application of the elicitor Acibenzolar-S-methyl (ASM) could only reduce infection in mineral soils. Thus, a more efficient strategy may be to promote elicitor applications to seeds where there is direct pathogen interaction, such as with seed- and soil-borne diseases. In this respect, some chitosan-based seed treatments have shown promising results as an organic alternative to control seedling blight and foot rot diseases caused by *Fusarium* species in wheat and barley (Reddy et al. 1999; Khan et al. 2006; Orzali et al. 2014).

5.5.2. Tolerance

It is particularly important to protect barley crops from early epidemics during the vegetative growth as barley yield largely relies on maximising tiller production and survival (Walters et al. 2012). Therefore, modelling of tolerance traits have suggested that a large canopy can be a trait for tolerating foliar diseases (Bingham and Topp 2009). A large canopy can reduce the impact of disease on growth as the remaining healthy tissue can potentially compensate for the loss of radiation interception (Bingham and Topp 2009). This mechanism of tolerance is also supported in this study as a larger canopy tended to have a lower proportion of senescent tissue under moderate and high disease severities of powdery mildew. Conversely however, it is also plausible that a larger canopy could increase the potential for trapping more spores or facilitate the spread to adjacent plants of both

wind-borne and splash-spread pathogens such as *Blumeria graminis* and *Rhynchosporium commune*. The fact that this relationship was strong under moderate severity but less prominent under high severity suggests that this may be possible in the event of very strong epidemics.

In these field trials, seed treatments did not increase canopy size, in fact, 'on-farm' seed priming resulted in a slightly reduced early vigour and presented more senescent tissue compared to plants sown from untreated seeds. A similar picture emerges when HAD is considered as a measure of plant fitness over time, indicating that some loss of vigour occurred overwinter and remained for the rest of the crop cycle. This loss of fitness is difficult to explain, although it is possible that 'on-farm' seed priming washes off important components of seed exudates, which are needed to establish beneficial associations with soil microbial communities such as rhizobacteria (Lamichhane et al. 2018). This may explain the magnitude of this lessened vigour at Balruddery, which has a richer environment in terms of microbial communities (as an arable field in a crop rotation) when compared to Mylnefield (in barley monoculture for over 30 years).

There seems to be a compromise between disease tolerance and attainable yield, particularly when the disease pressure is low (Parker et al. 2004; Bingham et al. 2009). This compromise is illustrated by the less tolerant cultivar (KWS Cassia) having the greatest attainable yield and *vice versa* for the less tolerant cultivar (KWS Tower) at high HAD. This is likely because modern varieties have been bred to perform near maximum radiation use efficiency under fungicide conditions, so that a loss in photosynthetically active tissue by disease translates into a more noticeable drop in yield (Parker et al. 2004). Although it might be tempting to suggest that chitosan may have some effect on overall tolerance, these differences were marginal and only evident in the most intolerant cultivar when compared to the non-primed control. Taken together, these results of overall tolerance suggest that elicitor seed treatments are only likely to benefit highly intolerant genotypes under high disease pressure.

Tolerance is the complex result of multiple traits operating at organ, plant and crop level (Ney et al. 2013) so that, complementary to particular candidate traits, the slope from representing yield unit against healthy tissue unit (HAD) was used to

more holistically evaluate tolerance. However, some caution must be taken when interpreting yield-HAD slopes. Although fungicides are useful to manipulate the disease severity and yield responses needed to fit reliable slopes, fungicides can have other physiological effects beyond controlling disease symptoms (Bingham et al. 2012; Ney et al. 2013). For example, triazoles and strobilurins have been found to alter N partitioning and increase yields even when disease symptoms are absent, which could bias the results (Ruske et al. 2003; Bingham et al. 2012).

The approach used in this study for estimation of overall tolerance included some modifications of methods previously applied in wheat (e.g., Parker et al. 2004; Collin et al. 2018). Firstly, HAD has been calculated from GS30, instead of from post-anthesis. Unlike wheat, barley tiller and spikelet formation are sensitive to variations in radiation interception (Arisnabarreta and Miralles 2008), hence, this approach allows an integration of canopy development stages into the calculation. Secondly, instead of constructing HAD from the integration of total planar area of individual sampled plants over time, HAD was calculated from in-field images taken above the canopy over time. This approach is non-destructive and at a field-scale provides a better representation of in-field crop architecture. In addition, zenithal images give more weight to the upper leaf layer, which intercept most of the incident radiation, than to the underlying leaf layers in the calculation, and thus represents a more realistic picture of radiation interception. However, this method should be tested on a larger number of cultivars and environments in order for it to be more widely validated.

5.5.3. Disease escape

Disease ‘escape’ can constrain the spread of late epidemics (from ear emergence onwards) to the upper leaves, which contribute the most sink tissue for ear formation and grain filling (Walters et al. 2012). In this study, it was found that rapid vertical growth may provide a certain degree of disease escape against powdery mildew but not necessarily to rhynchosporium. Successful attachment of powdery mildew primary germ tube to the leaf surface requires high humidity (Newton and Dashwood 1998). Frequent irrigation created conditions of high humidity at ground level, which in combination with the warm temperatures during late April 2019, provided the ideal microclimate for powdery mildew conidia

germination. Thus, it is likely that crops with rapid stem extension developed their upper leaves away from this optimal microclimate and before the pathogen became established, which resulted in fewer powdery mildew lesions post-anthesis. Similarly, height-related traits such as rapid stem elongation, final height or the distance of the leaf layers to the soil surface have been found negatively associated with amounts of the hemi-biotrophic pathogens *Mycosphaerella graminicola* and rhynchosporium in winter wheat and in spring barley respectively (Lovell et al. 1997; Bingham et al. 2008). However, this relationship between stem elongation and disease lesions may not be so straightforward for rhynchosporium in winter barley. Pathogen load is not only determined by splash dispersed conidia from lower infected leaves during the early spring precipitation. Earlier overwinter infection may represent another source of pathogen load, as rhynchosporium growths also systemically while remaining asymptomatic and, thus, hamper this relationship.

Whether seed priming can consistently increase height rate and/or other height-related traits is still unclear. The effect of 'on-farm' seed priming on plant height is either associated with positive effects (Murungu et al. 2004a; Harris 2006; Harris et al. 2007) or no effect (Farooq et al. 2008; Aune and Ousman 2011). However, it seems clear that potential effects on phenology are simply the result of quicker establishment that enables a faster growth rate throughout the crop cycle (Murungu et al. 2004a) and, thus, exploiting escape benefits will be conditional upon having this prior effect on establishment.

5.5.4. Yield

'On-farm' seed priming and/or chitosan seed dressing have limited scope for improving winter barley yields and even may result in lower yields. These results contrast with those obtained in Chapter 4 with spring barley where both 'on-farm' seed priming and chitosan seed dressing substantially increased grain yields. The mechanism for yield benefits in spring barley was the improved emergence and seedling vigour that lead to a greater number, and more vigorous, tillers being retained for grain filling. However, the same mechanism to enhance winter barley yields does not seem as effective. Although positive effects on emergence density can be gained (chitosan seed dressing seems to provide improved final emergence

more consistently than ‘on-farm’ seed priming), those were not sufficiently high to prevail until advanced tillering.

The mismatch between emergence and canopy cover at advanced tillering in winter crops may be due to the extent of the benefit of earlier emergence, which may be more limited under typically more humid conditions of autumn-sown crops than those for spring crops. Although crops grown from ‘on-farm’ primed seeds can attain some earlier emergence, the benefits associated with having moisture already within the seed will be rapidly offset if sown in a damp seedbed. Winter barley is also a more plastic crop than spring barley (García del Moral and García del Moral 1995), and the extended canopy formation period (typically from October to beginning of April) and lower rate of growth imposed by colder temperatures, favours tillering and may allow crops with less initial vigour to catch up. In agreement with these observations, seed priming or chitosan seed dressing have shown limited practical use for enhancing establishment of winter cereals in temperate climatic zones (Giri and Schillinger 2003; Subedi and Ma 2005; Wang et al. 2015). However, there could be considerable benefits for winter cereals grown in semi-arid regions (Rashid et al. 2006; Farooq et al. 2008). In contrast to temperate zones, winter crops are sown at the beginning of the dry period using the residual water from the rainy season. It is under these circumstances where planting hydrated seeds can make the difference between securing or aborting emergence (Wojtyla et al. 2016).

5.6. Conclusions

Providing sustainable disease control from seed treatments is attractive for practical and sustainable reasons when compared to spraying fields with fungicides. However, the extent of how seed treatments can complement IPM in conventional temperate agricultural systems seems limited. Inducing resistance from the seed is burdened by continuous interactions with biotic and abiotic elements that offset the expression of induced resistance in field crops. Seed treatments can deliver disease tolerance and escape traits, but these benefits will be conditional upon conferring successful establishment and vigour first. Thus, chitosan-based and ‘on-farm’ seed priming treatments may be better placed for using with spring crops or in semi-arid agriculture where the added vigour at emergence can more clearly surpass other interactions and facilitate the expression of tolerance and/or escape traits. A better

understanding of the spermosphere and the impact of seed treatments in seed exudates is also required to design more effective treatments for conventional agriculture.

Chapter 6: General discussion

6.1 Scope of the research

The aim of this thesis was firstly, to determine the potential contribution of 'on-farm' seed priming to increase food production in the developing world by holistically analysing the accumulated knowledge of low-input agricultural systems in the developing world (where it has been so far utilised). Scientific reports have generally supported its adoption; however, there was a need to quantitatively put into perspective its potential role in sustainably improving food security in the developing world. Secondly, this thesis aimed to determine the effectiveness of 'on-farm' seed priming to sustainably intensify barley production in conventional agricultural systems of the UK and Europe. This has never before been investigated and, with the increasing pressure to reduce chemical use (including those used in chemical seed treatments), non-chemical treatments and biopesticides are set to increasingly gain importance in more agroecological cropping schemes. Pivotal for achieving this aim was providing fundamental data on how seeds behave during 'on-farm' seed priming and how this adds value to the seed, as well as methods for optimisation of 'on-farm' seed priming. This was motivated by evidence of underuse and misuse of 'on-farm' seed priming by farmers due to a lack of information. At the same time, it was foreseeable that optimisation was a requirement if 'on-farm' seed priming is to be implemented in the high standards of conventional or organic agriculture in the developed world.

6.2 Can 'on-farm' seed priming significantly contribute to enhance crop yields in the developing world?

After the first scientific report about 'on-farm seed priming (Harris 1996), there has been a steady stream of research accumulating a large number of independent case studies around the world. Although these reports have generally reported positive outcomes, whether this form of priming can significantly enhance crop yields and, thus, actually contribute to food security had never been holistically examined. However, tackling this question in an experimental way, i.e. setting trials in representative locations with a wide range of crops would require an enormous amount of resources. Therefore, a quantitative meta-analysis was used and showed a remarkable average yield increase of 21 % across a representative number of

agri-environments relative to the non-primed (farmers practice) confirming the large potential of ‘on-farm’ seed priming to intensify crop production in developing countries (Chapter 2). Gains in yield can be mainly attributed to enhanced emergence, i.e. rapid emergence leads to better crop establishment, which is conducive to higher yields. This was also experimentally demonstrated for spring barley (Chapter 4) where improved emergence and seedling vigour conferred by seed priming treatments enabled crops to attain a greater number, and more vigorous tillers for grain filling stage. Overall, these results also emphasise the importance of seedling emergence for determining the rest of the crop development and, thereby, the value of seed priming treatments (Paparella et al. 2015; Lamichhane et al. 2018).

Seedbeds with inadequate moisture or subjected to high temperatures are likely to benefit the most from ‘on-farm’ seed priming and the greatest benefits can be expected for crops grown in arid and semi-arid climates (between 14 % and 34 % in yield). Likewise, under high saline or nutrient-deficient environments yield benefits were estimated between 17 % and 30 %. ‘On-farm’ seed priming reproduces the early stages of germination so that, at the moment of sowing, primed seeds have two direct agronomical advantages relative to non-primed seeds: (1) seeds are more advanced in the germination process, and (2) seeds are already hydrated. As demonstrated in Chapter 3, this partial hydrated state at sowing, commonly represents the biggest contribution of the two. Thus, under the low and unpredictable rain of arid and semi-arid climates this partial hydration can become crucial to secure completion of germination without suffering from a discontinuous water availability. Under saline soils, it follows a similar mechanism. Salinity produces both negative water potentials and oxidative stress which increases the time to emergence and, in turn, the vulnerability of the seed (Ibrahim 2016). Being hydrated and proximal to germination completion, primed seeds have a head start to quickly become a seedling before the salt stress comes insurmountable or at a fitness cost for the plant (Ibrahim 2016). Similarly, earlier rooting may facilitate absorption of nutrients before they are leached down in deficient-nutrient soils (Harris et al. 2001a).

‘On-farm’ seed priming benefits are not confined to adverse environments. Primed crops grown in warm temperate regions can be expected to attain significant

yield increases (between 9 % and 14 %), as well as those under more conventional management (i.e. fertilisers use and irrigated) are also likely to perceive significant benefits (between 5 % and 15 %). It is in these environments where developmental advantages are more pronounced. When the advantage of partially hydration is kept out of the equation, significant seedling vigour benefits can be obtained if priming is properly optimised (Chapter 3). It is hypothesised that part of the invigorating effect of 'on-farm' priming is due to the moderate abiotic stress generated during the soaking. The hypoxic conditions and/or membrane damage caused by rapid uncontrolled imbibition can trigger accumulation of proteins, enzymes and mRNA that leads to adaptative responses during subsequent stress events encountered during seedling growth (Rajjou et al. 2012; Chen and Arora 2013; Wojtyla et al. 2016).

6.3 The barley case: can 'on-farm' seed priming enhance yields in a European conventional agricultural system?

It follows from the above discussion that there is no reason why 'on-farm' seed priming would not deliver similar benefits to temperate agriculture of industrialised countries. Commercial seed priming treatments have not been adopted for arable crops in temperate agriculture because they are not commercially viable, i.e. too costly and too much handling for seeds with low economic margin (Taylor and Harman 1990; Paparella et al. 2015). In this situation, low-cost farmer-managed 'on-farm' seed priming may have a niche for sustainable intensification. This question was addressed in this research through field trials using barley (both spring and winter sown) as model crop. Barley was chosen for its socio-economic importance in both developing and developed countries, i.e., it is used as a staple food in marginal areas of the developing world and is of economic importance in European agriculture (Newton et al. 2011). In conjunction with 'on-farm' seed priming, chitosan was also applied to seeds to provide a positive control and, potentially, another environmentally sustainable seed treatment. Chitosan is an abundant biodegradable polymer which can elicit plant defences and stimulate growth so that also fitted the remit of sustainable intensification (Kashyap et al. 2015).

Table 6.1. Percentage change of equivalent 'on-farm' seed priming (OSP) and chitosan (CHP) treatment in spring and winter barley relative to non-primed (farmers' practice).

Barley type	Treatment	Final emergence	Canopy cover at tillering	Canopy at stem elongation	Canopy cover at booting	Yield	Reference
Spring[a]	OSP	6.9	6.3	9.3	5.3	11.5	Chapter 4
	CHP	23.6	19.3	19.8	10	14.9	
Winter[b]	OSP	2.2	-2.4	-4.3	-0.9	-2.1	Chapter 5
	CHP	6.2	0.4	-2.2	-1.2	-2.5	

[a]Average across two cultivars
[b]Average across three cultivars and two sites

The results from these trials showed marked differences between spring and winter crops in their response to chitosan-based and 'on-farm' seed priming treatments. Spring barley responded with enhanced emergence to treatments that later continued with comparably proportional increments in vegetation cover throughout canopy development (Table 6.1). The yield improvement due to 'on-farm' seed priming in this trial agreed with the predicted yield effect of 'on-farm' primed crops relative to non-primed for warm temperate climates in Chapter 2. Chitosan treatments largely exceeded the values of 'on-farm' seed priming for canopy cover, however, this was not translated into a proportional yield gain. This is possibly because the relationship between canopy size and radiation interception is nonlinear and, up to certain canopy size, further gains in light interception are progressively smaller (Bingham et al. 2007). By contrast, winter barley does not seem to respond positively to chitosan-based or 'on-farm' seed priming seed treatments. Although both treatments can give a slight boost to emergence relative to untreated seeds, this advantage is neutralised or reverted by advanced tillering indicating that growth of treated crops are potentially affected over the winter period. This is in line with a paucity of literature where commercial seed priming treatments have also shown limited practical use for enhancing establishment of winter cereals in temperate climates (Giri and Schillinger 2003; Subedi and Ma 2005).

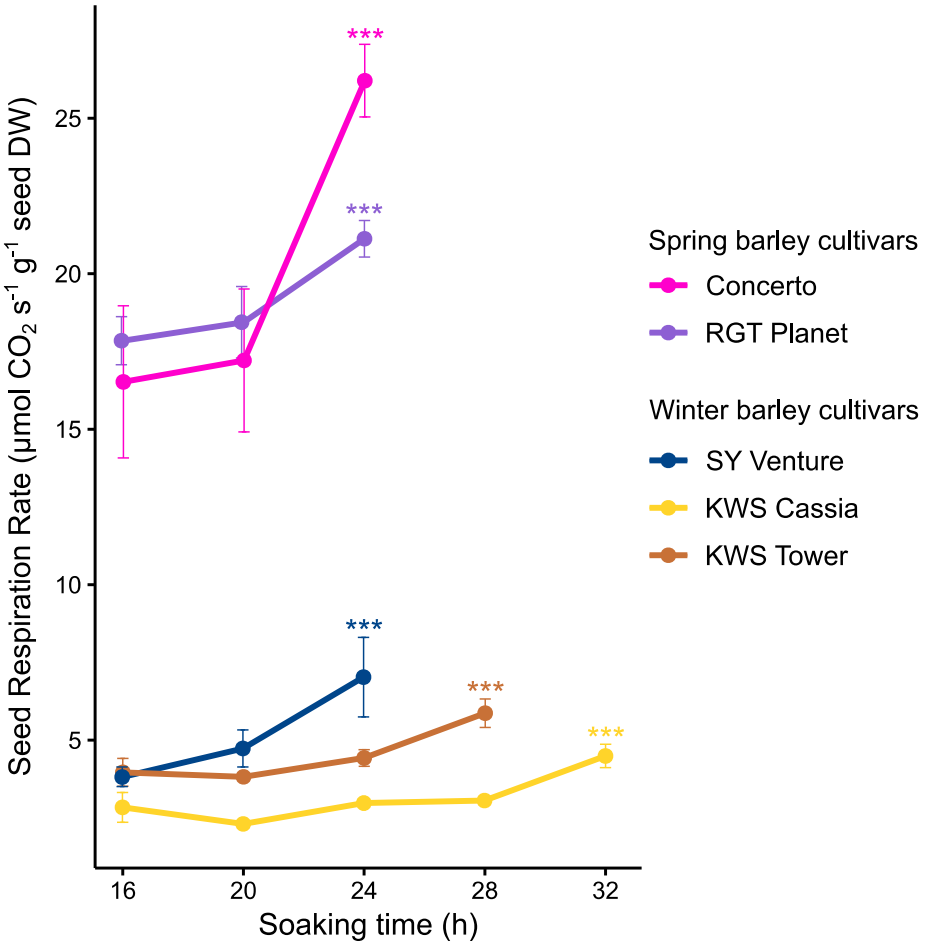


Figure 6.1. Changes in seed respiration rate during 'on-farm' seed priming for each cultivar. Asterisks denote significant differences in seed respiration (*** $P < 0.001$) at a soaking time relative to its immediate previous soaking time within each cultivar (LSD test). The soaking interval prior to the significant increase in respiration was taken as the optimal priming duration. Error bars show ± SE.

Whilst stimulating emergence and early growth may be beneficial in spring barley, this may actually be counterproductive for winter cereals. Interestingly, the much higher respiration rate of spring cultivars relative to winter cultivars also supports such a hypothesis (Figure 6.1). Seed respiration rate has previously been used as a proxy of seedling vigour (Wang et al. 2016), and the distinct respiration rates may reflect an active genetic selection towards cultivars with higher seedling vigour for spring-sown cultivars, however, this may not have been a trait of interest for breeders of winter cultivars. Thus, it is possible that a slow-paced growth habit prior to winter is part of the adaptative mechanism for overwinter acclimation and

altering this process through seed treatments may result in vigour penalties at the end of the cold period.

Overall, there are several reasons to advise against the use of seed priming in winter barley in particular, and likely to be generalised to other winter crops of temperate climates. Firstly, it is clear that prompt emergence associated with sowing partially hydrated seed will be limited under typically more humid conditions of autumn-sown crops than those for spring crops. Secondly, the plasticity of winter barley, i.e. the greater capacity to produce more tillers and to adjust their number according to resources available, will allow crops with less initial vigour to catch up (García del Moral and García del Moral 1995). There is also a risk that, if sowing is followed by substantial rain that results in flooded soils, primed seeds may suffer from more prolonged hypoxic conditions (Rashid et al. 2006). Lastly, it is also possible that accelerating emergence may be undesirable as it could somehow alter the mechanics of overwinter acclimation. However, these conditions do not seem to apply to winter cereals grown in tropical and subtropical regions, which do not overwinter (Rashid et al. 2006; Farooq et al. 2008). In contrast to temperate zones, winter crops are sown at the beginning of the dry period (*rabi* season) using the residual water from the rainy season. It is under these circumstances where planting hydrated seeds can make the difference between securing or aborting emergence (Wojtyla et al. 2016).

In spring barley, by contrast, both ‘on-farm’ seed priming and chitosan treatments seem to confer an early vigour advantage that enables them to accumulate biomass more rapidly throughout the growing season. Spring barley yield is predominantly sink-limited in temperate climates, so that N-fertilisers are commonly applied at sowing and prior to stem elongation to encourage tillering and the development of well-sized shoots before stem elongation. In such high-input systems, it is likely that the initially more vigorous crops from treated seed are able to capture more N before it is lost, through leaching or volatised, at each N application and, thus, maintaining greater canopies (with more viable tillers) for grain filling. Vigour is an effective trait for higher N uptake during early growth in wheat so that it is likely to be similarly effective in barley (Liao et al. 2004; Pang et al. 2014). These results are promising and data on the effects of contrasting climates, soil types and genetic background are now needed to more clearly define the potential

benefits that these seed treatments can deliver. If confirmed, it would be opportune to suggest the inclusion of seed priming and elicitor treatments as one more management practice to ensure that yield potential is not restricted early in the crop season.

6.3.1 Can 'on-farm' seed priming enhance host defences in a European conventional agricultural system?

Yield improvements delivered by 'on farm' seed priming often exceed the expected gain due to better establishment, i.e., its direct agronomical advantage (Chapter 2). Increased disease tolerance, escape from pests and diseases or induction of plant defences have been postulated within these studies as indirect priming effects to explain this establishment-yield mismatch in several occasions e.g., Rashid et al. (2004; Harris et al. (2005). This information, added to the need for more sustainable integrated disease management strategies, motivated the assessment of the interaction between seed treatments and disease.

The absence of disease to detectable levels in spring barley and the general poor response of winter barley to seed treatments restricted the evaluation of potential disease tolerance and/or escape. These field trials, however, enabled identification of candidate traits to deliver disease tolerance and escape. It was found that a greater canopy size can provide certain degree of tolerance to pre stem elongation powdery mildew infection in winter barley (Chapter 5). The greater remaining healthy tissue can potentially compensate for this loss of radiation interception (Bingham and Topp 2009). Interestingly, it was also found that rapid stem elongation can limit secondary spreads of powdery mildew and, hence, provide certain level of disease escape. Thus, it remains to be resolved whether the vigour benefits shown in spring barley, which were manifested as both greater canopy and height, would also provide a certain degree of tolerance and escape in the event of primary and secondary powdery mildew infections or other diseases.

Evaluation of the induction of generational and transgenerational defences through seed treatments in the field have also been addressed in this work (Chapters 4 and 5); however, no evidence was found to suggest that chitosan or 'on-farm' seed priming produce such effects. There is still the possibility that these effects were minor and remained unseen. As it has been previously suggested for

other elicitors, the continuous interactions with biotic and abiotic elements hinders the expression of potential induced resistance in field crops (Walters et al. 2013; Alexandersson et al. 2016; Iriti and Varoni 2017). Importantly, climatic conditions also play a major role in the detection of induced resistance and will influence the occurrence and intensity of the triggering stimuli (the external factor(s) that activates a stress response). This escalates another level of uncertainty in transgenerational effects as conditions can be very different from season to season. Thus, it is appropriate to suggest that, for the detection of these generational and transgenerational effects, field phenotyping needs to be coupled with molecular and epigenetic markers specifically involved in the defence induction (Ramírez-Carrasco et al. 2017). This would allow the reliable linking of defence priming and transgenerational memory in the field.

6.4 Optimisation of 'on-farm' seed priming is key for greater exploitation and adoption

Finding the optimal seed priming protocol for each crop species and genotype is key to getting the most out of the technology and, in turn, maximise seed performance (Paparella et al. 2015). However, this can be both expensive and time consuming as it involves performing numerous germination assays and mini-plot trials that can only provide retrospective indications of the effectiveness (Rajjou et al. 2012; Paparella et al. 2015). For simplicity, farmers preforming 'on-farm' seed priming have used conservative soaking times, commonly "overnight", despite this most likely being far from the optimum (Harris 2006). Therefore, alternative methods that allow the rapid determination of optimal soaking times are critical to enable both economic and practical exploitation of this technology.

The findings in Chapter 3 confirmed that soaking for a few hours, e.g. 8 h as equivalent to the "overnight" practice proposed for most tropical crops (Harris 2006), is enough to obtain the benefits from planting hydrated seeds. However, to obtain the additional developmental advantages requires longer soaking times. In barley, the maximum seedling vigour was acquired when the priming process was stopped just before the beginning of the differentiation of embryo tissues into coleoptile and coleorhiza (20 h) after which vigour began to decrease. This was consistently demonstrated both by seedling vigour testing in controlled environment cabinets and

in the field. The onset of embryonic axes elongation represents the optimal point of seed development and can be understood as the milestone marking the transition from seed to seedling. At this point, the advancement of several pre-germinative processes characteristic of phase II, i.e., gene transcription, synthesis of new proteins and amino acids, mitochondrial and DNA repair can be attained (He et al. 2015; Wojtyla et al. 2016; Ma et al. 2017). Beyond this point, further embryo development also entails loss of desiccation tolerance and accumulation of toxic fermentative, which can compromise the vigour of the future seedling.

The development of novel cost-effective methods for the determination of the 'optimal' soaking time was an important objective of this thesis. Accurate identification of developmental stages of germinating seeds can be very challenging, although the monitoring of seed respiration during 'on-farm' seed priming was shown to be an effective approach. The onset of embryonic axes elongation is typically preceded by a second burst of CO_2 flux (indicative of the activation of starch mobilisation that enable radicle emergence), which can be used as a marker. Figure 6.1 highlights how the timing of the peak is specific to each cultivar and, correspondingly, it is the 'optimal' priming treatment. The major disadvantage of this method is the need for specialised equipment and staff, which makes it only within the reach of agricultural research institutions. Alternatively, observation of embryo morphology can be also used for relatively large seeds such as grains. This method is simple and affordable, although still difficult to be reproducible in an 'on-farm' context as specific training for the identification of subtle embryo differences would be required.

Although these approaches could not be used reliably by farmers to optimise their own priming protocols for their own seeds, the methods presented in Chapter 3 could be carried out by extension workers and research agricultural institutions to provide recommended 'safe' and 'optimal' soaking times for the common varieties within a specific region. These methods represent a much more rapid and cost-effective alternative to the current optimisation approach through a series of germination assays and mini-plot trials (e.g., Harris et al. 1999; Rashid et al. 2004, 2006; Virk et al. 2006). Therefore, if properly exploited by extension workers and research agricultural institutions, these methods could facilitate the widescale adoption of 'on-farm' seed priming.

6.5 Concluding remarks

This research has provided robust and quantitative evidence to confirm ‘on-farm’ seed priming as a valuable technology to increase crop yields and, hence, food security in the developing world. The benefits of ‘on-farm’ seed priming come at almost no financial cost, which together with the simplicity of the method allows all farmers access to this seed priming technology. These characteristics makes ‘on-farm’ seed priming an excellent entry point for resource-poor farmers to take part in agricultural intensification (Aune and Bationo 2008). These findings are of significant relevance and can provide the evidence to governmental institutions and policymakers in developing countries to promote ‘on farm’ seed priming as a recommended practice.

Farmers using ‘on-farm’ seed priming need to be able to distinguish between ‘optimal’ and ‘safe’ soaking times to attain maximum benefit from this technology. When conditions allow seeds to be sown within a few hours after priming, ‘optimal’ soaking times produce maximal moisture content and seed advancement benefits would be the best strategy. When there is a risk of delayed sowing, shorter (‘safe’) soaking times must be used. Observation of seed respiration patterns by CO_2 flux seems an especially effective tool to rapidly find cultivar-specific ‘optimal’ soaking times without the need for a cumbersome series of germination assays and mini-plot trials. Therefore, these findings can contribute to a better exploitation of ‘on-farm’ seed priming and, thus, enhance its adoption.

‘On-farm’ seed priming technology and chitosan elicitor treatments are promising practices to sustainably intensify spring barley production in a European agriculture context. Crops from primed seeds show improved emergence and seedling vigour that lead to a greater number, with more vigorous tillers being retained for grain filling. It is hypothesised that this additional vigour is maintained throughout canopy expansion due to enabling a greater uptake of N-fertilisers. Thus, it may be interesting to include seed treatments and elicitors as one more management practice to ensure that yield potential is not restricted at an early stage in the crop season. Further research should explore indirect beneficial effects derived from enhanced growth such as greater disease tolerance or greater competition with weeds for light which may reduce tiller mortality.

By contrast, does not seem likely that winter barley benefits from such invigorating seed treatments. Although ‘on-farm’ seed priming and chitosan treatments can promote emergence, this advantage is reversed by advanced tillering and may imply a fitness cost that continues for the rest of the crop cycle resulting in small yield penalties. It is suspected that seed treatments alter somehow the adaptative mechanism for overwinter acclimation resulting in a fitness cost. By extension, seed treatments offer limited scope to increase disease tolerance or escape in winter barley, either alone or as a complement to fungicides.

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Supplementary material

Table S2.1. Levels within each potential variable affecting priming performance.

Moderator variables	Levels	Short description
Study type	Field	Research stations and farmers' plots
	Pots	Pots placed on field and greenhouses
	Labs	Incubators and controlled environment chambers
Climate[a]	Temperate	Cfb, Csa and Cwa
	Equatorial	Aw
	Semi-arid	BSh and BSk
	Arid	BWh
Yield-limiting factor	Non-stressed	No major nutrient or water limitations identified
	Nutrient deficient	Nutrient deficiencies identified as the major constraint
	Salinity	Saline water or soil identified as the main constraint
Plant type	Monocots	Barley, sorghum, wheat, rice, pearl millet, maize, korarima and finger millet
	Dicots	Chickpea, cotton, cowpea, groundnut, sesame, *Dracocephalum kotschyi* Boiss, fennel, mungbean and horsegram

[a]Koppen climate classes (Kottek et al. 2006).

Table S2.2. Measures used for publication bias characterisation of each effect size.

Response variables	Summary effect		Publication bias			
	No. studies	*LRR*[a]	Kendall's tau	*P* value (Kendall's tau)	No. impute[b]	'Corrected' *LRR*[c]
Time to 50% emergence	24	-0.244	-0.228	0.147	11	-0.434
Final emergence	41	0.104	0.067	0.560	0	0.104
Yield	65	0.191	0.139	0.110	15	0.205

[a]Natural log of weighted summary effect size across case studies. [b]Number of case studies imputed by the Duval and Tweedie 'trim and fill' method. [c]Corrected summary effect after imputing missing case studies using Duval and Tweedie 'trim and fill' method.

Figure S2.1. Funnel plots for each of the three datasets. The vertical line indicates the fixed effect estimate. Open circles represent case studies imputed by the Duval and Tweedie ‘trim and fill’ method.

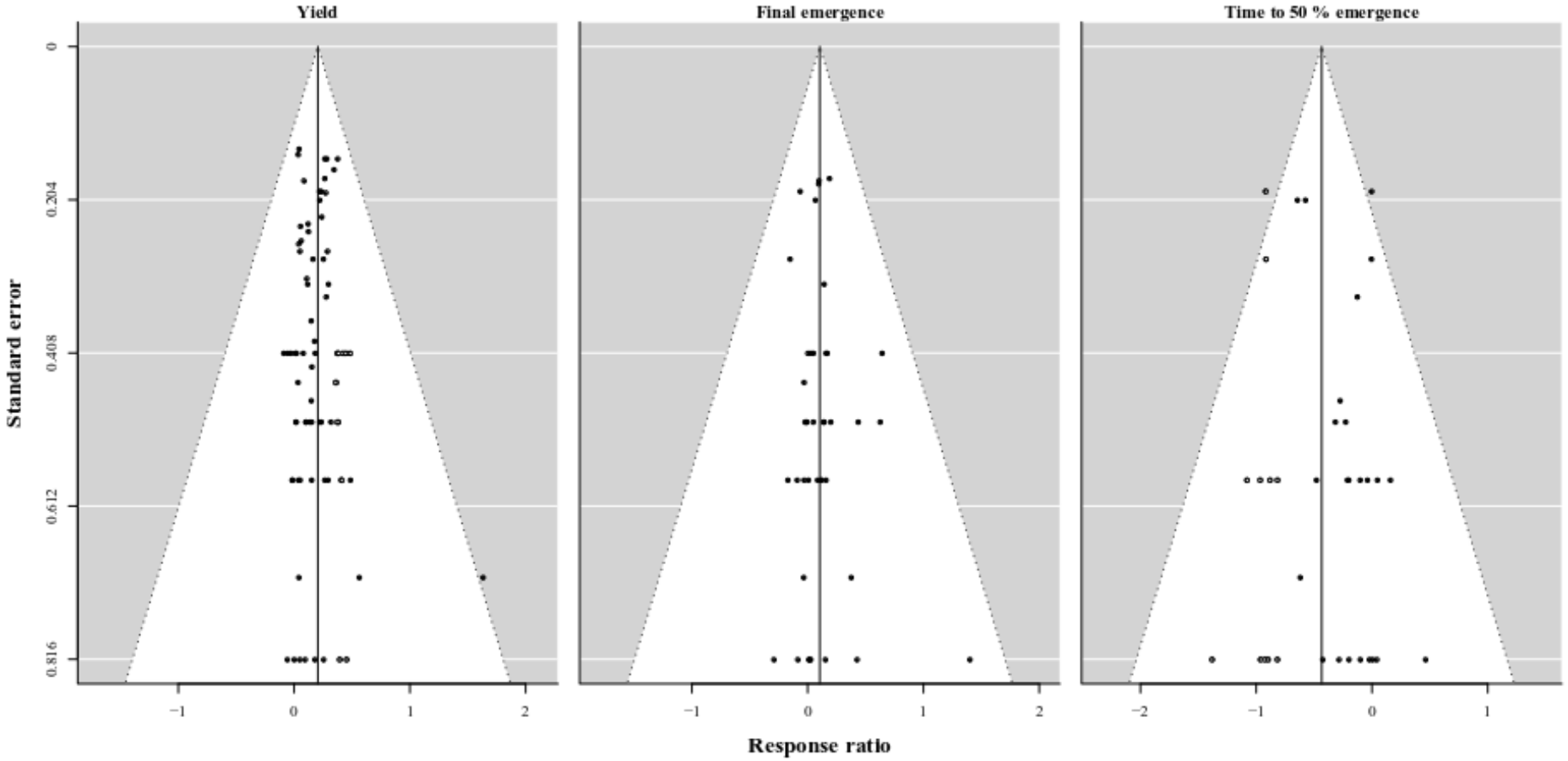

Table S3.1. Effect of seed priming soaking time on time to 50 % emergence (E_{50}) and the percentage of healthy emerged seedlings (%TE).

Cultivar	Treatment	E_{50} (h)	%TE[a]
Concerto	0 h	188.6	99.7 (1.51)
	16 h	186.5	98.3 (1.44)
	20 h	187.8	98.1 (1.43)
	24 h	185.9	97.9 (1.43)
RGT Planet	0 h	186.8	96.7 (1.39)
	16 h	188.0	95.2 (1.35)
	20 h	187.8	97.7 (1.42)
	24 h	186.3	97.1 (1.40)
$LSD_{Cv \times Tr}$		3.5	(0.16)
df		62	62

LSD: least significant differences for the interaction; df: degrees of freedom for the residual term.
[a]Back-transformed means and means on the transformed scale (between brackets).

Table S4.1. Mean values of grean area (GA), grain yield (GY), grain number (G no.), thousand grain weight (TGW), and grain nitrogen (GN) for all the seed treatments during 2018. grain yield (GY), grain number (G no.), thousand grain weight (TGW), grain nitrogen (GN) and percentage of grain retention (Retention %) on 2018 trial. Only LSD values for significant main effects or interaction are shown. Treatment abreviations are as in Table 4.1.

Cultivar / Treatment	GA_{Ti} (%)	GA_{SE} (%)	GA_{Bo} (%)	GY (t ha^{-1})	G no. (m^{-2})	TGW (g)	GN (%)	Retention % > 2.5 mm[a]
Concerto	**12.3**	**40.9**	**56.5**	**4.02**	**7,914**	**49.7**	**1.57**	**93.5**
NP	11.3	33.3	52.6	3.76	7,154	51.4	1.58	93.6
NP+0.5	14.9	47.5	62.0	4.39	8,682	49.4	1.56	93.6
NP+5	12.7	44.0	58.6	4.13	8,205	49.1	1.57	93.4
P20	13.0	42.2	58.6	4.29	8,414	49.8	1.57	93.9
P20+0.5	12.1	41.2	57.8	3.98	7,898	49.2	1.55	93.7
P20+5	12.7	41.5	56.2	4.17	8,084	50.3	1.49	93.5
P24	14.2	44.0	55.6	3.76	7,562	48.6	1.58	93.3
P24+0.5	10.6	35.8	50.5	3.57	7,153	48.9	1.59	93.2
P24+2.5	11.9	42.6	57.7	4.14	8,250	49.0	1.58	93.4
P24+5	9.4	37.1	55.0	4.05	7,737	51.4	1.61	93.2
RGT Planet	**12.9**	**44.4**	**60.7**	**4.49**	**8,733**	**50.1**	**1.56**	**93.8**
NP	12.3	44.9	59.7	4.15	8,101	50.0	1.54	92.8
NP+0.5	13.5	46.4	61.9	4.70	9,009	51.0	1.53	93.5
NP+5	10.7	45.0	60.0	4.56	8,744	50.7	1.54	94.0
P20	9.6	40.1	57.9	4.54	8,741	50.6	1.54	94.3
P20+0.5	13.4	47.1	62.0	4.72	9,362	49.2	1.55	94.0
P20+5	14.8	44.6	61.8	4.51	8,794	50.1	1.52	94.2
P24	15.3	45.5	61.5	4.43	8,633	50.0	1.56	93.8
P24+0.5	14.3	44.9	60.6	4.44	8,707	49.7	1.59	94.0
P24+2.5	12.5	43.4	61.5	4.25	8,414	49.4	1.60	93.5
P24+5	12.5	42.4	59.7	4.57	8,828	50.5	1.60	94.1
Grand mean	**12.6**	**42.7**	**58.6**	**4.26**	**8,324**	**49.9**	**1.56**	**93.7**
LSD_{Cv}		2.9	2.2	0.14	268			0.2
LSD_{Tr}				0.32	300	0.6	0.05	
$LSD_{Cv \times Tr}$								

[a]Percentage of screened grain after passing through a 2.5 mm mesh.
Cultivar means and Grand mean in bold.

Table S4.2. Mean values of disease score for yellow rust (DSY), disease score for rhynchosporium (DSR), grain yield (GY), grain number (G no.), thousand grain weight (TGW), and grain nitrogen (GN) for all the seed treatments during 2018. grain yield (GY), grain number (G no.), thousand grain weight (TGW), grain nitrogen (GN) and percentage of grain retention (Retention %) on 2019 trial. Only LSD values for significant main effects or interaction are shown. Treatment abreviations are as in Table 4.1.

Cultivar / Treatment	DSY (%)	DSR (%)	GY (t ha^{-1})	G no. (m^{-2})	TGW (g)	GN (%)	Retention % > 2.5 mm[a]
Concerto	**5.47**	**1.03**	**5.26**	**11,030**	**44.0**	**1.57**	**90.6**
NP	5.50	1.22	5.29	10,951	44.5	1.56	90.0
NP+0.5	5.50	0.94	5.40	11,274	44.2	1.56	91.3
NP+5	5.75	1.14	5.26	10,953	44.2	1.58	91.6
P20	5.75	0.74	5.41	11,412	43.9	1.55	90.2
P20+0.5	5.00	0.69	5.30	11,040	44.2	1.60	91.9
P20+5	6.00	1.47	5.19	11,253	42.6	1.58	87.1
P24	5.00	1.66	4.95	10,256	44.2	1.57	91.8
P24+5	5.25	0.38	5.33	11,103	44.2	1.59	90.5
RGT Planet	**5.58**	**0.76**	**5.27**	**11,169**	**43.6**	**1.57**	**89.9**
NP	5.38	0.83	5.20	11,091	43.3	1.60	89.7
NP+0.5	5.75	1.39	5.33	11,503	42.8	1.57	88.8
NP+5	5.25	0.60	5.55	11,756	43.6	1.57	91.2
P20	5.50	0.39	5.33	11,089	44.4	1.56	91.0
P20+0.5	5.50	0.58	5.27	11,069	43.8	1.58	91.3
P20+5	5.00	0.48	5.15	10,913	43.5	1.56	90.1
P24	7.00	0.66	5.05	11,056	42.0	1.59	86.6
P24+5	5.25	1.14	5.31	10,873	45.0	1.53	90.9
Grand mean	**5.52**	**0.90**	**5.27**	**11,100**	**43.8**	**1.57**	**90.3**
LSD_{Cv}							
LSD_{Tr}							
$LSD_{Cv \times Tr}$					1.4		1.9

[a]Percentage of screened grain after passing through a 2.5 mm mesh.
LSD: least significant differences for the interaction; df: degrees of freedom for the residual term.
Cultivar means and Grand mean in bold.

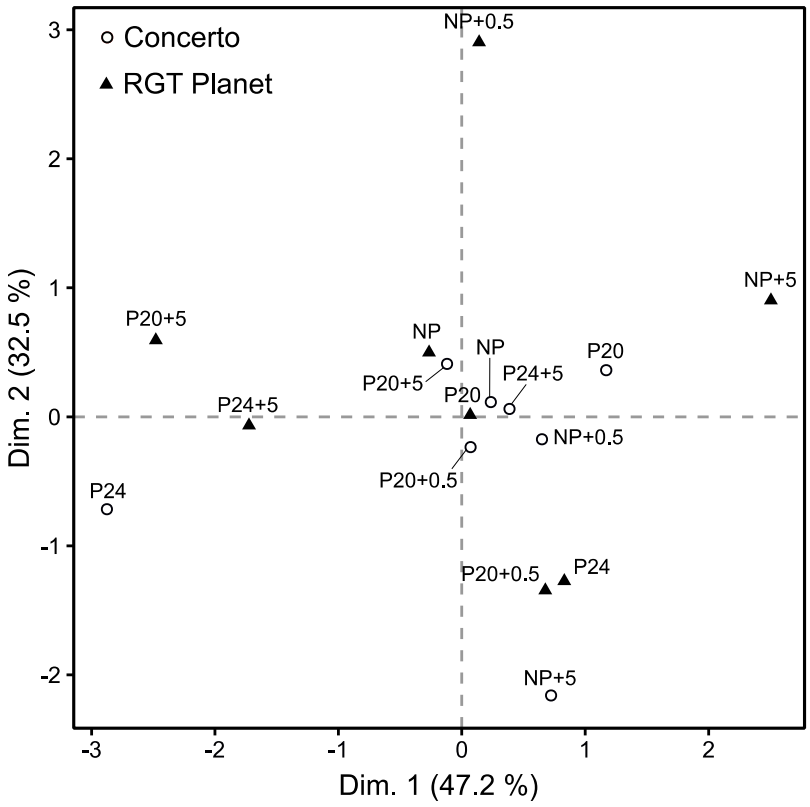

Figure S4.1. Representation of individual treatments on the basis of the first two dimensions by cultivar in 2019.

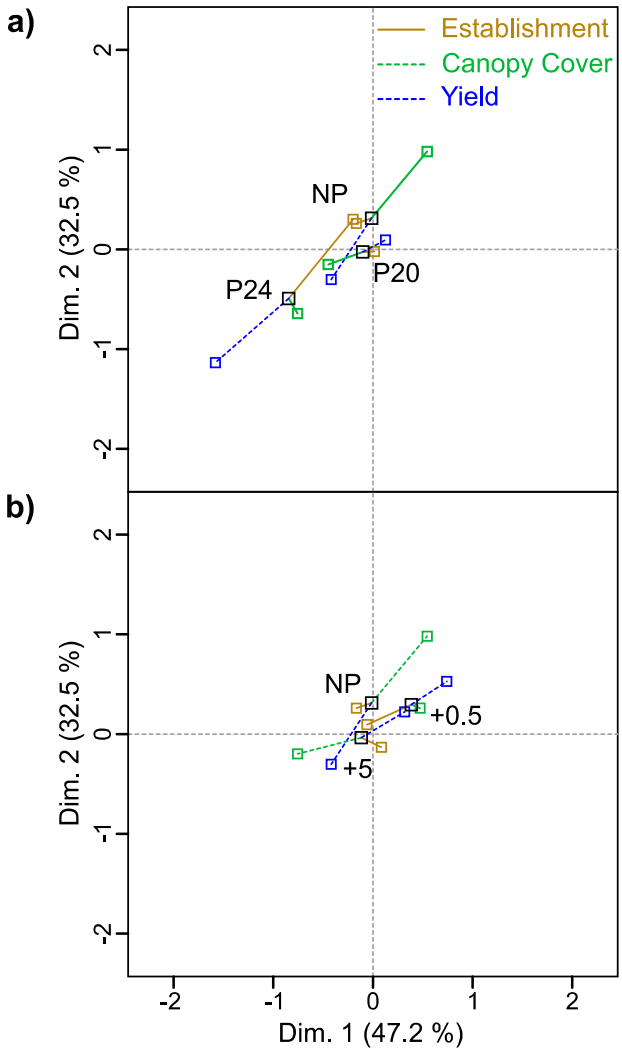

Figure S4.2. Projection of the groups of variables (coloured squares) onto the global analysis according to (a) 'on-farm' seed priming levels, 20 h (P20) and 24 h priming (P24); and (b) chitosan concentrations levels, 0.5 (+0.5), 2.5 (+2.5) and 5 g l^{-1} (+5) against untreated (NP) in 2019. Each dark square of a given factor level is the centroid of the treatments belonging to this level.

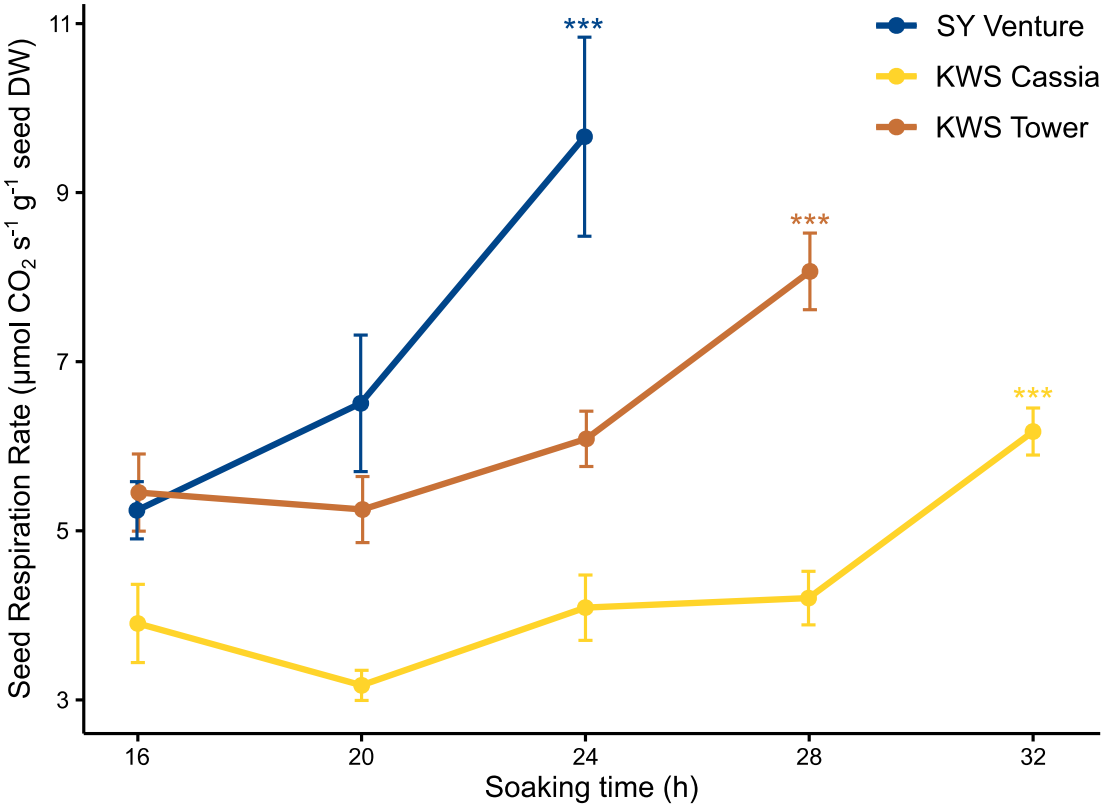

Figure S5.1. Changes in seed respiration rate during 'on-farm' seed priming for each cultivar. Data are means ± SE (n = 3 replicates of 150 seeds soaked in distilled water (1:6 (w/v)) in 100 ml plastic pots, at 20 °C in the dark) for each soaking time and cultivar. Asterisks denote significant differences in seed respiration (*** $P < 0.001$) at a soaking time relative to its immediate previous soaking time within each cultivar (LSD test). The soaking interval prior to the significant increase in respiration was taken as the optimal priming duration.

Figure S5.2. Illustration of seedling counting method. a) Shows the area of the picture counted and, (b) shows the zoom at which seedlings are counted using the Cell Counter plugin in FIJI to record the counts.

Figure S5.3. Flowchart of image processing for total plant tissue and percentage of senescent tissue estimation.

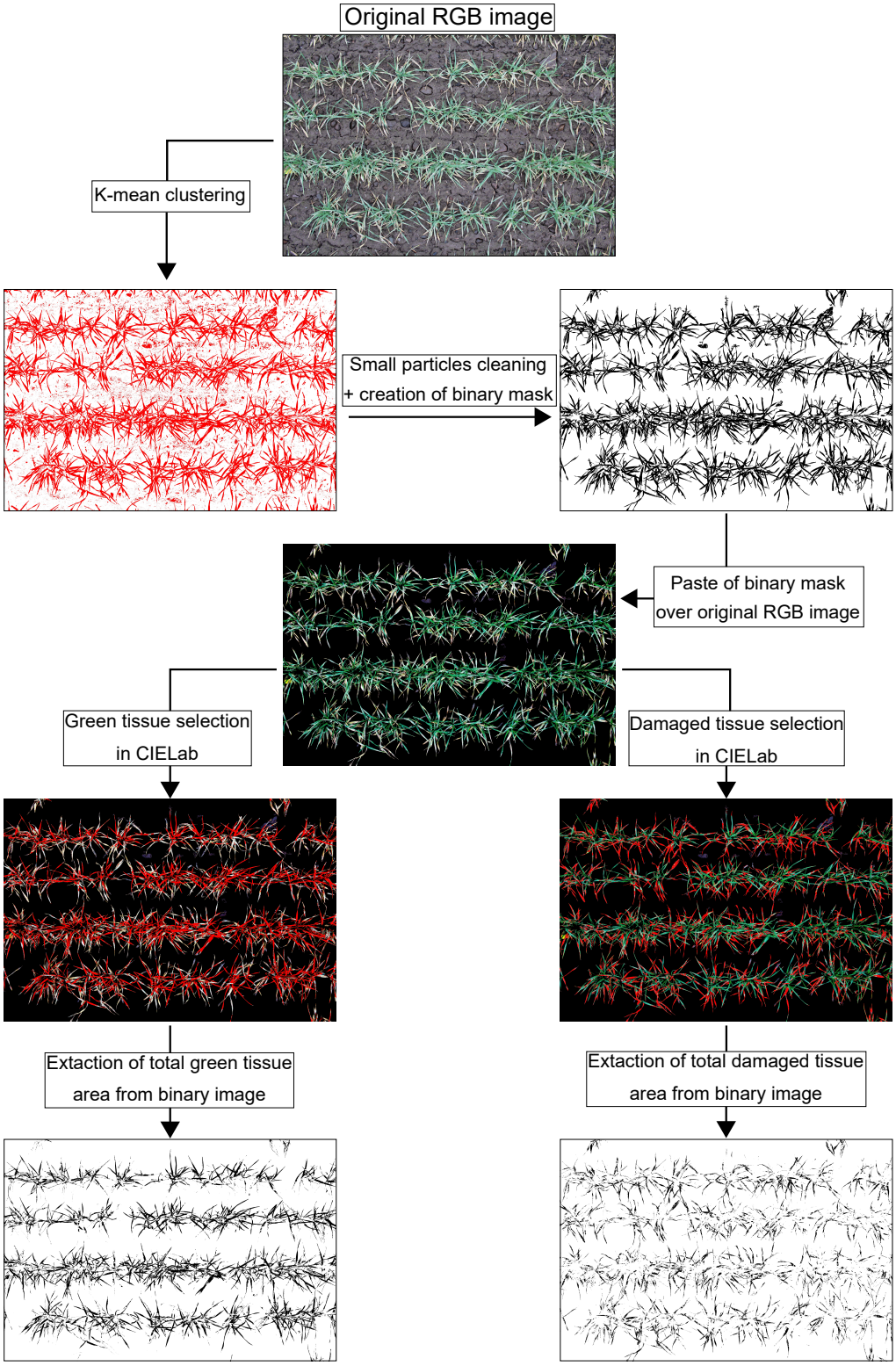

Table S5.1. Final height and time to 50 % GS49 averaged by fungicide and seed treatment. Values in each row followed by different letters differ significantly from each other: LSD test (P > 0.05).

	F0			F1		
Site	NP	WP	CP	NP	WP	CP
Stem elongation rate (cm d^{-1})						
Balruddery	-	-	-	-	-	-
Mylnefield	2.39[a]	**2.49[b]**	2.39[a]	2.44[a]	2.39[a]	2.42[a]
Final height (cm)						
Balruddery	71.3[a]	72.3[a]	71.9[a]	74.3[a]	73.1[a]	73.0[a]
Mylnefield	72.2[a]	72.6[a]	71.3[a]	74.9[a]	73.4[a]	72.9[a]
Time to 50 % GS49 (d)						
Balruddery	211.5[a]	212.0[a]	211.3[a]	211.3[a]	211.3[a]	211.4[a]
Mylnefield	209.1[a]	209.2[a]	208.8[a]	209.3[a]	208.9[a]	208.9[a]

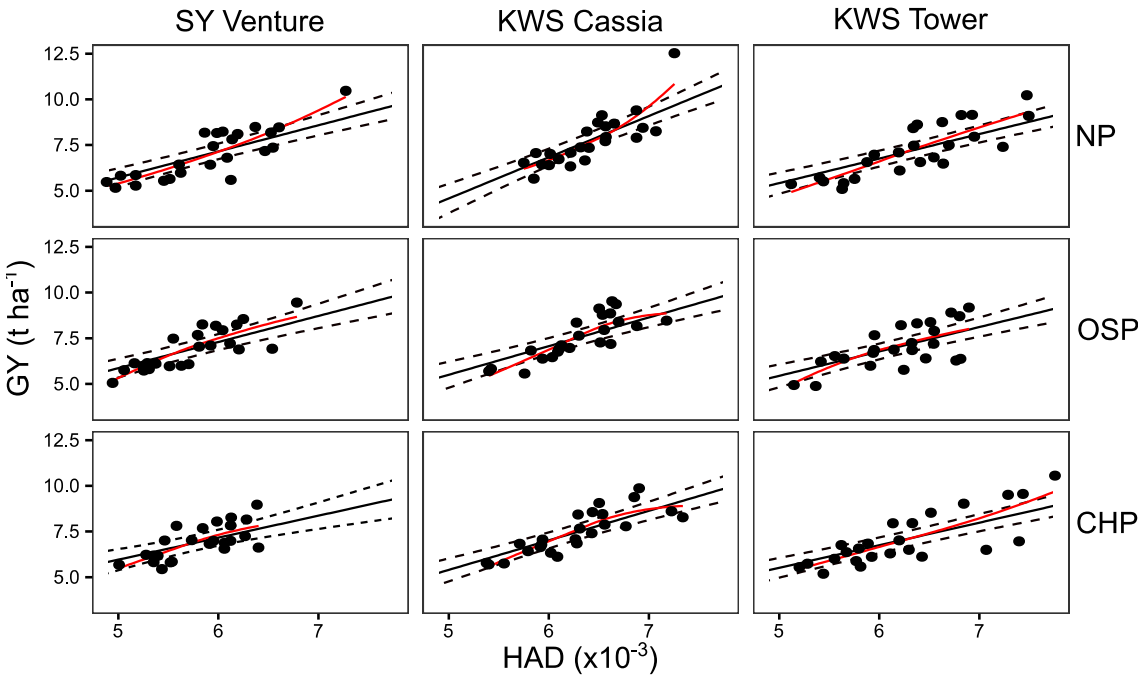

Figure S5.4. Visual diagnosis of linearity by loess smoothed line (in red). As data was dispersed along the fitted line (i.e., does not show fungicide/untreated clusters), and the loess smoothed line did not excessively deviate from the fitted tolerance line, the dataset was considered suitable for tolerance analysis.