Ultrafine particles in inhabited areas in the Arctic - From very low to high concentrations

Pétursdóttir, Una; Kirkelund, Gunvor Marie; Press-Kristensen, Kåre; Hertel, Ole; Mikkelsen, Teis Nørgaard

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Highlight

- A 10-day heatwave was superimposed to elevated temperature and CO₂ around flowering
- The applied heatwave decreased barley yield by 52%
- Aboveground vegetative biomass increased from heatwave exposure
- 22 barley accessions showed variation in decreased yield and stability of yield

1 **How a 10-day heatwave impacts barley grain yield when superimposed onto future**
2 **levels of temperature and CO₂ as single and combined factors**

3

4 **Cathrine H. Ingvorsen**

5 cathrine.ingvorsen@csiro.au

6 Technical University of Denmark, Risø Campus, Frederiksborgvej 399, DK-4000

7 Roskilde, Denmark

8 Present address: Black Mountain Laboratories, GPO Box 1600, Canberra, ACT, 2601,

9 Australia

10 **Corresponding author:** T +61 0402 475 053, M cathrine.ingvorsen@csiro.au

11

12 **Michael F. Lyngkjær**

13 mlyn@plen.ku.dk

14 University of Copenhagen, Section for Plant Biochemistry, Thorvaldsensvej 40, DK-

15 1871 Frederiksberg C, Denmark

16

17 **Pirjo Peltonen-Sainio**

18 pirjo.peltonen-sainio@luke.fi

19 Natural Resources Institute Finland (Luke), Management and Production of Renewable

20 Resources, Latokartanonkaari 9, FI-00790 Helsinki, Finland

21

22 **Teis N. Mikkelsen**

23 temi@env.dtu.dk

24 Technical University of Denmark, Dept. Environmental Engineering, AIR,

25 Bygningstorvet 115, DK-2800, Kgs. Lyngby, Denmark.

26

27 **Anders Stockmarr**

28 anst@dtu.dk

29 Technical University of Denmark, Dept. Applied Mathematics and Computer Science,
30 Matematiktorvet, Bld. 324, 2800 Kgs. Lyngby, Denmark

31

32 **Rikke B. Jørgensen**

33 rijq@env.dtu.dk

34 Technical University of Denmark, Dept. Environmental Engineering, AIR,
35 Bygningstorvet 115, DK-2800, Kgs. Lyngby, Denmark

36 **Abstract**

37 Heatwaves pose a threat to crop production and are predicted to increase in frequency,
38 length and intensity as a consequence of global warming. Future heatwaves will occur
39 in addition to the ongoing increase of mean temperature and CO₂. To test effects of
40 heatwaves superimposed to future climate scenarios, 22 barley accessions were
41 cultivated with elevated temperature (+5°C) and CO₂ (700ppm) as single factors and in
42 combination. The control treatment mimicked ambient Scandinavian early summer
43 conditions (19/12°C, day/night; 400ppm CO₂). Around flowering a 10-day heatwave of
44 33/28°C (day/night) was superimposed to all treatments. The lowest average grain yield
45 was observed when the heatwave was superimposed onto the combined elevated
46 temperature and CO₂ treatment. Here the yield decreased by 42% compared to no
47 heatwave and 52% compared to ambient conditions. When the heatwave was
48 superimposed onto ambient conditions the average grain yield decreased by 37%
49 compared to no heatwave. There was no significant difference between the relative
50 grain yield decrease caused by the heatwave in the ambient and future climate scenarios.
51 In contrast, the vegetative aboveground biomass increased upon heatwave exposure,
52 leading to a strong decline in the harvest index. Our results strongly emphasize the need
53 to produce heatwave resilient cultivars.

54

55 **Keywords:** Biomass, extreme events, genotype differences, heat exposure, *Hordeum*
56 *vulgare* L., multifactor treatment, stability

57

58

59 **1. Introduction**

60 Extreme weather events like heatwaves, floods, droughts and storms cause acute
61 changes in growth conditions determining primary production (Fischer and Schär, 2009;
62 Hajat *et al.*, 2010; Collins *et al.*, 2013). Collected data from recent decades together
63 with results from simulation studies suggest that the variability within seasons can be
64 more unfavorable for crop production than the general changes from season to season
65 (Reyer *et al.*, 2013; Gourdji *et al.*, 2013, Tack *et al.*, 2015). In a statistical study, inter-
66 annual climate variability was shown to account for >60% of maize, rice, wheat and
67 soybean yield variability (Ray *et al.*, 2015). Hence, large variations in the climate within
68 the crop seasons, such as a heatwave, are detrimental for the end result.

69 In the 2012-2013 growth season Australia experienced what became known as the
70 ‘angry summer’, where over 100 temperature records were broken (BoM, 2014). An
71 extreme heatwave caused large scale yield failures in Russia in 2010 (Trenberth and
72 Fasullo, 2012), and Europe experienced extreme heatwaves in 2003 and 2006. In 2003,
73 the European heatwave caused a 21% decrease in the French wheat production as
74 temperatures were up to 6°C above long-term means and precipitation being less than
75 50% of the average (Ciais *et al.*, 2005). Losses in cereal crop production from heat and
76 drought in the period from 1964 to 2007 were showed to reach 9-10% globally with the
77 highest losses in recent years (Lesk *et al.*, 2016). Unfortunately, predictions are that
78 global warming will make summer heatwaves more frequent and severe together with
79 decrease in precipitation during the summer period (Meehl and Tebaldi, 2004; Fischer
80 and Schär, 2010; Collins *et al.*, 2013).

81 In the north of Europe, barley (*Hordeum vulgare* L.) - especially spring barley - is the
82 cereal species occupying most of the cultivated area (19%), and the grains are

83 predominantly used for feed and malt (FAOSTAT 2017). The annual average increase
84 in grain yield of barley and wheat (*Triticum aestivum* L.) observed up to 1995 has
85 ceased in Scandinavia (FAOSTAT, 2017). Stagnation of grain yield might, at least
86 partly, is alleviated by the development of climate resilient cultivars. However, to
87 develop climate resilient cultivars, assessing the effects of the most likely and relevant
88 climate changes to a range of genotypes is essential. Studying the effects of future
89 extreme events are challenging due to the high complexity of their timing, frequency
90 and intensity, and the fact that they will be superimposed on the seasonal changes.

91 The effect of elevated temperature (eTmp) and elevated atmospheric carbon dioxide
92 concentration (eCO₂) on grain yield have been evaluated as single-factors and
93 combined-factors under experimental conditions in FACE (free air carbon dioxide
94 enrichment) and in enclosure studies as well as in simulation studies (Lawlor and
95 Mitchell, 1991; Conroy *et al.*, 1994; Jablonski *et al.*, 2002; Ainsworth and Long, 2005;
96 Lobell *et al.*, 2011; Challinor *et al.*, 2014; Ingvordsen *et al.*, 2015a; Cai *et al.*, 2016).

97 The numerous studies generally report decreasing grain yield by eTmp and increasing
98 grain yield from eCO₂. In combinations, the harmful effect of eTmp is not fully
99 complemented by eCO₂, and therefore, grain yield generally decreased (Conroy *et al.*,
100 1994; Long *et al.*, 2006; Ingvordsen *et al.*, 2015a). The above mentioned studies all
101 reported results from a maximum of four accessions, and crop responses to climate
102 change are almost exclusively reported from studies including a very limited number of
103 genotypes. In contrast the present study includes 22 accessions representing a diverse
104 genetic origin and thereby widening our knowledge on genotypic effects in response to
105 eTemp and eCO₂.

106 Temperature stress caused by exposure to constantly increased eTmp affects cereal
107 yield differently than exposure to an extreme temperature event like a heatwave. The

negative effect of a heatwave on grain yield is mainly determined by the timing in relation to the cereal development stage, with the most susceptible stage being around flowering (Barnabas *et al.*, 2008, Barber *et al.*, 2017). In turn, the physiological response mechanisms of individual cultivars vary and are associated with their final yield (Stone and Nicolas 1994; Hakala *et al.*, 2012). Under field conditions, the differences observed in development between the accessions together with time of sowing for each accession would have influenced at which development stage the heatwave would have had its effect. Sufficient variability in cultivar earliness/lateness, cultivation of mixed cultivars and agricultural management can enable partial escape from the deleterious effects of heatwaves (Tewolde *et al.*, 2006).

Few studies have so far investigated the effect on crop production caused by heatwaves superimposed to projected future levels of temperature and/or CO₂. One study applied a 15-day heatwave of maximum 35°C, 8 hours a day during grain filling on three wheat cultivars under simultaneous exposure to eCO₂ (750ppm; Bencze *et al.*, 2004). However, none have, to our knowledge, applied a heatwave under the realistic future climate scenario of eTmp and eCO₂ in combination and assessed a large number of genotypes.

In the present study a 10-day heatwave of 33/28°C (day/night) was induced around the time of flowering to 22 spring barley accessions. The heatwave was timed around flowering, which is known to be the most critical developmental phase of barley yield determination at high latitudes (Peltonen-Sainio *et al.*, 2011). The heatwave was superimposed to projected future levels of temperature and CO₂ as single factors and combined, conditions close to IPCC's worst case scenario for the end of this century (~RCP8.5; Collins *et al.*, 2013). We ask if heat waves will be more or less devastating

132 when superimposed on future growth conditions with eTmp and eCO₂ considering grain
133 yield, biomass, calculated harvest index (HI) and stability of grain yield.

134

135

136 **2. Material and Methods**

137

138 *2.1 Plant material*

139 Based on their performance, 22 barley (*Hordeum vulgare* L.) accessions were selected
140 from a previous study on production under eTmp, eCO₂, and eCO₂ combined with
141 eTmp (Ingvordsen *et al.*, 2015a). The accessions represent both high and low yielding
142 lines and include landraces, old (1924-1962) and new (1978-2010) cultivars. Details on
143 the 22 accessions can be found in Table 1. The accessions were supplied by NordGen
144 (the Nordic Genetic Resource Center; <http://www.nordgen.org/>) and Nordic breeding
145 companies.

146

147 *2.2 Growing conditions*

148 The accessions were cultivated in the RERAF (Risø Experimental Risk Assessment
149 Facility) phytotron at the Technical University of Denmark, Campus Risø, Roskilde.
150 RERAF has the advantage of six identical 24 m² (6 m × 4 m × 3 m) gastight chambers
151 individually programmed and with continuous measurements of the experimental
152 conditions. The light regime mimicked the long days of southern Scandinavia (May-
153 July) with 16 hours of light and 8 hours of dark. Lamps were controlled to imitate

154 sunrise and sunset (one hour each) in the beginning and end of the light regime. Light
155 intensity was in PAR (photosynthetically active radiation) averaged at approximately
156 400 mol photons m⁻² s⁻¹ at canopy height. Each accession was grown in 11 L pots filled
157 with 4 kg of sphagnum substrate (Pindstrup Substrate No. 6, Pindstrup Mosebrug A/S,
158 Denmark) supplemented with 10 g NPK fertilizer (21-3-10, Yara) at sowing. Per
159 accessions twelve seeds were sown in each of two pots, and at the seedling stage
160 thinned to eight experimental plants per pot. The pots were placed on wheeled growing
161 tables; one pot remained in the basic treatment the other was moved to the heatwave
162 treatment at Zadoks growth stage 49 (first awns visible; ZS, Zadoks *et al.*, 1974; Fig. 1).
163 Throughout the experiment 4.4 L m⁻² day⁻¹ of water was applied in all treatments at the
164 beginning of the daytime regime by an automated surface dripping system. At the early
165 stages of growth (seedling stage), excess water was drained from the pots. Watering was
166 reduced stepwise, when plants in a given treatment started ripening, ZS 90, and
167 watering was ended at maturity, ZS 99. The growth stage was determined in the control
168 treatments. The amount of water supplied was sufficient to avoid water limitation
169 during growth under ambient conditions. Relative humidity was constant at 55/70 %
170 (day/night) for all treatments. At growth day 27, about ZS 15-17, all plants were treated
171 prophylactically against aphids with Confidor WG 70 (Bayer A/S). To avoid any
172 unintended chamber specific effects the treatments were rotated between chambers once
173 a week. When chamber rotations took place, conditions in all chambers were set to
174 ambient, and the batches of plants were moved to their new chamber, and the
175 corresponding treatment applied again. The chamber rotation was accomplished within
176 one hour (all inclusive). Concurrent with the chamber rotation all tables were rotated
177 within the treatments according to a scheme, so that any table/pot received a new
178 position in the chamber (e.g. pots facing the outer rim were moved to the center etc.).

179 Further, the positions of the accessions were identical between treatments. The rotation
180 between chambers was ended 68 days after sowing to avoid plant damage, when
181 moving the wheeled tables through the chamber doors.

182

183 2.3 Treatments

184 The barley accessions were cultivated under four climatic scenarios referred to as basic
185 treatments. The basic treatments were: 1) ambient (amb), mimicking south
186 Scandinavian summer conditions with 19/12°C (day/night) and CO₂ concentration at
187 400ppm; 2) constantly elevated temperature (eTmp) with +5°C day/night; 3) constantly
188 elevated CO₂ (eCO₂) with +300ppm; 4) combined constantly elevated temperature and
189 CO₂ (eTmp&eCO₂). The eTmp and eCO₂ were set close to those projected by IPPC for
190 the Nordic region at the end of the 21st century (Collins *et al.*, 2013).

191 The heatwave treatments were superimposed to the basic treatments (then designated
192 amb+H, eTem+H, eCO₂+H, eTmp&eCO₂+H) as constant hot temperature 33/28°C
193 (day/night) for 10 days and applied individually to the accessions around anthesis.

194 When half of the plants of a given accession had reached ZS 49 (first awns visible), one
195 of the pots was moved to the heatwave treatment. The CO₂ concentration in the
196 heatwave treatment followed the climatic scenarios and was 400ppm for amb+H and
197 eTem+H and 700ppm for eCO₂+H, eTmp&eCO₂+H. Throughout the heatwave
198 treatment, watering was applied in a volume of 4.4 L m⁻² day⁻¹ as in the basic
199 treatments. After the heatwave treatment the pot was transferred back to its original
200 basic treatment. The experimental setup is shown in Fig. 1.

201

202 2.4 Data collection and analyses

203 Plants were harvested individually and dried for a minimum of three weeks (20°C,
204 continuous high air flow, 55% relative humidity). After threshing, grain yield (g plant⁻¹)
205 and aboveground vegetative biomass (g plant⁻¹) were sized. Harvest index (HI; grain
206 yield proportional to total aboveground biomass, %) was calculated from grain yield
207 and aboveground biomass. Stability measures over the eight treatments were calculated
208 by the static environmental variance (S^2 ; Roemer, 1917) and the dynamic Wricke's
209 ecovalence (W^2 ; Wricke, 1962) according to

$$210 \quad S^2_i = \sum (R_{ij} - m_i)^2 / (e - 1) \quad [1]$$

$$211 \quad W^2_i = \sum (R_{ij} - m_i - m_j + m)^2 \quad [2]$$

212 where R_{ij} is the observed yield of the accession i in the treatment j , m_i is mean yield of
213 the accession across treatments, e is number of environments, m is the average of all m_i
214 termed the grand mean. To the raw data a mixed effects model with randomized
215 accession and pot effects was applied to verify treatment effects, of eTmp, eCO₂, H, and
216 their interactions. Random interactions between accession and treatments eTmp, eCO₂
217 and H were investigated and rendered insignificant prior to analysis of treatment effects.
218 To investigate potential impact of the individual accessions, a derived mixed effects
219 model with fixed effects of accessions instead of random was constructed, including
220 fixed effects of accessions and interaction effects between accessions and the eTmp,
221 eCO₂ and H treatments. The pot effect was initially investigated through Maximum
222 Likelihood estimation, which considered potential within-pot-competition effects. After
223 establishing a positive within-pot correlation, the analysis was carried out with standard
224 software for mixed effects models. This model formula and parameter estimates are
225 described in detail in the Supplementary material. Furthermore, accessions were

grouped into landraces, old and modern cultivars, and the mixed effects model with random pot effect and accessions replaced with the grouping was analyzed. A similar model was applied to above ground vegetative biomass, and analyzed. Presented parameters (see supplementary material) were constructed with the REML procedure. Estimates, confidence intervals and p-values in for ratios Table 2 and ratio comparisons for relative heatwave effects were constructed through simulation. Significance levels are $p < 0.001$:***; $p < 0.01$:**; $p < 0.05$:*. All modelling and correlation analysis was carried out using the software package R, version 3.2.3 (R Core Team, 2015).

3. Results

3.1 Experimental levels of temperature, CO₂ and humidity

The levels of temperature, CO₂ and humidity applied in RERAF throughout plant cultivation were according to set points (Supplementary Table S1), except for the ambient CO₂ treatment. Here the averaged value reached 452.71ppm (± 33.53) around 50ppm higher than set value. The RERAF facility lacks the mechanism to absorb CO₂, hence plant and especially soil respiration have potentially caused the higher values. The hypothesis on respiration being the responsible factor was supported by the overshoots of the set-point value being most prominent within the first hours of the day and during the night regime (data not shown).

248 3.2 Effects of the basic climate treatments

249 Comparing the elevated CO₂ and temperature treatments with ambient conditions
250 (Table 2), the effect of eCO₂ was found to increase overall grain yield by about 26%,
251 while eTmp decreased overall grain yield by 43%. When the single factors were
252 combined in eTmp&eCO₂ treatment, grain yield decreased by about 18%. Interestingly,
253 the effect of the combined factors seemed to be additive with a non-significant
254 interaction ($p=0.25$). Total aboveground vegetative biomass was also increased (35%)
255 by eCO₂, whereas eTmp caused a moderate decrease of almost 5%. In the two-factor
256 eTmp&eCO₂ treatment the aboveground vegetative biomass increased 20%. The effects
257 of eCO₂ and eTmp were not additive for aboveground vegetative biomass ($p=0.03$);
258 thus, the effect of the two individual factors combined could not be predicted from the
259 effect of the individual factors. The treatment effects on grain yield and aboveground
260 vegetative biomass were reflected in the Harvest Index (HI), which was significantly
261 reduced in the two treatments with eTmp, while eCO₂ had no effect on HI. Hence,
262 increase in grain yield and aboveground vegetative biomass was proportionally similar
263 to the that in the amb treatment. The modelled production parameter estimates for the
264 accessions are presented in Table 2. The basic treatments resemble the set up in our
265 previous study that also included the same accessions (Ingvordsen *et al.*, 2015a), and
266 Pearsons correlation coefficient on grain yield between the previous and present
267 experiments was high (74-82 %, $p<0.001$).

268 The accessions reached the transfer-stage (ZS 49) earliest in the two-factor
269 eTmp&eCO₂ treatment, on average 45.5 days after sowing, 5 days earlier than in the
270 ambient treatment (Supplementary Fig. S1).

271

272 3.3 Effects of the heatwave

273 All 22 accessions survived the 10-day heatwave applied around anthesis (due to faulty
274 watering in the eTmp+H treatment of cultivar Drost P., data for this accession and
275 treatment were excluded from the measured results). From the modelled estimates in
276 Table 2, average grain yield was found to decrease 52%, when comparing the future
277 eTmp&eCO₂+H scenario with the basic amb scenario, indicating severe production loss
278 under future conditions ($p<0.0001$). The relative production loss caused by the
279 heatwave is not significantly different in the future and ambient scenario ($p=0.71$). The
280 relative effect of the heatwave was independent of the basic treatment in all scenarios,
281 with $p>0.13$. On the basis of the similar relative decrease caused by the superimposed
282 heatwave, we estimated the relative effect on overall grain yield as a 39% decrease.
283 Given the similar relative effect of the superimposed heatwave, the highest grain yield
284 was found in the treatment of eCO₂+H at 5.23g plant⁻¹, pooling all accessions (Table 2).
285 The production of aboveground vegetative biomass was in all basic treatments increased
286 by heatwave exposure, however not significantly for eTmp (Table 2). As the heatwave
287 decreased yield and increased aboveground vegetative biomass in all basic treatments,
288 HI was decreased accordingly suggesting change in allocation from grain to vegetative
289 biomass (Table 2).

290

291 3.4 Accession specific effects

292 The days for the individual accession to reach the transfer-state at ZS 49 was influenced
293 by the basic treatments. The first accession to reach ZS 49 and being transferred to the
294 heatwave was the old Swedish cultivar ‘Mari’ grown under eTmp, and the last
295 accession was the landrace ‘Griechische’, transferred 35 days later, also grown under

296 eTmp (Supplementary Fig. S1). The modern Danish cultivar ‘Sebastian’ and the
297 landraces ‘Solenbyg’ and ‘Grenoble I’ spanned only 2-5 days in reaching ZS 49 over all
298 four of the basic treatments, demonstrating stable rate of development. Early accessions,
299 reaching the transfer-state first under all basic treatments, were two modern Norwegian
300 cultivars, ‘Arve’ and ‘Brage’, two old Swedish cultivars, ‘Brio’ and ‘Mari’ and the
301 landraces ‘Bjørne’ and ‘Kushteki’ (Supplementary Fig. S1).

302 The analysis showed that in our experimental setup heatwave effects were not
303 significantly dependent on the barley accession ($p=0.08$). However, the negative grain
304 yield effects by the heatwave treatments varied among the 22 accessions and ranged
305 from 32% to 54% reduction in the amb+H and from 55% to 72% reduction in the
306 eTmp&eCO₂+H treatments (Fig. 2). Some accessions like the landraces, Königsberg
307 and Vilm, and the Danish modern cultivar Alliot seemed to be substantially more
308 affected by the eTmp&eCO₂+H treatment than the amb+H treatment. In contrast, the
309 old Swedish cultivar Mari and the modern Danish cultivar Anakin showed stronger
310 decrease in grain yield under the amb+H treatment than under the eTmp&eCO₂+H
311 treatment, showing potential to resist heatwaves under future climate conditions. As
312 expected, the analysis revealed that the group of modern cultivars yielded significantly
313 higher than the group of landraces ($p<0.0001$), but they did not produce more
314 aboveground vegetative biomass compared to the landraces.

315 The different responses in grain yield of the set of accessions over the eight applied
316 treatments revealed a static environmental variance, S^2 , ranging from 1.80 to 9.35,
317 where lower values indicate static stability to environmental changes (Table 1).
318 According to S^2 , the landrace ‘Oslo’ and the French cultivar ‘Prestige’ were identified
319 as most stable over all applied treatments. In addition, ‘Prestige’ holds the seventh
320 highest mean grain yield across treatments, whereas ‘Oslo’ ranked 20th in mean yield.

321 Wricke's ecovalence, W^2 , for grain yield ranged from 2.37 to 23.32 over the eight
322 treatments, where a high ecovalence indicates larger fluctuation across the treatments
323 compared to the other accessions. The modern Danish cultivar 'Evergreen' had the
324 second highest mean grain yield over the eight treatments, however, a considerable
325 decrease in grain yield under eTmp most likely caused 'Evergreen' to be identified as an
326 accession responding differently from the majority, and ranking only 20th out of the 22
327 for W^2 . Low values of W^2 were presented by the landraces 'Kushteki' and 'Moscou' and
328 the cultivar 'Arve' released in Norway.

329 Calculating S^2 and W^2 separately over the four basic treatments and the four +H
330 treatments showed that the accessions were either stable over the basic treatments or
331 over the +H treatments (Supplementary Fig. S2). The only exceptions were the landrace
332 'Oslo' and the Danish cultivar 'Alf', as they showed stable grain yield according to S^2
333 under both the four basic treatments and the four heatwave treatments. However, both
334 accessions were among the bottom three accessions for mean grain yield. No correlation
335 between grain yield in the treatments with and without heatwave could be found for
336 either S^2 ($p=0.16$) or W^2 ($p=0.08$). The lack of correlation between treatments with and
337 without heatwave suggests that the underlying mechanisms causing stability to a
338 heatwave and to eTmp and eCO₂ are not the same.

339

340

341 **4. Discussion**

342 *4.1 Response to the single-factor basic treatments*

343 Our results support the generally observed trend of decreased grain yield in response to
344 eTmp and increased grain yield under eCO₂ (Morison and Lawlor, 1999; Bokszczanin
345 and Fragkostefanakis, 2013) with the observed 26% increased grain yield by eCO₂ and
346 the 43% decreased grain yield by eTmp for the 22 barley accessions in this study.
347 However, the magnitude of the observed trend varies between studies. From a FACE
348 experiment Manderscheid *et al.* (2009) observed eCO₂ at 550ppm to increase grain
349 yield by 9-18% for the barley accession ‘Theresa’. A 2°C temperature increase in the
350 soil (4 cm depth) under field conditions caused a 4% yield decrease in the barley
351 accession ‘Quench’ (Högy *et al.*, 2013). In a phytotron study on four barley accessions
352 (‘Gl. Dansk’, ‘Lazuli’, ‘Anakin’ and ‘Barke’) overall grain yield was increased by 57%
353 when exposed to eCO₂ at 700ppm, whilst grain yield was decreased by 27% under +5°C
354 eTmp (Clausen *et al.*, 2011). The differences may result from variation in several
355 factors such as crop species, level, timing and length of exposure to eTmp and eCO₂.

356

357 4.2 Response to the combined-factor basic treatment

358 Only few experimental studies have addressed effects of combined eTmp and eCO₂
359 treatments on grain yield and biomass. In rice, the combination of eTmp and eCO₂ at
360 levels predicted for the end of the 20th century (+4°C and +200 to 300ppm CO₂) has
361 been reported to affect grain yield negatively (Ziska *et al.*, 1997). A modelling study of
362 rice, wheat and soybean also reported decreased grain yield from the combined eTmp
363 and eCO₂ (Long *et al.*, 2006). Our results on barley support these earlier findings from
364 other crops, which bodes ominously for the production in a future climate, where CO₂
365 and temperature will increase concerted. For grain yield we found that the effect of
366 these two individual factors was additive and this additivity could predict the response

367 in their combined treatment. This finding is in contrast to previous findings in a larger
368 dataset including 138 barley accessions (Ingvordsen *et al.*, 2015a). However, interaction
369 between factors might still be present, but significance may not be detected due to the
370 smaller set of 22 accessions. In contrast, for vegetative biomass the effect of the
371 individual factors was not additive, when compared to the combined treatment, as also
372 found by Ingvordsen *et al.* (2015a). The incongruence in identified additive effect of
373 single factor treatments between the two studies can also reflect the variation in
374 genotypic responses in the two sets of accessions. In any case, it is crucial to screen
375 diverse sets of numerous cultivars to help fill the knowledge gap on genotype effect,
376 information of value for breeding and in modelling studies.

377

378 *4.3 Level, timing and frequency of the superimposed heatwave*

379 A heatwave is an extreme event where timing, intensity and length are difficult to
380 predict and therefore it is challenging to include such extreme events in the forecast for
381 the cropping season and in choice of cultivar (Ben-Ari *et al.*, 2016). In Southern
382 Scandinavia (Denmark) a heatwave is today defined as when average of maximum
383 temperatures registered over three consecutive days exceed 28°C (DMI, 2014). No
384 information could be found describing most likely timing, temperature regime and
385 length of future heatwaves in this northern region. In southern Europe, model prediction
386 of future heatwaves forecasts that by the end of this century heatwaves will occur with a
387 frequency between 1.7 and 2.3 times per growing season, last between 11 and 17 days,
388 and be up to 3°C warmer than previous heatwaves (Meehl & Tabaldi, 2004). Hence the
389 applied heatwave in the present study is +5°C warmer and 7 days longer than the
390 current Nordic definition, and likely represents a realistic future extreme temperature

391 event. The timing with the heatwave striking at ZS49 (first awns visible) targeted the
392 sensitive stages of pollen development, anthesis and ovule formation (Sakata *et al.*,
393 2000; Hakala *et al.*, 2012; Gourdji *et al.*, 2013) and had increased effect on yield. A
394 study in maize, identified 13% decreased yield under a 3-day heatwave of +6°C applied
395 at early reproductive stage (silking), but no decrease in yield when applied at early
396 vegetative stage (Siebers *et al.*, 2017). Heat tolerance around flowering has been
397 identified as a key trait to improve the European primary production of wheat in the
398 future climate (Stratonovitch and Semenov, 2015).

399

400 *4.4 Response in grain yield to the superimposed heatwave*

401 The heatwave superimposed in the most realistic future climate treatment, eTmp&eCO₂,
402 halved the grain yield (52%) when compared to production in the climate of today
403 (amb). Halving the eatable harvest is a considerable impact potentially leading to
404 insufficient food supply. Interestingly, the results showed consistent relative decrease in
405 grain yield on all basic treatments, in response to heatwave exposure. With the
406 consistent relative heatwave response in all four basic treatments, the differences found
407 in final grain yield and aboveground biomass after superimposed heatwave were caused
408 by the basic treatments, i.e. the already present increases in temperature and CO₂. One
409 can speculate if the superimposed heatwave was so severe that it caused physiological
410 processes to operate at an absolute minimum during its duration, and therefore affected
411 all basic treatments relatively uniformly. Counter to that speculation Fitzgerald *et al.*,
412 (2016) identified eCO₂ to ameliorate the effect of an 8-day naturally occurring heatwave
413 in a semi-arid environment with temperature >35°C (FACE experimental setup). The
414 eCO₂ increased the yield by 17-40% during the heatwave in a modern and an old wheat

415 cultivar respectively. However, due to the heatwave occurring naturally, there are no
416 data for the yield at ambient temperature. A study by Bencze *et al.* (2004)
417 superimposing a less extreme heatwave, supports our suggestion that the final grain
418 yield is determined by the basic treatment. Bencze *et al.* (2004) cultivated three wheat
419 cultivars at 375ppm and 750ppm CO₂ and superimposed a heat event consisting of a
420 temperature increase 8 hours a day for 15 days around grain filling at maximum 35°C in
421 contrast to ambient temperature at maximum 24°C. The highest grain yield post
422 heatwave exposure was identified in the accessions exposed to the high CO₂
423 concentration (Bencze *et al.*, 2004) as observed in our study (Table 2).

424

425 *4.5 Response in biomass to the superimposed heatwave*

426 The aboveground vegetative biomass was increased by the heatwave at all basic
427 treatments, whereas grain yield decreased, hence, the allocation between vegetative
428 biomass and grain changed, as was observed in the HI (Table 2). Considering the
429 disruptive effect the heatwave had on flowering, with less grain formed, it is likely that
430 grains were not filled making resources available for vegetative biomass. Allocation
431 from reproductive biomass to vegetative biomass was also identified by Batts *et al.*
432 (1998) in one of two wheat cultivars exposed to a temperature gradient. As plants from
433 the basic eTemp treatment came from an environment with elevated temperature before
434 heatwave exposure, plants from the amb and eCO₂ treatments experienced the highest
435 temperature increases of +14°C (eTemp and eTemp&eCO₂ +9°C) when transferred to the
436 heatwave, and also showed the largest gain in vegetative biomass suggesting
437 temperature to be the dominant factor for the shift in allocation. Numerous plant
438 processes are involved in the allocation of resources to organs, and HI is a good

439 indicator for shifts in resource allocation. In addition, HI has been shown to have a high
440 heritability (Hay, 1995) and therefore, grain production under future climate conditions
441 could benefit from identification of genotypes with stable and high HI in a variable
442 climate. With an apparent future increase in aboveground vegetative biomass, its
443 functionality should be explored (Ghaly and Alkoaik, 2010).

444

445 *4.6 Genetic resources for future resilient cultivars*

446 Our analysis of 22 spring barley accessions showed that heatwave effects did not appear
447 to be significantly dependent on accession. One can only speculate if an accession
448 specific response would have shown if the applied heatwave had been less extreme, or if
449 other accessions e.g. accessions adapted to warm drought prone environments had been
450 included.

451 The recorded complexity in the response patterns to applied treatments (Fig. 2) will not
452 simplify the task for plant breeders. Various studies have represented CO₂-
453 responsiveness as a potential breeding target that has not previously been exploited
454 (Manderscheid and Weigel, 1997; Ziska *et al.*, 2004; Franzaring *et al.*, 2013). In the
455 present study, the heatwave response was independent of the basic treatment, and
456 consequently the higher grain yield from eCO₂ continuously lead to the highest grain
457 yield being produced under eCO₂+H suggesting that CO₂-responsiveness is beneficial in
458 a future climate with higher occurrence of heatwaves. Correlating CO₂-responsiveness
459 (increased grain yield under eCO₂) with yield in the eTmp&eCO₂+H scenario in the
460 present study was though non-significant.

461 The optimal climate resilient cultivar is high yielding in environments with climate
462 stresses over its life cycle. Yet, among accessions in the present study, stability and high

463 grain yield were rarely characteristics of the same accession. Stability over treatments
464 were combined with low yield in the eTmp&eCO₂+H treatment, and poor stability
465 combined with high yield in the eTmp&eCO₂+H treatment. Accessions that were stable
466 and/or high yielding in the eTmp&eCO₂+H treatment are potential candidates in
467 breeding programs aiming at diminishing climate caused losses in production,
468 especially if the desired traits can be easily exploited using marker assisted selection
469 (Ingvordsen *et al.*, 2015b).

470

471 **5. Conclusion**

472 Translation of findings from studies of environmental effects under artificial conditions
473 to actual field conditions needs to be carefully considered. Environmental factors like
474 light, water, nutrients and temperature conditions are much more variable under field
475 conditions and plants are exposed to a verity of beneficial and pathogenic
476 microorganism and will possible influence the magnitude of the findings. However,
477 manipulating temperature and mimicking heatwaves under field conditions is only
478 partly possible so far (Kimball et al., 2007; Bruhn et al., 2013). Therefore results from
479 studies using controlled growth conditions are sometime the best option to gain
480 knowledge about future climate change related effect on crop production, and relative
481 differences between temperature associated treatments. .

482 Under future climate conditions decreased summer precipitation is projected for
483 southern Scandinavia (IPCC, 2007). In the present study water allocation was identical
484 in all treatments and pots. However, in the treatments of eTmp and under heatwave
485 conditions increased vapor pressure deficit has despite equal relative humidity changed
486 evapotranspiration conditions from those of the ambient treatment. Therefore, reported

487 effects of the treatments with eTmp can be concerted responses to heat and water
488 availability. From today's climate to a climate with a 10-day heatwave superimposed to
489 IPCC's worst case scenario around year 2100, we measured an immense 52% decrease
490 in grain yield over all 22 accessions. Modern accessions were highest yielding and
491 effects of the superimposed heatwave did not depend significantly on accession. With a
492 similar relative yield decrease from the superimposed heatwave in the four basic
493 treatments, final grain yield was predominantly determined by the basic treatments.

494 The 52% decrease in grain yield strongly emphasize that future temperature extremes
495 exert a great threat to crop production systems and stress the need to continuously
496 identify genotypic variation for breeding future climate resilient cultivars. Together with
497 new better cultivars also diversified cropping systems and management should be
498 prioritized to feed the future world population.

499

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511

512

513 **Appendix A. Supplementary data**

514 Supplementary data associated with this article can be found, in the

515 online version, at xxxx

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Table 1. The tested barley accessions with mean yield and stability across all treatments; ambient, elevated temperature, elevated CO₂, elevated temperature and elevated CO₂ in combination and all four treatments +/-heatwave. Modern cultivar (mCV), old cultivar (oCV), landrace (LR), genebank number (NGB), environmental variance (S^2) and Wricke's ecovalence (W^2). The accessions are sorted after the mean grain yield across the eight treatments (m_i), and numbers in brackets are the ranking based on their stability indices. *Values for Drost P. based on seven of the eight treatments (eTemp+H excluded due to faulty watering).

Accessions name	NGB / Breeder	Culton type	Sub type	Country of origin / Country of breeding	Year of release	m_i	S^2	W^2
Bjørne	NGB9326	LR	6	unknown		6.25	6.19(18)	5.23(6)
Evergreen	Nordic Seed A/S Plant Breeding	mCV	2	Denmark	2010	6.19	9.35(22)	17.46(21)
Brio	NGB9327	oCV	6	Sweden	1924	6.13	6.04(16)	5.93(9)
Brage	Graminor Plant Breeding	mCV	6	Norway	2010	5.97	5.15(8)	5.89(8)
Anakin	Sejet Plant Breeding I/S	mCV	2	Denmark	2006	5.91	5.83(12)	5.09(5)
Solenbyg	NGB13402	LR	6	Norway		5.90	7.38(19)	8.41(14)
Prestige	NGB16750	mCV	2	France	2000	5.88	2.68(2)	9.00(15)
Kushteki	NGB6288	LR	6	Afghanistan		5.87	5.48(10)	2.37(1)
Moscou	NGB9482	LR	2	unknown		5.83	5.93(14)	4.00(3)
Drost P.*	NGB6281	oCV	2	Denmark	1951	5.81	4.90(7)	7.30(13)
Alliot	NGB16757	mCV	2	Denmark	1999	5.73	4.42(6)	15.03(19)
Sebastian	Sejet Plant Breeding I/S	mCV	2	Denmark	2002	5.72	3.89(4)	5.98(10)
Griechische	NGB9333	LR	6	Greece		5.56	5.57(11)	4.11(4)
Arve	NGB11311	mCV	6	Norway	1990	5.36	5.90(13)	3.72(2)
Grenoble I	NGB9378	LR	6	France		4.63	8.67(21)	23.32(22)
Edvin	Boreal Plant Breeding	mCV	6	Finland	2008	4.54	7.95(20)	11.76(17)
Vilm	NGB9435	LR	2	Germany		4.21	5.98(15)	5.34(7)
Anita	NGB15250	oCV	6	Norway	1962	4.20	6.15(17)	7.27(12)
Mari	NGB1491	oCV	2	Sweden	1960	4.12	5.36(9)	12.03(18)

Oslo	NGB9315	LR	6	Norway	4.09	1.80(1)	10.25(16)
Königsberg	NGB9310	LR	6	unknown	3.65	3.12(3)	16.71(20)
Alf	NGB4707	mCV	2	Denmark	1978	3.41	4.20(5)
							6.32(11)

Table 2. Model estimates for grain yield, aboveground vegetative biomass and harvest index (HI) for the 22 barley accessions with 95%

standard confidence intervals, when cultivated under ambient (amb), elevated levels of temperature (eTmp) and CO₂ (eCO₂) as single factors or in combination (eTmp&eCO₂) and with (+H) and without a 10-day heatwave around anthesis. The *p*-values indicate the difference to the production parameter under ambient conditions (amb). ****p* < 0.001; ***p* < 0.01; **p* < 0.05. Difference in % is relative to ambient conditions (amb).

Treatment	Grain yield (g plant ⁻¹)	Difference in %	Aboveground vegetative biomass (g plant ⁻¹)	Difference in %	HI (%)	Difference in %
amb	7.16±0.51	-	8.33±0.86	-	45.31±2.51	-
eTmp	4.06±0.51 ***	-43.3 (±5.6)	7.94±0.86	-4.6 (±7.7)	35.76±2.53 ***	-21.1 (±4.9)
eCO ₂	9.00±0.51 ***	25.8 (±7.3)	11.21±0.86 ***	34.7 (±9.,9)	45.31±2.51	0
eTmp&eCO ₂	5.90±0.51 ***	-17.5 (±8.1)	9.99±0.86 ***	20.1 (±10.2)	35.75±2.51 ***	-21.1 (±4.9)
amb+H	4.51±0.51 ***	-37.0 (±6.7)	10.27±0.86 ***	23.4 (±9.1)	27.50±2.53 ***	-39.3 (±4.7)
eTmp+H	2.70±0.53 ***	-62.3 (±6.6)	8.11±0.86	-2.6 (±9.0)	23.66±2.53 ***	-47.8 (±4.7)
eCO ₂ +H	5.23±0.51 ***	-26.9 (±6.9)	14.33±0.86 ***	72.3 (±14.1)	27.50±2.53 ***	-39.3 (±4.7)
eTmp&eCO ₂ +H	3.42±0.51 ***	-52.2 (±6.5)	11.34±0.86 ***	36.3 (±9.9)	23.66±2.53 ***	-47.8 (±4.7)

Fig. 1. Schematic overview of the four climatic scenarios and the superimposed 10-day heatwave treatment, illustrated for one accession. For the basic treatments it was one treatment per chamber and during heatwave treatments, two basic treatments in each of the heatwave chambers. amb: ambient conditions, eCO₂: elevated CO₂, eTmp: elevated temperature, eTmp&eCO₂: elevated temperature and elevated CO₂ in combination, +H: heatwave superimposed to basic treatment. For each accession one pot in the basic treatment was transferred to the heatwave treatment as indicated with arrows.

Fig. 2. Reduction in grain yield of 22 barley accessions after a 10-day heatwave superimposed around flowering to plants grown under climatic conditions corresponding to (a) ambient conditions (amb) or (b) futur senario with elevated temperature and CO₂ (eTmp&eCO₂).

Supplemental

Table A.1. Set point and experimental levels of temperature, atmospheric concentration of CO₂ ([CO₂]) and humidity $\pm$ standard deviations in the six chambers; four chambers mimicking basic treatments and two chambers mimicking basic treatments with a superimposed heatwave (+H).

	<u>Temperature (day/night)</u>		<u>[CO₂] (constant)</u>		<u>Humidity (day/night)</u>	
	<u>Set point</u>	<u>Experimental</u>	<u>Set point</u>	<u>Experimental</u>	<u>Set point</u>	<u>Experimental</u>
Ambient	19/12°C	18.36 $\pm$ 2.18/13.02 $\pm$ 2.48	400 ppm	451.54 $\pm$ 40.65	55/70	56.55 $\pm$ 4.53/67.43 $\pm$ 5.41
eTmp	24/17°C	22.97 $\pm$ 2.18/18.20 $\pm$ 2.45	400 ppm	459.72 $\pm$ 40.85	55/70	56.64 $\pm$ 4.61/67.25 $\pm$ 5.43
eCO ₂	19/12°C	18.22 $\pm$ 2.23/13.04 $\pm$ 2.45	700 ppm	700.27 $\pm$ 23.18	55/70	56.63 $\pm$ 4.65/67.51 $\pm$ 5.38
eTmp & eCO ₂	24/17°C	23.05 $\pm$ 2.24/18.08 $\pm$ 2.44	700 ppm	693.89 $\pm$ 23.76	55/70	56.66 $\pm$ 4.69/67.32 $\pm$ 5.42
+H	33/28°C	32.41 $\pm$ 2.63/28.01 $\pm$ 2.52	400 ppm	446.88 $\pm$ 19.09	55/70	56.47 $\pm$ 4.41/67.59 $\pm$ 5.24
+H & eCO ₂	33/28°C	32.42 $\pm$ 2.52/28.02 $\pm$ 2.50	700 ppm	703.14 $\pm$ 21.00	55/70	56.43 $\pm$ 4.55/67.55 $\pm$ 5.20

Fig. A.1. Number of growing days before transfer to the heatwave treatment. An accession was transferred when four of eight plants reached Zadoks growth stage 49. Ambient conditions: amb, elevated CO₂: eCO₂, elevated temperature: eTmp and elevated CO₂ and temperature in combination: eTmp & eCO₂.

Fig. A.2. Environmental variance (S_i^2), Wricke's ecovalence (W_i^2) and mean grain yield across either the four basic treatments (top) or four basic+Heatwave (+H) treatments (bottom). Pearson's correlation of S_i^2 between the basic and basic+H treatments was -0.335 with p-value at 0.135. Drost P. not included, as watering in the eTemp+H treatment was defective.

Fig. A.3. Change (%) in grain yield of the 22 spring barley accessions exposed to ambient climate treatment of 19/12°C (day/night) and 400ppm CO₂ and no heatwave exposure and the future climate scenario with elevated temperature (24/17°C) and elevated CO₂ (700ppm) in combination and a superimposed 10-day heatwave around flowering. In brackets is the rank (1: highest, 22: lowest) according to grain yield under basic ambient conditions.

Model description

The model used for generating estimates for Table 2 is of the form

$$Y_i = \alpha + \beta_T + \beta_{CO_2} + \beta_H + \beta_{T:CO_2} + \beta_{T:H} + \beta_{CO_2:H} + Z_{ACC|POT} + \varepsilon_i, \quad (1)$$

Where the response Y is one of Grain Yield, Biomass or Harvest Index. The treatment types elevated Temperature (T) and elevated CO₂ (CO₂) together with the superimposed Heatwave (H) enters into the parameters according to the treatment of the *i*'th observation and presence of heatwave or not. Z denotes randomized effects of Accessions and Pots, nested within Accessions. Absence of treatment refers to the ambient conditions. Thus, if observation *i* has been subject to elevated Temperature but no Heatwave and no elevated CO₂, the model for observation *i* is

$$Y_i = \alpha + \beta_T + Z_{ACC|POT} + \varepsilon_i,$$

While an observation *i* that has been subject to both elevated Temperature and a superimposed Heatwave, but not elevated CO₂, has the model

$$Y_i = \alpha + \beta_T + \beta_H + \beta_{T:H} + Z_{ACC|POT} + \varepsilon_i.$$

The estimated difference between these two scenarios is thus described by the parameter sum $\beta_H + \beta_{T:H}$.

Parameters estimates in the basic model (1) is listed in Table A.2. below for all three response types.

Table A.2. Parameter estimates in the random effects model (1), for responses Grain Yield (GrY), Biomass (BiM) and Harvest Index (HI) and SD for estimates.

	GrY			BiM			HI		
	estimate	sd	p	estimate	Sd	p	estimate	sd	p
α	7.16	0.26	<0.0001***	8.33	0.44	<0.0001***	45.31	1.28	<0.0001***
β_{CO_2}	1.84	0.23	<0.0001***	2.88	0.33	<0.0001***	-	-	NS
β_T	-3.10	0.23	<0.0001***	-0.39	0.33	0.25	-9.56	1.23	<0.0001***
β_H	-2.65	0.28	<0.0001***	1.93	0.33	<0.0001***	-17.81	1.24	<0.0001***
$\beta_{CO_2:T}$	-	-	NS	-0.83	0.38	0.03*	-	-	NS
$\beta_{CO_2:H}$	-1.12	0.32	0.0006***	1.18	0.38	0.002**	-	-	NS
$\beta_{T:H}$	1.29	0.32	0.0001***	-1.77	0.38	<0.0001***	5.72	1.75	0.001**