Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Characterization of a new dwarf watercress (Nasturtium officinale R Br.) 'Boldrewood' in commercial trials reveals a consistent increase in chemopreventive properties in a longer-grown crop

Voutsina Nikol
University of Southampton
Robert D. Hancock
The James Hutton Institute
Felipe Becerra-Sanchez
University of Southampton
Yufei Qian
University of California at Davis
Gail Taylor (gtaylor@ucdavis.edu)
University of California at Davis

Research Article

Keywords: watercress, Brassicaceae, germplasm collection, crop morphology - antioxidant capacity, chemoprevention, glucosinolates, commercial trial

Posted Date: October 3rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3386421/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

We describe ‘Boldrewood’, a new accession of watercress (*Nasturtium officinale* R. Br.) that was initially found to be of short stature with high antioxidant capacity (Payne, Clarkson, Rothwell, & Taylor, 2015). This was of particular commercial interest because it offered the potential to develop a novel watercress product with fork-friendly size and improved health-benefits. In two commercial trials comparing Boldrewood to a control, we confirmed that Boldrewood exhibits a dwarf phenotype with a significantly shorter stem and consistently produced more leaves per stem area alongside comparable crop biomass. The antioxidant and chemopreventive capacity of Boldrewood were comparable to the commercial crop. For the first time, we observed a novel increase in glucosinolate concentrations and cytotoxicity to cancer cells, characterised as decreased IC_{50} (half-maximal concentration of an inhibitor), associated with increased crop age at harvest. This suggests that a slower-growing and longer to harvest crop provides a significant improvement in health benefits gained in this leafy crop which is already known to be highly nutrient dense and with considerable chemopreventive ability.

Short informative

This report characterizes a new dwarf watercress (Nasturtium officinale, Brassicaceae) through commercial trials. The report focuses on key traits of crop morphology and high consumer health benefits, specifically antioxidant capacity, chemopreventive potency and glucosinolate content, and identifies previously-unreported temporal effects on the crop’s nutritional value.

Introduction

Watercress (*Nasturtium officinale* R. Br.) has a long history of medicinal and food crop use by humans, detailed by Manton (1935). It is produced on a large scale as a leafy green for salads and soups across the globe although exact market data is hard to find. In the U.K., watercress cultivation is inescapably linked with the chalk streams of the southern counties, particularly those of Hampshire, Dorset, Devon and Wiltshire. Typically, seedlings are produced in glasshouses or polytunnels and then transferred to purpose-built gravel beds (Natural England, 2009; Kaner-White, 2021). The crop is flood-irrigated for several weeks until it reaches the desirable size, at which point it is mechanically harvested and sent to market, most commonly as part of pre-washed and bagged salad products (Palaniswamy & McAvoy, 2001).

Watercress is a member of the *Brassicaceae* plant family, which includes a large range of economically-important edible plants, such as broccoli, cabbage, and mustard; oilseed rape, one of the largest oil crops; and the prominent research model species, *Arabidopsis thaliana* (L.) Heynh. Many of these *Brassicaceae* plants have significant markets or applications driving the development of extensive resources to facilitate the production of new and improved cultivars. For example, Beneforté® broccoli was recently developed and released to market as a more nutrient-dense cultivar, with culinary trials to support customer health (Traka et al., 2013; Costa-Pérez, et al., 2023). However, this is not the case with

watercress, which in the UK holds a modest 5% of the total salads market (personal communication, Dr Steve Rothwell). To date, limited resources exist to enable crop improvement in response to consumer demands or increased production pressures (Solankey et al.,2021).

Overall, efforts in crop breeding are driven by a variety of targets, such as increasing yield - for example, the development of modern high-yielding bread wheat cultivars (Slafer, Satorre, & Andrade, 1994) - or introducing cosmetic improvement to a crop to increase sales, as with the standardization of the orange colour in carrots (Simon, 2010). The University of Southampton watercress breeding program aims to improve the current watercress cultivar and discover new cultivars that might be successful in the market. Guided by the grower input, the focus has been set on three particular breeding targets: 1) above-ground morphology, 2) consumer health benefit, and 3) resource-use efficiency (Hibbert & Taylor, 2022). Here we are concerned with targets 1) and 2) – developing a novel watercress crop of improved morphology and health benefits. The first is focused on the development a leafier product with a shorter stem, more suited to bagged salads as opposed to the traditional product of bunched watercress. The second breeding target is to capture, preserve and potentially enhance the high phytonutrient profile in the commercial cultivar. Watercress is known to carry a considerable health benefit for the human consumer and was recently ranked as the most powerful fruit or vegetable to consume for health (Di Noia, 2014). Research has shown the crop to be linked with detoxification of carcinogens (Hecht et al., 1995); reduction of cancer risk (Gill et al., 2007); decreased damage by reactive oxygen species during strenuous exercise (Fogarty, Hughes, Burke, Brown, & Davison, 2013); and reduced inflammation (Sadeghi et al., 2013). In this report, we will focus on two key aspects of the phytonutrient profile of watercress: antioxidant (AO) capacity and the anti-cancer properties, quantified as glucosinolate (GLS) content and the cytotoxicity of the plants to cancer cells (IC_{50} – the concentration required to kill 50% of cells present in culture).

The AO profile of watercress is thought to be composed primarily of phenolic compounds, carotenoids and ascorbic acid (commonly Vitamin C), and several studies have looked at the concentrations and composition of the crop for these compounds in watercress (Martínez-Sánchez, Gil-Izquierdo, Gil, & Ferreres, 2008; Payne, Mazzer, Clarkson, & Taylor, 2013; Santos, Oliveira, Ibáñez, & Herrero, 2014; Qian et al., 2023). In a recent publication comparing AO capacity of a number of *Brassicaceae* leafy crops, watercress contained the highest concentrations in both AO and ascorbic acid content, highlighting the important role this crop can play in delivering human nutrition (Martínez-Sánchez et al., 2008). GLS are thought to be particularly beneficial to humans (Manchali, Chidambara Murthy, & Patil, 2012; Traka & Mithen, 2008) and are produced in response to invertebrate predation on watercress (Newman, Hanscom, & Kerfoot, 1992). Upon tissue damage to the plant, glucosinolates are hydrolysed by the enzyme myrosinase and one of the products of this reaction are isothiocyanates (Bones & Iversen, 1985; Bones & Rossiter, 1996). In watercress, primarily gluconasturtiin is converted to phenethyl isothiocyanate (PEITC) and the quality of watercress nutritional profile can be enhanced by a controlled environment using a hydroponic system (Newman et al., 1992; Qian et al., 2022). PEITC is not only considered to have potent cancer-fighting properties (Manchali et al., 2012; Traka & Mithen, 2008), but is also responsible for the highly-marketable peppery flavour of this leafy salad.

Despite the outstanding promise of modern agrobiotechnology, such as Next Generation Sequencing and genome editing, pre-existing genetic diversity in the natural world remains central for crop improvement and is the single largest, and arguably the most straightforward, resource to draw from in crop breeding (Dwivedi, Sahrawat, Upadhyaya, & Otriz, 2013). A germplasm collection, containing known variants and wild relatives or progenitors of a crop, holds the potential not only to discover pre-existing variants, but also the genetic information to begin understanding a crop and building the resources necessary to establish a breeding program. Therefore, for understudied watercress the establishment of a germplasm collection, beginning in 2009, by the Taylor Lab group was a major stepping stone (Payne et al., 2015). The collection contains approximately 50 accessions of seed and clonal material, collected globally by individuals from watercress growers and donated by Warwick HRI. The collection was assessed for variation in above-ground morphology and tissue AO capacity and showed promising variation across the 48 accessions (Payne et al., 2015). It has been utilized further to sequence the transcriptome of watercress and perform differential expression analysis for AO capacity and GLS content (Voutsina et al., 2016).

A particular accession, named ‘Boldrewood’, exhibited the top-ranking AO capacity of the collection (Payne 2011) leading to its selection for further comparisons. Boldrewood has also repeatedly produced a dwarf phenotype. The gluconasturtiin and PEITC content in Boldrewood were examined and although the concentration of gluconasturtiin was lower than that of the commercial cultivar, the concentration of PEITC was similar, leading the researchers to suggest a more efficient conversion in the Boldrewood material (Payne, 2011). Payne (2015) examined differences in gene expression between the two accessions and concluded that the Boldrewood watercress showed a reduction in gene expression of biochemical pathways critical to plant growth, specifically brassinosteroids and phenylpropanoid/lignin synthesis, which could explain its dwarf phenotype. In addition, Payne et al. (2015) noted an increase in expression of genes associated with plant defences, such as GLS and ascorbic acid biosynthesis, which could contribute to the consistent high phytonutrient profile identified.

The potential of this accession to address two of the breeding targets of the program have led to commercialization trials to test practical aspects of its cultivation and marketing and to confirm this phenotype in a commercial field setting, because the work detailed above was conducted in controlled laboratory conditions lacking natural variation or stressors encountered by a commercial crop. Thus, the aim of this research was to compare the performance for marketable traits of the Boldrewood accession to the current cultivar under commercial conditions.

Materials and Methods

The ‘Boldrewood’ accession was compared to an active commercial U.K. cultivar, referred to as ‘Control’ hereafter, across two consecutive summer growing seasons on commercial farms in Dorset, U.K. In both trials, the seed used had been fixed through several generations of selfing and the seedlings were produced through the standard commercial procedures, thus a consistent material quality can be assumed that is comparable to the usual commercial production for this particular grower. In addition,

both trials were grown to organic farming specifications for the commercial production of organic watercress.

Trial 1 - A commercial trial in 2015

Boldrewood was commercially tested during the U.K. growing season in 2015 on a commercial watercress farm located in Dorset, U.K. (50°44’24.7”N, 2°12’40.5”W). In late June, the following material was harvested from watercress beds that were close in proximity: 1) a Boldrewood at 28 d post-planting, 2) a Control at 28 d post-planting, and 3) a second Boldrewood at 42 d post-planting. The final group represents the size at which the commercial grower classified the Boldrewood plants as ‘ready to harvest’ and was, thus, comparable to the ‘ready to harvest’ Control crop at 28 d.

Five 30 cm by 30 cm quadrats were randomly sampled and photographed for Boldrewood and Control crops. Five stems were randomly taken from each quadrat, cut from the base, and measured immediately for stem length, stem diameter (in the middle of the stem), and number of leaves. Leaf number index was calculated as the ratio of leaves to stem. Within each quadrat replicates were averaged to produce a quadrat mean value for further analysis. As an assessment of yield, the entire biomass in each quadrat was collected, dried at 80° C to constant weight, and weighed.

Five foil pouches were filled with tissue, randomly from across the bed, and snap-frozen for the evaluation of AO capacity and GLS concentration. Tissue AO capacity was assessed using a ferric reducing ability of plasma (FRAP) assay modified for plant sap (Benzie & Strain, 1996; Payne et al., 2013). Quantitative GLS analysis was carried out on lyophilized and finely ground tissue as follows: Approximately 0.02 g of lyophilized material was rehydrated in 1ml solution of 75% methanol and 0.1% formic acid, then briefly vortexed, sonicated for 15 minutes and mixed for 10 min on a shaker. Samples were then centrifuged (21000 g, 5 min) and the supernatant transferred to High Performance Liquid Chromatography (HPLC) vials. Samples were placed in a random order and then sequentially injected (5 µl) onto a Phenomenex Luna 3 m C18 (2) 150 x 2 mm column. Solvents were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), flow rate was 190 µl/min and the gradient was 0 min, 5% B; 45 min, 5% B; 47 min, 75% B; 52 min, 75% B; 54min, 5% B; 60min, 5% B. GLS were identified by parent and fragment mass spectra and in the case of gluconasturtiin and glucobrassicin by coelution with commercial standards. GLS concentration was estimated using the peak area of the parent ion in the mass spectrometer compared to a standard curve of the same compound (for 4-Me glucobrassicin the standard curve of glucobrassicin was used to estimate concentration).

As the aim was to compare the groups for performance in particular marketable traits, a single-factor Analysis of Variance (ANOVA) was used to test group differences ($a \leq 0.05$), with ‘accession’ treated as a fixed factor. All data were examined for normality and homogeneity of variance. Where ANOVA assumptions were not met, specifically for the glucobrassicin and gluconasturtiin concentrations, a Welch’s Test and a pairwise comparison were used instead. Further comparisons were made using a

Tukeys's HSD test. Statistical analysis and graphs were completed in R, version 3.2.2 (R Development Core Team 2013).

Trial 2 – A commercial trial in 2016

Trial 2 was undertaken at a commercial watercress farm in Dorset U.K (50°48'46.8"N, 2°08'47.9"W). A watercress bed was cleared and dressed, according to standard commercial practice for organic watercress. Both Control and Boldrewood accessions were germinated and transplanted to the bed as week-old seedlings. A randomized plot design was used containing ten 4 m x 4 m plots of each accession and equal volumes of seedlings were transplanted to each plot by hand.

After 21 d of growth the farm manager deemed the Control accession was "ready to harvest" and the following measurements were taken: stem length, stem diameter, and number of leaves, dry weight (of a 30 cm x 30 cm quadrat), and tissue was snap-frozen in liquid nitrogen for AO and cancer cell cytotoxicity assays. The morphological measurements were taken in the same way as in Trial 1: 5 replicate plant stems were harvested from within plots, staying at least 50 cm within the plot boundary to avoid edge effects, and the mean was used for statistical comparisons. AO capacity was measured as in Trial 1. Additionally, the toxicity of the watercress sap to cancer cells was assessed by carrying out MTS cell proliferation assays (Cell Titer 96® Aqueous One Solution, Promega, U.K.) on MCF-7 breast cancer cells, similarly to Cavell et al. (2012), and an IC_{50} value was extrapolated from the fitted survival curves. To make this dataset relevant to the grower and because the two accessions vary in size, these measurements were repeated the following two weeks, at 28 and 35 d, to cover the entire period during which either one or the other accession were deemed as "ready to harvest" by the farm manager (Fig. 1).

Data were analysed using a two-factor Analysis of Variance (ANOVA), investigating differences in group means of fixed factors 'accession' and 'date', and their interaction. For most variables, the dataset was balanced and so they were analysed using Type I sum of squares (SS). The IC_{50} and AO capacity datasets had missing values and were analysed as a Type III SS. Normality and homogeneity of variances was checked and data was log transformed as necessary. Further comparisons were made using a Tukeys's HSD test. Statistical analysis and graphs were completed in R, version 3.2.2 (R Development Core Team 2013).

Results

Trial 1 in 2015

Table 1 shows the mean and standard error for agronomic traits collected in this study: stem length (cm), stem diameter (mm), number of leaves, leaf number index, and biomass (g). For stem length, the Control accession had longer stems ($p < 0.0001$) than both Boldrewood age groups; however stem diameter was not different. The number of leaves present was higher in the 42 d Boldrewood plants ($p = 0.0002$) but the 28 d plants of both accessions were not different to each other. Leaf number index was also found to be different between the accessions ($p = 0.0014$) with both Boldrewood age groups showing more leaves per

unit of stem than the Control. This mirrors the difference in stem length between the two accessions and suggests that the 'dwarf' Boldrewood plants are indeed producing a comparable amount of leaves on a smaller stem. However, biomass (Table 1) was taken as an indicator of crop yield and showed a significant difference between the 28 d Boldrewood and the other two treatments ($p < 0.0001$). This suggests that Boldrewood would require an additional 14 d of growth to produce the same biomass yield as the Control accession.

Table 1. The mean and standard error of the mean (n = 5) for morphological traits and biomass of Control and Boldrewood accessions at 28 and 42 d post-planting in Trial 1. Statistical significance is denoted as * $p \leq 0.05$, ** $p \leq 0.01$. *** $p \leq 0.001$, and **** $p \leq 0.0001$.

		28 d		*42 d*	
Trait		**Control**	**Boldrewood**	**Control**	**Boldrewood**
Stem length (cm)	****	9.32 (± 0.73)	2.88 (± 0.45)	N/A	3.85 (± 0.92)
Stem diameter (mm)	ns	1.35 (± 0.23)	1.27 (± 0.16)	N/A	1.47 (± 0.15)
No of leaves	***	13.94 (± 4.86)	15.28 (± 8.71)	N/A	28.84 (± 11.18)
Leaf index	**	1.48 (± 0.05)	6.02 (± 1.50)	N/A	7.64 (± 0.54)
Biomass (g)	****	14.03 (± 0.48)	7.23 (± 0.56)	N/A	15.71 (± 1.09)

For phytonutrient content and the key benefits to consumers, an AO assay was conducted and specific GLS concentrations were assessed using HPLC-MS. The AO potential of the Boldrewood accession, shown in Figure 2, decreased significantly from 28 to 42 d of growth ($p = 0.01$) but the Control accession was not different to either of the other groups. An opposing trend was noted for gluconasturtiin, the primary glucosinolate found in watercress (Figure 3), which increased for Boldrewood at 28 to 42 d of age ($p = 0.006$). In this case again, the gluconasturtiin concentrations of the Control accession did not vary from either Boldrewood age group. The concentrations of glucobrassicin were not statistically different, however the concentrations of 4-methoxy-glucobrassicin were higher in both ages of the Boldrewood accession to those measured in the Control accession ($p = 0.0016$).

In summary, during Trial 1 the Control plants had longer stems but Boldrewood plants produced more leaf per stem unit. Despite this, the Boldrewood biomass lagged approximately two weeks behind the Control. The accessions did not differ for the primary glucosinolate, gluconasturtiin, but Boldrewood samples contained more 4-methoxy-glucobrassicin.

Trial 2 in 2016

The agronomic traits assessed during this trial are presented in Figure 4. Stem length was consistently longer in Control plants (accession, $p < 0.0001$; date, $p < 0.0001$), confirming the results of Trial 1. Stem

diameter also tested for significant ANOVA factors (accession, $p = 0.001$; date, $p < 0.0001$) but post hoc testing showed that true differences were due to an increase in stem diameter in both accessions at 35 d. Therefore, the results remain consistent with Trial 1; the two accessions do not differ significantly in stem diameter. There were significant differences in both factors for the number of leaves (accession, $p = 0.03$; date, $p < 0.0001$), however after post hoc testing, the significant p values are explained by an increase in leaf number for both accessions from 21 to 28 d (see Figure 4). As in Trial 1, leaf number index was consistently higher for the Boldrewood plants (accession, $p < 0.0001$; date, $p < 0.0001$) and it universally decreased over time, as stem elongation was faster than new leaf formation and expansion. Crop productivity was compared again through an assessment of biomass set by each accession. Mean dry weight, shown in Figure 4, was significantly different for both factors accession ($p = 0.00026$) and date ($p < 0.0001$), but post hoc analysis revealed that differences were relevant to time and that the accessions yielded comparable biomass at each time point.

The phytonutrient quality of the crops was also tested. Sap was extracted and used in a serial dilution to treat cancer cells in order to estimate the concentration required to inhibit cancer cell growth by 50%, or IC_{50}. The results from this assay are shown in Figure 5, and although accession was not a significant factor, date was highly significant for this variable ($p = 0.00016$). The IC_{50} concentration decreased with time implying that the watercress extract increased in potency against cancer cell growth as the plants aged. The potent chemopreventive compound in watercress is phenethyl isothiocyanate (PEITC) which is derived from gluconasturtiin (Newman et al., 1992). As gluconasturtiin was found to increase with age in Trial 1, we can conclude that this result is consistent to Trial 1. For AO, the assay revealed no differences between accessions or date in Trial 2, with the mean AO capacity of the Control plants ranging from 464.2 to 547.1 mmol of Fe^{2+} equivalent per g fresh weight and that of Boldrewood plants from 508.8 to 592.4 mmol of Fe^{2+} equivalent per g fresh weight.

In summary for Trial 2, the Control accession maintained a longer stem than Boldrewood and Boldrewood plants again produced more leaves per stem unit (Figure 1). In contrast to Trial 1, biomass did not differ significantly between the two accessions suggesting a minimal loss in yield if replacing one accession with the other in production. An interesting increase in cytotoxic capacity against cancer cells with crop age was noted for a second time here likely linked to the GLS/isothiocyanate system. Table 2 includes key traits comparisons between both trials.

Table 2. Key result synthesis for marketable traits across both Trials. Results expressed in market-relevant wording and “Comparable” indicates that the two accessions were not found to be statistically different in the particular case.

	Trial 1	Trial 2
Stem length	Control: longer	Control: longer
Stem diameter	Comparable	Comparable
No leaves	Boldrewood: leafier	Comparable
Leaf index	Boldrewood: leafier per stem unit	Boldrewood: leafier per stem unit
Biomass	Boldrewood: 2-week lag to current market size	Comparable
Antioxidant capacity	Comparable	Comparable
Glucosinolates:		N/A
Gluconasturtiin	Comparable: Increasing with time	-
4-methoxy-glucobrassicin	Boldrewood: higher	-
IC50 cancer cell inhibition	N/A	Comparable: Increasing with time

Discussion

Our results confirm that the Boldrewood accession exhibits a dwarf phenotype in comparison with the commercial Control watercress. The Control used in this research is a watercress cultivar that is grown widely across the south of England by many commercial companies and which each company continues to produce yearly from selfed seed. Thus, Boldrewood represents a new source of novel germplasm with a distinct dwarf phenotype now observed over several years and in contrasting environments. Boldrewood plants produced a higher ratio of desirable leaves to a smaller ratio of stem, which is in line with the breeding targets set by this program. Thus, this new watercress has the more 'forkable' phenotype of particular interest for use in bagged salads.

For phytonutrients, Control and Boldrewood appeared to be mostly comparable in these trials. AO capacity did not vary between the accessions in either trial and this is in agreement with the previous comparisons (Payne et al., 2015). The indolic glucosinolate (GLS) 4-methoxy-glucobrassicin was significantly higher in Boldrewood than in Control watercress. This glucobrassicin derivative has been reported in watercress previously and is involved in plant defences (Kopsell, Barickman, Sams, & McElroy, 2007). In a quantification study of watercress GLS, gluconasturtiin concentrations were decisively higher (63% in aromatic form) compared to any other GLS (1% in aliphatic and 36% in indole forms) detected and should be the focus of such comparisons in watercress (Liang et al., 2018). This observation is in agreement with the results of Rose et al. (2000), where the derivative of gluconasturtiin, phenethyl isothiocyanate (PEITC) was found to be in concentrations three times higher than any other isothiocyanate. In human nutrition, both these GLS appear to play a role in cancer cell apoptosis and

prevention (Cartea & Velasco, 2008; Gupta et al., 2014) and are therefore important breeding targets for watercress. The results presented here suggest that Boldrewood shows no loss of gluconasturtiin compared to the Control and has higher amounts of indolic glucosinolates, suggesting that the health benefits to the consumer are at the very least preserved.

Perhaps our most intriguing finding is the observation that stage of crop maturity appears to have a significant effect on the phytonutritional profile. In Trial 1, AO capacity declined in the older Boldrewood samples, whereas gluconasturtiin concentrations increased. This increase in GLS was mirrored with a significant increase in the potency of the watercress as an inhibitor of cancer cell growth as the crop aged in Trial 2 – a novel finding which was unknown in watercress. Previously, studies have primarily linked AO capacity to polyphenolic compounds and ascorbic acid in watercress (Ninirola et al., 2014). AO capacity has shown variation in response to numerous factors. In watercress, AO capacity differed between spring and winter growing seasons (Ninirola et al., 2014) and contrasting growing systems (Qian et al., 2023) ; between parts of the plant and increased across 6 post-harvest days while refrigerated (Payne, 2011). GLS concentrations in watercress have been shown to increase in response to stressors/stimulants, specifically longer days, poor light quality, higher temperatures, soil nutrients and water availability (Schreiner et al., 2005; Engelen-Eigles, Holden, Cohen, & Gardner, 2006; Kopsell et al., 2007). Thus, we can postulate that variability in GLS in harvested leaves is commonplace and suggests these 'natural plant defecne traits' are not highly heritable and may help to explain the variability seen in this study.

A single study alone has measured change in growing watercress phytonutrients over time and charted a consistent increase in both ascorbic acid (an AO) and PEITC to a plateau at 40 days (Palaniswamy, McAvoy, Bible, & Stuart, 2003). This study however was limited to controlled conditions, which do not accurately reflect the commercial environment (Payne et al., 2015). The current study is the first to examine change in AO and chemopreventive capacity over time in the watercress crop within a commercial setting. As the variation in GLS concentrations in plant tissue seems particularly species-specific across Brassicaceae's examined (Booth, Walker, & Griffiths, 1991; Velasco, Cartea, Gonzalez, Vilar, & Ordas, 2007; Wentzell & Kliebenstein, 2008), this is an important finding for watercress. The implication is that eating watercress harvested from a more mature crop, versus the popular baby-leaf salads, could be linked to a greater chemopreventive effect. We speculate that extending the growth period could be further investigated as a method to increase the health benefit seen by the consumer for watercress.

In conclusion, through extensive testing, we have established the dwarf growth form and consistently high and beneficial phytonutrient character of the new Boldrewood watercress in the agricultural setting. Boldrewood could be marketed to meet consumer demand for a salad leaf with less stem. In the commercial environment, Boldrewood was often harvested up to 14 d later that the usual crop, due to its smaller size and that these additional days of growth resulted in a crop with increased chemopreventive benefits in trials using human cancer cells. However, we have not completed a cost benefit analysis in this study to consider how increased growing costs might be off-set by a premium product. This new watercress offers significant potential to grow a slower crop, for up to two weeks longer than the

traditional harvest date, but with a potential significant improvement in phytonutrition, whilst conforming to current market size specifications.

Declarations

Acknowledgements

This research was funded by Vitacress Salads Ltd. and the University of Southampton. The authors thank farm manager, Mark Newton (Vitacress Salads Ltd.), for facilitation and technical oversight of the trials and invaluable advice. The authors are also grateful for assistance with field trials by Andy Elmsworth (Vitacress Salads Ltd.), Billy Valdes, Libby Arnold, Guillaume Zinberlin, and Ivan Greffet; and to Professor G. Packham, Dr J. Cowan, Dr M. Coldwell and Dr E. Papadakis for their advice and training in mammalian tissue culture. Dr M. Coldwell kindly supplied the MCF-7 breast cancer cells used.

Conflict of Interest

The University of Southampton (Professor Gail Taylor) shares breeder's rights to Boldrewood watercress with Vitacress Salads Ltd. The other authors have no conflict of interest to declare.

References

1. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76.
2. Bones, A. M., & Iversen, T.-H. (1985). Myrosin cells and myrosinase. *Israel Journal of Botany*, *34*(2–4), 351–376.
3. Bones, A. M., & Rossiter, J. T. (1996). The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum*, *97*(1), 194–208.
4. Booth, E. J., Walker, K. C., & Griffiths, D. W. (1991). A time-course study of the effect of sulphur on glucosinolates in oilseed rape (*Brassica napus*) from the vegetative stage to maturity. *Journal of the Science of Food and Agriculture*, *56*(4), 479–493.
5. Cartea, M. E., & Velasco, P. (2008). Glucosinolates in Brassica foods: bioavailability in food and significance for human health. *Phytochemistry Reviews*, *7*(2), 213–229. https://doi.org/10.1007/s11101-007-9072-2
6. Cavell, B. E., Syed Alwi, S. S., Donlevy, A. M., Proud, C. G., & Packham, G. (2012). Natural product-derived antitumor compound phenethyl isothiocyanate inhibits mTORC1 activity via TSC2. *Journal of Natural Products*, *75*(6), 1051–1057.

7. Costa-Pérez, A., Núñez-Gómez, V., Baenas, N., Di Pede, G., Achour, M., Manach, C., Mena, P., et al. (2023). Systematic Review on the Metabolic Interest of Glucosinolates and Their Bioactive Derivatives for Human Health. Nutrients, 15(6), 1424. MDPI AG. Retrieved from http://dx.doi.org/10.3390/nu15061424
8. Di Noia, J. (2014). Defining powerhouse fruits and vegetables: a nutrient density approach. *Prev Chronic Dis*, *11*, 130390.
9. Dwivedi, S., Sahrawat, K., Upadhyaya, H., & Otriz, R. (2013). Food, Nutrition and Agrobiodiversity Under Global Climate Change. In D. L. Sparks (Ed.), *Advances in Agronomy* (pp. 1–128). Elsevier Inc.
10. Engelen-Eigles, G., Holden, G., Cohen, J. D., & Gardner, G. (2006). The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.). *Journal of Agricultural and Food Chemistry*, *54*(2), 328–334.
11. Fogarty, M. C., Hughes, C. M., Burke, G., Brown, J. C., & Davison, G. W. (2013). Acute and chronic watercress supplementation attenuates exercise-induced peripheral mononuclear cell DNA damage and lipid peroxidation. *The British Journal of Nutrition*, *109*(2), 293–301.
12. Gill, C. I. R., Haldar, S., Boyd, L. a, Bennett, R., Whiteford, J., Butler, M., … Rowland, I. R. (2007). Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. *The American Journal of Clinical Nutrition*, *85*(2), 504–510.
13. Gupta, Wright, S. E., Kim, S.-H., & Srivastava, S. K. (2014). Phenethyl isothiocyanate: A comprehensive review of anti-cancer mechanisms. Biochimica et Biophysica Acta. Reviews on Cancer, 1846(2), 405–424. https://doi.org/10.1016/j.bbcan.2014.08.003
14. Hecht, S., Chung, F., Richie, J. J., Akerkar, S., Borukhova, A., Skowronski, L., & Carmella, S. (1995). Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers. *Cancer Epidemiol. Biomarkers Prev.*, *4*(8), 877–884.
15. Hibbert, L.E., Taylor, G., Improving phosphate use efficiency in the aquatic crop watercress (Nasturtium officinale), Horticulture Research, Volume 9, 2022, uhac011, https://doi.org/10.1093/hr/uhac011
16. Kaner-White, Y. (2021). *An English market town celebrates the punchy plant with an extravagant annual watercress festival and pristine heritage railway*. Retrieved from BBC: https://www.bbc.com/travel/article/20210926-englands-beloved-nose-twisting-salad
17. Kopsell, D. a, Barickman, T. C., Sams, C. E., & McElroy, J. S. (2007). Influence of nitrogen and sulfur on biomass production and carotenoid and glucosinolate concentrations in watercress (*Nasturtium officinale* R. Br.). *Journal of Agricultural and Food Chemistry*, *55*(26), 10628–10634.
18. Liang, Lee, H. W., Li, Z., Lu, Y., Zou, L., & Ong, C. N. (2018). Simultaneous Quantification of 22 Glucosinolates in 12 Brassicaceae Vegetables by Hydrophilic Interaction Chromatography–Tandem Mass Spectrometry. ACS Omega, 3(11), 15546–15553. https://doi.org/10.1021/acsomega.8b01668
19. Manchali, S., Chidambara Murthy, K. N., & Patil, B. S. (2012). Crucial facts about health benefits of popular cruciferous vegetables. *Journal of Functional Foods*, *4*(1), 94–106. https://doi.org/10.1016/j.jff.2011.08.004

20. Manton, I. (1935). The cytological history of Watercress (*Nasturtium officinale* R. Br.). *Zeitschrift Für Induktive Abstammungs- Und Vererbungslehre*, *69*(1), 132–157.
21. Martínez-Sánchez, A., Gil-Izquierdo, A., Gil, M. I., & Ferreres, F. (2008). A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. *Journal of Agricultural and Food Chemistry*, *56*(7), 2330–2340.
22. Newman, R. M., Hanscom, Z., & Kerfoot, W. C. (1992). The watercress glucosinolate-myrosinase system: a feeding deterrent to caddisflies, snails and amphipods. *Oecologia*, *92*, 1–7.
23. Niñirola, D., Fernández, J. A., Conesa, E., Martínez, J. A., & Egea-Gilabert, C. (2014). Combined effects of growth cycle and different levels of aeration in nutrient solution on productivity, quality, and shelf life of watercress (*Nasturtium officinale* R. Br.) plants. *HortScience*, *49*(5), 567–573.
24. Palaniswamy, U. R., & McAvoy, R. J. (2001). Watercress: A salad crop with chemopreventive potential. *HortTechnology*, *11*(4), 622–626.
25. Palaniswamy, U. R., McAvoy, R. J., Bible, B. B., & Stuart, J. D. (2003). Ontogenic variations of ascorbic acid and phenethyl isothiocyanate concentrations in watercress (*Nasturtium officinale* R.Br.) leaves. *Journal of Agricultural and Food Chemistry*, *51*(18), 5504–5509.
26. Payne, A. C. (2011). *Harnessing the Genetic Diversity of Watercess (Rorippa nasturtium - aquaticum) for Improved Morphology and Anti- cancer Benefits: Underpinning Data for Molecular Breeding [doctoral thesis]*. Southampton: University of Southampton.
27. Payne, A. C., Clarkson, G. J. J., Rothwell, S., & Taylor, G. (2015). Diversity in global gene expression and morphology across a watercress (*Nasturtium officinale* R. Br.) germplasm collection: first steps to breeding. *Horticulture Research*, *2*, 15029. https://doi.org/10.1038/hortres.2015.29
28. Payne, A. C., Mazzer, A., Clarkson, G. J. J., & Taylor, G. (2013). Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. *Food Science & Nutrition*, *1*(6), 439–44.
29. Qian, Y., Hibbert, L. E., Milner, S., Katz, E., Kliebenstein, D. J., & Taylor, G. (2022). Improved yield and health benefits of watercress grown in an indoor vertical farm. *Scientia Horticulturae*, *300*, 111068. doi:10.1016/j.scienta.2022.111068
30. Qian, Y., Hibbert, L. E., Katz, E., Smith, H. K., Kliebenstein, D. J., & Taylor, G. (2023). Watercress yield and quality vary depending on both genotype and environment: Results from highly contrasting growing systems of California and UK. Scientia Horticulturae, 319, 112154. doi:10.1016/j.scienta.2023.112154
31. R. (2013). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
32. Rose, P., Faulkner, K., Williamson, G., & Mithen, R. (2000). 7-Methylsulfinylheptyl and 8-methylsulfinyloctyl isothiocyanates from watercress are potent inducers of phase II enzymes. *Carcinogenesis*, *21*(11), 1983–1988.
33. Sadeghi, H., Mostafazadeh, M., Sadeghi, H., Naderian, M., Barmak, M. J., Talebianpoor, M. S., & Mehraban, F. (2013). In vivo anti-inflammatory properties of aerial parts of *Nasturtium officinale*.

Pharmaceutical Biology, (2008), 1–6. https://doi.org/10.3109/13880209.2013.821138

34. Santos, J., Oliveira, M. B. P. P., Ibáñez, E., & Herrero, M. (2014). Phenolic profile evolution of different ready-to-eat baby-leaf vegetables during storage. *Journal of Chromatography. A*, *1327*, 118–131.
35. Schreiner, M. (2005). Vegetable crop management strategies to increase the quantity of phytochemicals. European Journal of Nutrition, 44(2), 85–94
36. Simon, P. W. (2010). *Domestication, Historical Development, and Modern Breeding of Carrot*. *Plant Breeding Reviews* (Vol. 19). https://doi.org/10.1002/9780470650172.ch5
37. Slafer, G. A., Satorre, E. H., & Andrade, F. H. (1994). Increases in grain yield in bread wheat from breeding and associated physiological changes. In *Genetic Improvement of Field Crops* (pp. 1–68).
38. Solankey, S.S., Kumari, M., Akhtar, S., Singh, H.K., Ray, P.K. (2021). Challenges and Opportunities in Vegetable Production in Changing Climate: Mitigation and Adaptation Strategies. In: Solankey, S.S., Kumari, M., Kumar, M. (eds) Advances in Research on Vegetable Production Under a Changing Climate Vol. 1. Advances in Olericulture. Springer, Cham. https://doi.org/10.1007/978-3-030-63497-1_2
39. Traka, M. H., & Mithen, R. (2008). Glucosinolates, isothiocyanates and human health. *Phytochemistry Reviews*, *8*(1), 269–282.
40. Traka, M. H., Saha, S., Huseby, S., Kopriva, S., Walley, P. G., Barker, G. C., … Mithen, R. F. (2013). Genetic regulation of glucoraphanin accumulation in Beneforté broccoli. *The New Phytologist*, *198*(4), 1085–1095.
41. Velasco, P., Cartea, M. E., Gonzalez, C., Vilar, M., & Ordas, A. (2007). Factors affecting the glucosinolate content of kale (*Brassica oleracea* acephala group). *Journal of Agricultural and Food Chemistry*, *55*(3), 955–962.
42. Voutsina, N., Payne, A. C., Hancock, R. D., Clarkson, G. J., Rothwell, S. D., Chapman, M. A., & Taylor, G. (2016). Characterization of the watercress (*Nasturtium officinale* R. Br.; Brassicaceae) transcriptome using RNASeq and identification of candidate genes for important phytonutrient traits linked to human health. *BMC Genomics*, *17*, 378. https://doi.org/DOI 10.1186/s12864-016-2704-4
43. Wentzell, A. M., & Kliebenstein, D. J. (2008). Genotype, age, tissue, and environment regulate the structural outcome of glucosinolate activation. *Plant Physiology*, *147*(1).

Figures

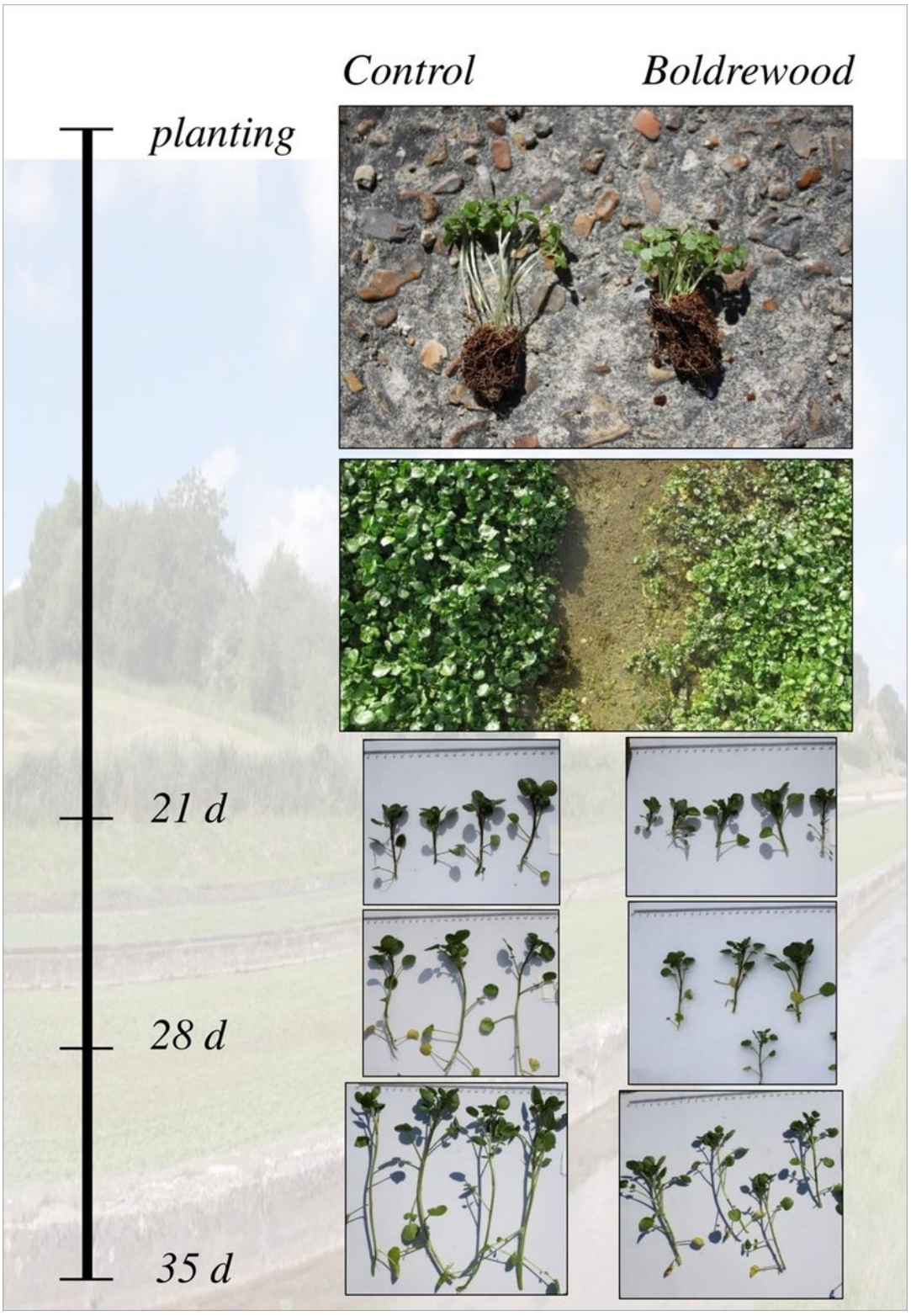


Figure 1

Images of the Control and Boldrewood accessions across the time course of Trial 2 in 2016 showing the differences in morphology of the two accessions from seedling to harvest-ready plants

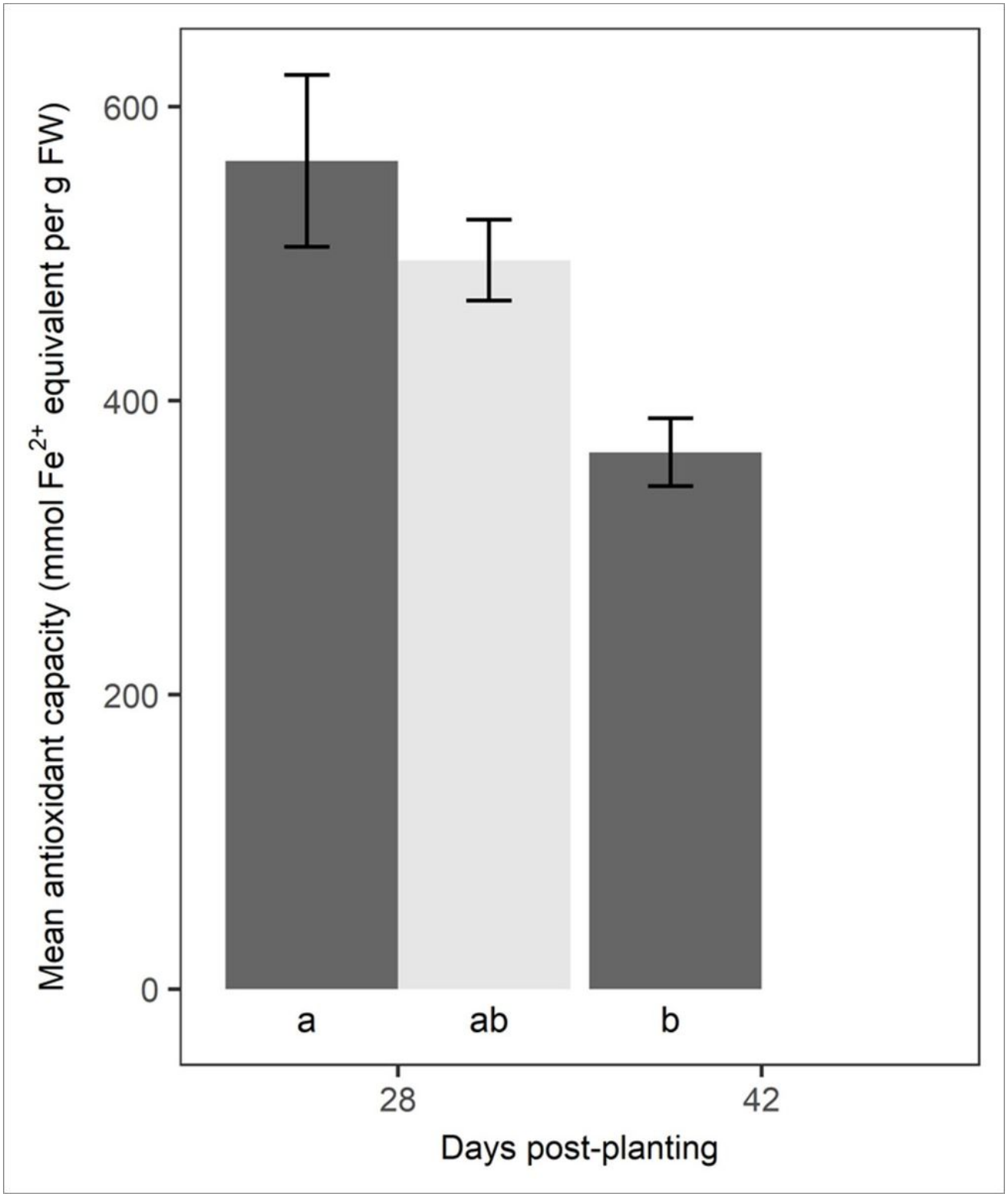


Figure 2

Mean antioxidant capacity (mmol of Fe^{2+} equivalent per g fresh weight) of Boldrewood (dark grey) and Control (light grey) accessions in Trial 1. Values are as measured by FRAP antioxidant assay. Error bars indicate the standard error of the mean (n = 5) and letters denote statistically significant differences ($p \leq 0.05$).

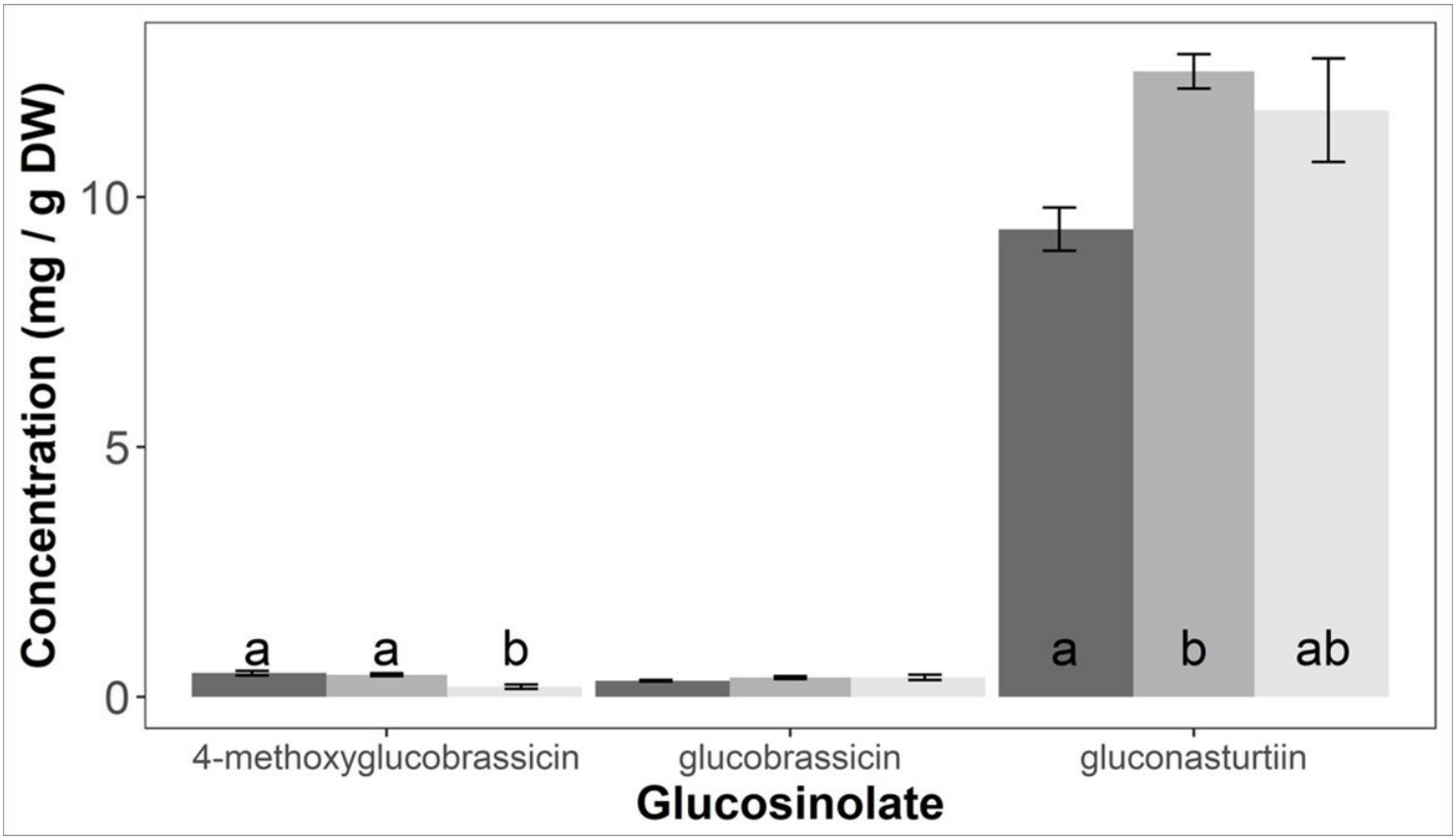


Figure 3

Specific glucosinolate concentrations (mg per gram of dry weight) for each accession in Trial 1. Boldrewood at 28 d post-planting is represented in dark, Boldrewood at 42 d in medium, and Control at 28 d in light grey. Error bars indicate the standard error of the mean (*n* = 5)

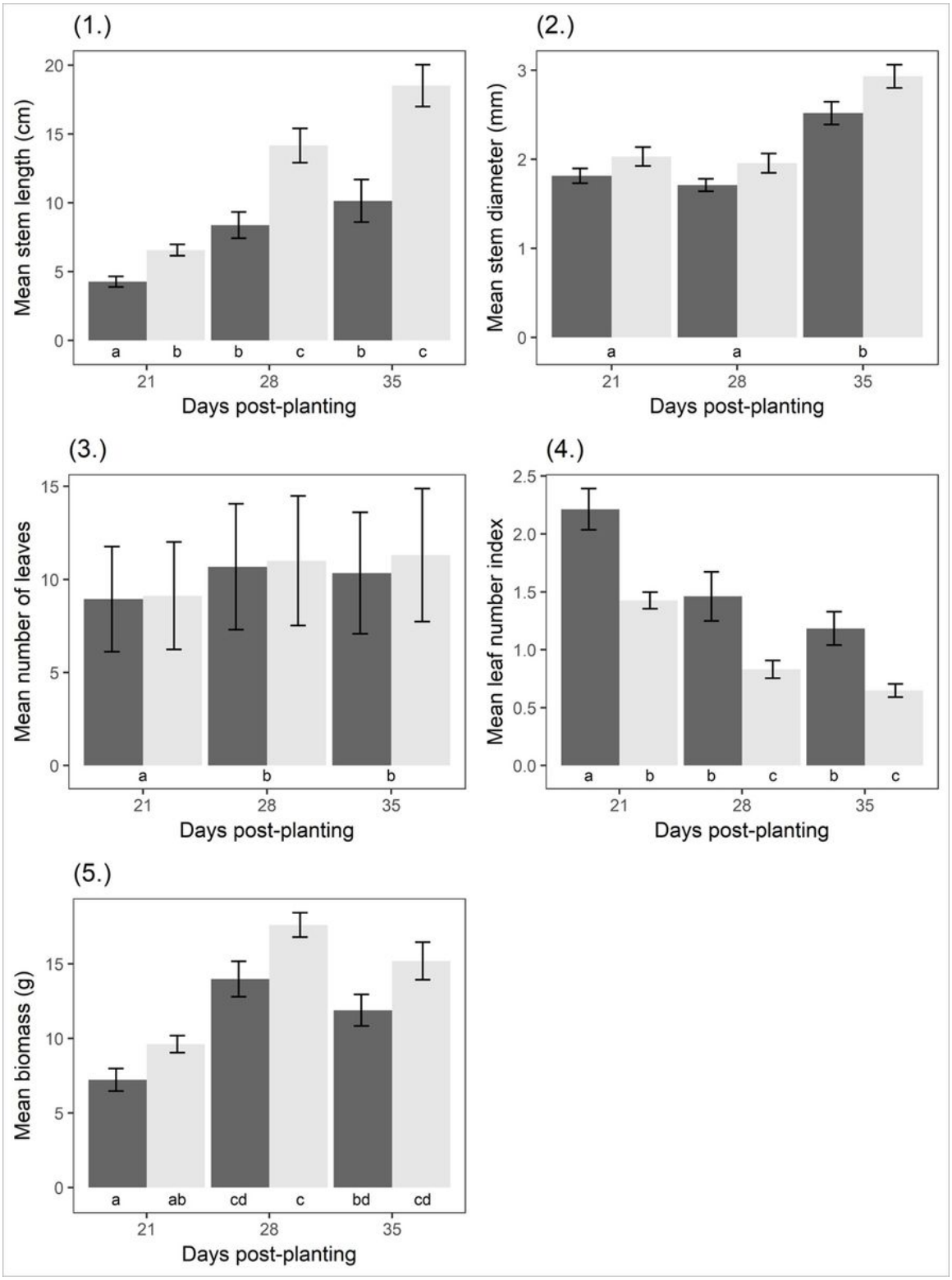


Figure 4

Mean values for stem length (1, cm), stem diameter (2, mm), number of leaves (3), leaf number index (4) and biomass (5, g) for Boldrewood (dark grey) and Control (light grey) accessions at 21, 28 and 35 days post-planting. Error bars signify the standard error of the mean (n=10) and letters denote statistically significant differences ($p \leq 0.05$).

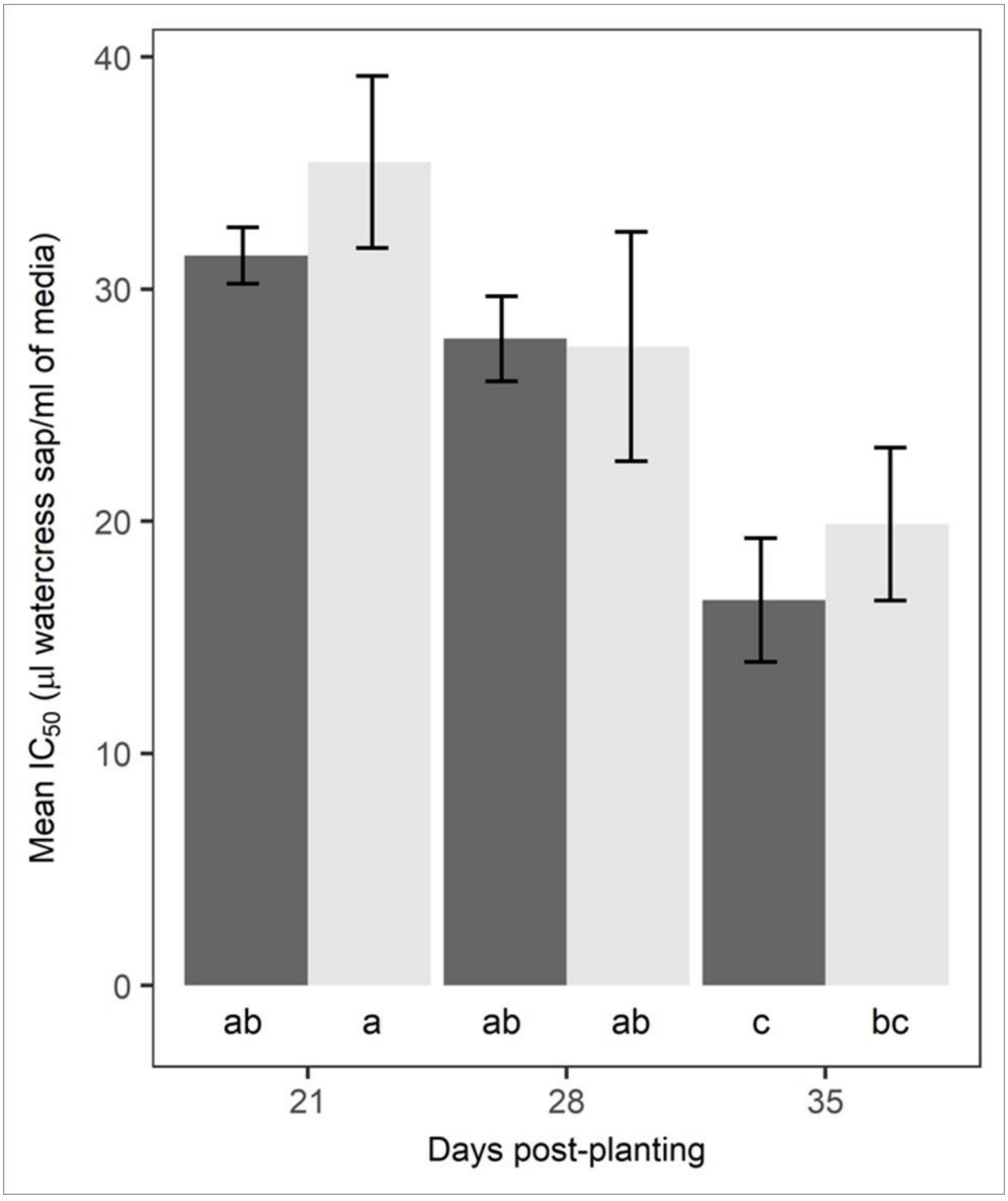


Figure 5

Mean inhibitor concentration required to cause lethal toxicity to 50 % of MCF7 breast cancer cells present (IC_{50}, µl of watercress sap per ml of medium) for Boldrewood (dark grey) and Control (light grey) watercress accessions in Trial 2. Error bars indicate the standard error of the mean (n=10) and letters denote statistically significant differences ($p \leq 0.05$).