Uv-b crops can be planted, so stem

UV-B acts as a source of stress, primarily due to DNA damage, and is an important environmental signal that regulatesa variety of processes in plants such as plant growth and the production ofprotective sunscreen compounds.

In this study, we analysed the architecture andflavonoid accumulation in oilseed rape (Brassica napus), and demonstrate thatUV-B inhibits hypocotyl elongation in response to a shade avoidance signal. Weadditionally demonstrate that UV-B increases the content of the flavonoidquercetin in B. napus cotyledons, and that UV-B may affect root architecture inArabidopsis thaliana, although more research is required for definitiveconclusions. Our results suggest that UV-B manipulation on the appearance andflavour of both field and glasshouse crops could overcome the negative impactsof dense planting. Introduction Plants are oftengrown in dynamic environments where they must adapt their growth and developmentin response to ambient light conditions and compete with surrounding neighboursfor light. They therefore have sophisticated sensing mechanisms to perceive lightsignals as an accurate indicator of neighbour proximity: infringing neighboursabsorb red (R) light and reflect far-red (FR) light, resulting in a reducedR: FR ratio that serves as a signal of neighbour proximity prior to shading (Franklin& Whitelam, 2005). In many plant species, this elicits a collectiveresponse known as shade avoidance, which involves the activation of phytochromeinteracting factors (PIFs) and auxin biosynthesis, resulting in rapid stem elongation to overtop competitors (Smith, 1982; Lorrain et al.

, 2008; Tao et al., 2008). Shade avoidance canbe detrimental to the aesthetic quality and therefore commercial success ofcrops, and can lead to reduced yields via processes such as stem lodging (Baker et al., 1998;

Franklin & Whitelam, 2005). These issues are further exacerbated becauseshade avoidance is a limiting factor to how densely crops can be planted, sostem length control is an extremely important agricultural focus. As a result, different methods have been carried out to study how shade avoidance could beimpeded (Robson et al.

, 1996; Wang et al., 2011), with the hopeto produce high-quality crops planted at high densities; a topic of special significancegiven the global challenge to feed 9 billion people by 2050 (Lee, 2011). UV-B regulates abroad range of both developmental and physiological processes in plants, and isan important environmental signal for plant growth, the production of sunscreencompounds, leaf morphology, and defence against pests and pathogens (Kakani etal., 2003; Jenkins et al., 2017). In higher plants, UV-B signals areperceived by the phytochrome photoreceptor UVR8 (Rizzini et al., 2011), where upon UV-B absorption the photoreceptor activates various signaltransduction cascades that converge to block auxin biosynthesis and elongation viadirect and indirect inhibition of PIF4/5 activity (Hayes et al.

, 2014; Hayes et al., 2017), thereby inhibiting shade avoidance. Light-controlled stem elongation and UV-B’srole in antagonising shade avoidance are well characterised in Arabidopsis thaliana (Kim et al., 1998; Boccalandro et al., 2001; Li et al., 2013), but our understanding of Brassica napus, or of other crops in the family Brassicaceae, is limited. One study, however, has described the control of UV-absorbingphotoreceptors in the inhibition of hypocotyl elongation in Brassicaoleracea (Lercari et al., 1989).

As well as antagonising shadeavoidance, UV-B can act as a source of stress (Müller-Xing et al., 2014), under which Arabidopsis has been seen to acquire a subfamily of flavonoidscalled flavonols (Li et al., 1993; Lois, 1994) that are regarded aseffective UV filters (Stapleton & Walbot, 1994) and have been suggested toprotect plants against UV-B-induced damage (Braun & Tevini, 1993), especiallysince flavonoid synthesis genes are efficiently activated by UV-B (Kubasek etal., 1992).

B. napus plants have been seen to acclimatise toincreases in UV-B through accumulation of flavonoids (Wilson et al., 2001), specifically kaempferol and quercetin glycosides (Olsson, 1998). Here, weinvestigated whether UV-B antagonises shade avoidance in Brassica napus(oilseed rape) the same way as in Arabidopsis, with the aim to determine ifUV-B supplementation could translate to crops.

Another aim of thisinvestigation was to explore how pigment content in B. napus is affectedby UV-B, and to discuss the possible implications of this to crop quality sinceflavonoids affect colour, taste, antioxidant properties (He & Giusti, 2010), and may provide health benefits (Yao et al., 2004; Stracke etal., 2010; Jaakola & Hohtola, 2010). Our final aim was to see if low R: FRand UV-B affect root architecture: One study has documented that plants areable to transmit sunlight directly down to roots (Lee et al., 2016), andsince shade avoidance suppresses shoot branching (Morelli & Ruberti, 2000; Casal, 2012), we hypothesized that roots would show reduced branching inlow R: FR. This idea has been further strengthened by another study explainingthat FR-light enrichment of the shoot organs of Arabidopsis

seedlings reducedthe formation of lateral roots (van Gelderen et al., 2018), even thoughthe roots were not themselves exposed to the light.

Given the fact that rootsseem to react to shade simulation in the same way as shoots, there is potentialthat UV-B could also antagonise reduced root branching. Materials and methods Plant Material. Brassicanapus seeds were from the cultivar Rv31. Wild type and uvr8-1 mutantArabidopsis thaliana seeds were inthe Landsberg erecta (Ler)background. Growth Conditions. Seeds were sowndirectly onto a 3: 1 mixture of all-purpose growth medium (Sinclair): horticultural silver sand (Melcourt). After 3 days of stratification indarkness at 4ºC, seedlings were germinated in a controlled growth cabinet (Microclima1600E, Snijder Scientific) in continuous white light (R: FR > 5) at 20ºC temperature, 70% humidity and under a 16-h light/8-h dark cycle for 3 days, after which theywere transferred to treatment conditions for 4 days.

To provide white light, white fluorescent tubes (400-700 nm) were used at a photon irradiance of 90µmolm-2 s-1. Supplementary far-red LEDs were placed overheadfor low R: FR experiments, and supplementary UV-B was provided at a photonirradiance of 400 mWm2 by Phillips TL100W/01narrow band UV-B bulbs. Layersof yellow heat-resistant tape (Lee filter) were used to control UV-B output(approx. 1 µmol m? 2s? 1). Polycarbonate filters (3 mmthick) were used to block UV-B for plants grown in –UV-B conditions. All lightmeasurements were performed using anOcean Optics FLAME-S-UV-VIS spectrometer with a cosine corrector(oceanoptics. com).

Plant Measurements. Arabidopsis and B. napus seedlings were mounted ontosquare plates with an activated charcoal and agar medium for viewing. To makecharcoal agar, 3.

75g 1. 5% agar (Sigma-Aldrich), 2. 5g 1%activated charcoal (Sigma-Aldrich), and 250 mldistilled water was microwaved until boiling and the agar was properlydissolved. Once the agar had been cooled and poured, a sterile toothpick wasused to move any bubbles to the side, and the agar was left to set for 10 minbefore being stored in the fridge. Measurements of seedling hypocotyl length (mm)and cotyledon area (mm2) were recorded using ImageJ software (1. 50i).

Hypocotyls were measured from the shoot apical meristem to the shoot-root junction, and visible leaf area was measured by drawing around leaf perimeters using the'freehand selection' tool. For Arabidopsis genotypes at least 20 seedlings weremeasured per condition, and for B. napusat least 10 seedlings were measured.

StatisticalAnalyses. Statistical analyses of quantitative data were carried out using SPSS v. 23 (IBM). Tukey's post hoc tests were used to deduce statistically significantmeans ($P < 0.05$), as indicated byletters in figures. Subsets a, b, c, d – in order of increasing means. FlavonoidExtraction.

Arabidopsis and B. napusseedlings were cultivated and grown as mentioned above. Mentholic extracts wereproduced from 10-100 mg plant material (whole seedlings of Arabidopsis andcotyledons of B. napus) harvested in 2ml tubes, with two 3mm chrome steel ball bearings added to each.

Plant materialwas ground in liquid nitrogen with a vortex before being homogenised andextracted in 100 µl 80% methanol (Sigma-Aldrich), and thenvortexed again to assure proper mixing. Samples were incubated for 15 min at 70ºC then centrifuged for 10 min (21 ºC; 13. 2 rpm) in a standard table centrifuge. Supernatants were then dried in a SpeedVac at 65 ºC, and dried pelletsdissolved in 1 µl 80% methanol mg-1 fresh weight.

High-PerformanceThin Layer Chromatography. 3 µl of methanolic extracts were spotted onto HPTLCsilica gel 60 glass plates (Millipore). Chromatography was performed ina closed glass tank with a mobile phase of ethyl acetate (Sigma-Aldrich), formic acid(Alfa Aesar) acetic acid (Sigma-Aldrich) and water(100: 11: 11: 26 v/v). After separation, plates were air-dried and flavonoidsdetected by spraying 2 ml of 1% (w/v) 2, 3-dibromopropanal (DPBA) (Sigma-Aldrich), in methanol 4times with 5 min between sprayings. Then 2 ml of 5% (w/v) PEG 4000 (AppliChem) andmethanol solution was sprayed 3 times, with 5 min between sprayings.

After 15min the stained plate was visualised on a UV illuminator (365 nm), andphotographed. The cited study used LC-MS to profile the flavonol glycosides andassign them to different colours as follows: green, kaempferol derivatives; orange, quercetin derivatives; blue, sinapate derivatives and unknownsubstances; dark red, chlorophyll (Stracke etal., 2010). Root analysis. 6 sterile square plates were preparedwith 1. 05g Murashige & Skoog growth medium (Duchefa Biochemie), 1g of 1%agar (Sigma-Aldrich), and 500ml water. The pH of the agar was then altered toreach 5. 7 using 0.

1M potassium hydroxide (Sigma-Aldrich). 60 Ler and 60 uvr8-1 seeds were sterilised with bleach and hydrogen chloride(100: 3), and then 10 Ler and 10 uvr8-1 sown onto a reference line 50 mmfrom the top of each plate. One plate was used for each treatment condition (WL±UV-B and low R: FR ±UV-B). Two extra plates were blacked out from the referenceline downwards with black electric tape for WL ±UV-B treatments. For thetreatments involving +UV-B, a hot scalpel was used to cut the plastic platecovers and the plates were sealed with a single layer of plastic wrap.

Allplates were sealed with microporous tape. After 3 days of stratification indarkness at 4ºC, seedlings were stored vertically to germinate in the growthcabinet (Microclima 1600E, Snijder Scientific) in continuous white light (R: FR> 5; 20ºC; 70%; 16-h light/8-h dark cycle), for 3 days. Plates were thenmoved into treatment conditions to grow vertically for 14 days, after which theywere photographed on black cloth.

Taproot length was measured using ImageJ (1. 50i), and qualitative observations of root branching were made. Results UV-B stronglyinhibits shade avoidance in B. napusin the same way as Arabidopsis. B. napus and Arabidopsisseedlings were grown in white light (WL) and low R: FR conditions with andwithout UV-B supplementation (Figs.

1a & 2a). Plants grown in low R: FRconditions were significantly elongated compared to plants grown in WL alone, confirming that B. napus displaysshade avoidance (Figs. 1a & 1b).

UV-B supplementation resulted insignificantly shorter hypocotyls in a background of WL, however theUV-B-mediated inhibition of hypocotyl

elongation was notably greater in lowR: FR. These data show that UV-B antagonises shade avoidance in B. napus correspondingly to Arabidopsis (Figs.

1 & 2). Hypocotyl inhibition was dependent on the UVR8 photoreceptorbecause the UVR8-deficient mutant, uvr8-1, showed similar shade avoidance responses in the presence and absence of UV-B(Fig. 2b). In contrast to hypocotylelongation data, it was unclear whether UV-B antagonised cotyledon area. Wequantified cotyledon area in both species, and in some cases cotyledon area didreduce in low R: FR compared to WL. UV-B appeared to slightly antagonise this(Figs.

1c & 2c), but overall cotyledon area was not a robust phenotype. Flavonoidproduction in B. napus and Arabidopsisis strongly influenced by UV-B We assayed B. napusand Arabidopsis seedlings for changes in flavonol glycoside content in responseto UV-B using thin layer chromatography as described previously (Stracke et al., 2010), to gain qualitativeinformation on flavonoid content.

Substantialincreases in the levels of flavonol derivatives, particularly quercetinglycosides, were detected upon exposure to UV-B in B. napus. There was also an indication of increases in kaempferolderivatives, although these were not as conclusive. In Arabidopsis, therewas a small increase in quercetin (orange) derivatives in both wild type and uvr8-1genotypes with UV-B supplementation, however the main difference seen was theincrease of kaempferol (green) derivatives in wild type seedlings with UV-Bsupplementation in both WL and low R: FR (Figs. 3a & 3b). These

resultsimply that the kaempferol pathway in Arabidopsis is UVR8-mediated, but that thequercetin pathway is not UVR8-mediated, despite being affected by UV-B. Low R: FR and UV-B affect root architecture in Arabidopsis. Wild type andmutant Arabidopsis seeds were sterilised and grown upright on agar plates incontinuous WL for 3 days, before being transferred to treatment conditions for14 days.

Low R: FR did not significantly affect taproot length in eithergenotype (Fig. 4a), however qualitative observations determined that low R: FRsignificantly reduced root branching in both genotypes (Supplementary Fig. 1).

UV-Bappeared to further reduce root branching in both WL and low R: FR conditions, witha seemingly greater reduction in low R: FR over WL. Tap root length only reducedsignificantly when low R: FR was supplemented with UV-B (Fig. 4a), and thisoccurred in both genotypes, implying that this result was independent of the UVR8photoreceptor. Taproot lengthsignificantly reduced when the WL +UV-B plate was covered, but this onlyoccurred in the uvr8-1 mutant (Fig.

4b). In the wild type genotype, covered plates showed no significant change inroot length for either WL or WL+UV. Both genotypes appeared to have reducedroot branching (Supplementary Fig. 2) when covered in WL, however no obviouschange to root branching was seen in either genotype when the WL+UV plate was covered. Discussion Consistent with studies onArabidopsis (Franklin & Whitelam, 2005; Keuskamp et al.

, 2010), hypocotyl elongation was induced in B. napus seedlings in low R: FR; aresult that is further supported by a study on B. napus (Rondanini etal.,

2014). This elongation was antagonised when low R: FR-treated plantswere simultaneously exposed to UV-B despite the detection of a strong shadeavoidance signal by the UVR8 photoreceptor (Fig. 1b). The plant growthregulator auxin has an established role in shade avoidance, and in ArabidopsisUV-B is presented as a suppressor of auxin biosynthesis and therefore of shadeavoidance, resulting in a compact plant (Jansen, 2002). The similarities in thehypocotyl responses between Arabidopsis and B.

napus to simulated shadeand UV-B suggest that similar signalling pathways exist in both species. In Arabidopsis, low R: FR has beenshown to reduce cotyledon size (Li et al., 2012; Martínez-García etal., 2014; Procko et al.

, 2014), and the introduction of UV-B hasbeen shown to promote leaf expansion (Hayes et al., 2014). In somerepeats of our investigation, cotyledon area reduced in low R: FR compared to WLin both species, and UV-B appeared to slightly antagonise this, which wasconsistent with the literature.

However, our data sets were too variable tomake any conclusions, including whether changes in cotyledon area areUVR8-mediated. It is possible that changes to cotyledons in low R: FR ±UV-Bconditions where due to leaf curling rather than overall surface area (Fierro etal., 2015), especially since it has been reported that a phenotypicresponse to UV-B in B.

napus is upward leaf curling (Wilson &Geenberg, 1993). Our cotyledon data most likely showed so much variabilitybecause our measuring method may not have been indicative of true cotyledonsurface area. Instead, cotyledon area should have been measured by counting thepixels on images of

flattened leaf blades rather than drawing free-hand onImageJ. Finally, in some repeats, the optimum number of seedlings was not used foranalysis due to late germination of seedlings, and as a result the sample sizeswe averaged were not always consistent. This is especially true in B. napussince the size of seedlings limited the number of seeds that could be sown ineach pot. If this investigation were to be repeated bigger pots would be usedand more B.

napus seeds would be sown. Despite these method limitations, however, our hypocotyl results were very consistent for all four true repeats. Although B. napus did notshow changes in hypocotyl length that were as dramatic as in Arabidopsis, thepatterns were still the same, which signifies the importance of shade avoidanceinhibition in improving crop quality and influencing how densely crops can beplanted. Our findings, as supported by the literature, suggest importantimplications for field crops such as B. napus, where plants grown insunlight receive naturally accompanying elevations in UV-B. Given humandepletion of the stratospheric ozone layer, shade avoidance in field crops maybe naturally reduced.

Moreover, one study has reported that lettuce pre-treatedwith UV-B outperformed control plants after transplantation to fields (Wargent etal., 2011), which as an example of an approach to maximise production ofgood-quality field crops, especially if natural UV-B wavelengths are notsufficient in inhibiting shade avoidance. Our findings could additionally applyto agricultural crops that can be grown glasshouses or commercial growingenvironments, including some members of the family Brassicaceae (Bailey etal., 2006).

This is because a number of novel glass materials that transmitUV-B are becoming available (Paul et al., 2005) to replace traditional glasshousematerials such as glass and polycarbonates that attenuate UV-B. As well as having credible impacts on shade avoidance, UV-Balso increased flavonoid accumulation in B.

napus, as well as Arabidopsis. Our findings of increased kaempferol glycosides and aslight indication of quercetin derivatives in wild type Arabidopsis wereconsistent with other studies (Ryan etal., 2001). Our analysis offlavonoid levels in B. napus cotyledons suggested that UV-B induces theaccumulation of quercetin (Fig. 3a & 3b) in both white light and low R: FR; information that is also supported in the literature (Wilson et al., 2001). Furthermore, workby Olsson and colleagues states that quercetin glycosides accumulates more thankaempferol glycosides with UV-B supplementation in B.

napus (Olsson, 1998), which further supports our findings. Stress caused by UV-B is known toenhance the production of reactive oxygen species (ROS) that can damage plantDNA and proteins (Sharma, 2012). Flavonoids such as quercetin, are reported tobe effective antioxidants in scavenging ROS and absorbing UV-B to protectagainst sun damage (Smith & Markham, 1998). It is therefore notsurprising that quercetin abundance in B. napus was induced by UV-B. Flavonoids have been linked toreduce risks for a number of diseases in large population studies (Cazarolli et al., 2008)and hence could play a crucial role in human nutrition when accounted for incommercial crop growth.

However, despite potential health benefits, flavonoidssuch as quercetin are associated with bitterness (Drewnowski &Gomez-Carneros, 2000), so growers using UV-B supplementation for aesthetic andplanting purposes will need to account for potential flavour alterations. It is important to note that our resultsonly provide a qualitative indication of flavonol accumulation; our method didnot involve mutant controls, which would have helped quantify results andenable a clearer understanding of the flavonoid biosynthesis pathway. Moreover, the bands on our plates for B. napus were not separated clearly enough, despite experimenting with a different mobile phase ratio and obtaining clearruns for analyses of Arabidopsis. Based on previous studies onshoot branching, we hypothesised that shade avoidance would reduce root branching, and that UV-B would antagonise this response. Our results showed no significantchange to taproot length in response to low R: FR, however root branching did reduce(Supplementary Fig. 1).

Unlike what we expected, UV-B seemed to exaggerate theshade avoidance response in both WL and low R: FR, rather than antagonise it, but upon further investigation into available literature, UV-B’s suppression ofroot extension has also been found in another study (Tong et al., 2008). Overall, our preliminary results imply that root shade avoidance does occur andthat UV-B may influence root architecture.

It makes sense, for example, that uvr8-1taproot length significantly reduced in low R: FR +UV because the seedlingswould be stressed if it couldn’t produce any protective compounds. However, itis not clear where UV-B fits in, if at all, because our data was extremelyvariable, we only made qualitative statements regarding roots, and ourinvestigation was only

repeated once due to time constraints. Furthermore, definitiveconclusions cannot be made because treatment conditions weren’t properly controlled; plates in +UV-B conditions were covered with cling film, whereas non-UV-Bplates were covered with the normal plate cover.

As a result, we cannot becertain if the condition of roots in cling-filmed plates was due to UV-Bdamage, moisture loss, or a combination of both. Finally, investigated if light istransmissible through plants as indicated in the literature (Lee et al., 2016; van Gelderen et al., 2018), by coveringWL ±UV-B with black electrical tape. Our results partially support the idea ofthe transmission of light signals through plants because covered and uncoveredWT treatments saw no significant change (Fig. 4b).

However, this experiment wasalso only repeated once due to lack of time so we were unable to make anyconclusions. It would be interesting to further explore if and how UV-B affectsroot architecture in Arabidopsis, and to then apply this research to othercrops because root quality affects crop value (Easson et al., 1993); plantswith smaller roots are likely to result in root lodging due to reduced root anchorage (Liu et al., 2012), and root lodging is likely to happen at high plant densities (Berry et al.

, 2000; Liu et al., 2012) because shade avoidancereduces resource allocation to roots (Sparkes & King, 2008). Collectively, our results suggest that UV-B supplementationmay have a use in providing a mechanism to adjust the harvest yield, aestheticquality, and flavour of oilseed rape. These findings could translate to othercrops as described, especially to commercially grown glasshouse crops, wherenovel glasshouse materials and UV-B-supplementing

LEDs could enable future large scale UV-B supplementation that iseconomically and environmentally desirable (Paul et al., 2005; Wargent etal.

, 2006). There were caveats to our investigation, which were primarily dueto time constraints; however further investigation into how crops respond toUV-B will promote optimal designs for plant growth management. Interestingleads for further development include UV-B’s affect on root architecture, andwhether UV-B-mediated flavonoid production is a key component of shadeavoidance inhibition in B. napus since there is evidence that auxin isregulated by flavonoid accumulation in Arabidopsis (Brown et al., 2001).