NERC Open Research Archive
sharing our research

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Article (refereed) - postprint

Hayes, Felicity; **Williamson, Jennifer**; **Mills, Gina**. 2012 Ozone pollution affects flower numbers and timing in a simulated BAP priority calcareous grassland community. *Environmental Pollution*, 163. 40-47. 10.1016/j.envpol.2011.12.032

Copyright © 2011 Elsevier Ltd.

This version available http://nora.nerc.ac.uk/16276/

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at http://nora.nerc.ac.uk/policies.html#access

NOTICE: this is the author's version of a work that was accepted for publication in *Environmental Pollution*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Environmental Pollution*, 163. 40-47. 10.1016/j.envpol.2011.12.032

www.elsevier.com/

Contact CEH NORA team at
noraceh@ceh.ac.uk

The NERC and CEH trademarks and logos ('the Trademarks') are registered trademarks of NERC in the UK and other countries, and may not be used without the prior written consent of the Trademark owner.

Ozone pollution affects flower numbers and timing in a simulated BAP Priority calcareous grassland community

Felicity Hayes[a*], Jennifer Williamson[a] and Gina Mills[a]

[a]Centre For Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor,
Gwynedd, LL57 2UW, UK
*Corresponding author. Felicity Hayes
E-mail address: fhay@ceh.ac.uk
Tel.: +44-1248-374500
Fax: +44-1248-362133

Research highlights

- An increase in ozone accelerated timing of maximum flowering in *Lotus corniculatus*
- Ozone reduced flower numbers in *Campanula rotundifolia* and *Scabiosa columbaria*
- Reduced water availability did not protect most species from the effects of ozone

Abstract

Mesocosms representing the BAP Priority habitat ‘Calcareous Grassland’ were exposed to eight ozone profiles for twelve-weeks in two consecutive years. Half of the mesocosms received a reduced watering regime during the exposure periods. Numbers and timing of flowering in the second exposure period were related to ozone concentration and phytotoxic ozone dose (accumulated stomatal flux). For *Lotus corniculatus,* ozone accelerated the timing of the maximum number of flowers. An increase in mean ozone concentration from 30 ppb to 70 ppb corresponded with an advance in the timing of maximum flowering by six days. A significant reduction in flower numbers with increasing ozone was found for *Campanula rotundifolia* and *Scabiosa columbaria* and the relationship with ozone was stronger for those that were well-watered than for those with reduced watering. These changes in flowering timing and numbers could have large ecological impacts, affecting plant pollination and the food supply of nectar feeding insects.

Capsule

Increased tropospheric ozone affected timing of flowering and maximum flower numbers in calcareous grassland mesocosms.

Keywords

Ozone; accelerated flowering; stomatal flux; drought; phenology

1. Introduction

Concentrations of tropospheric ozone have been increasing steadily over the last 150 years as a result of increasing emissions of precursor molecules such as oxides of nitrogen and VOCs from anthropogenic sources (Solberg et al., 2005; Volz and Kley, 1988). Current mean ozone concentrations are typically 35-40 ppb at Mace Head, Ireland (Derwent et al., 2007) and have increased at a rate of 0.16 ppb per annum over the period 2000 – 2007 (Tripathi et al., 2010). Tropospheric ozone concentrations across the northern hemisphere are predicted to increase further as emissions of precursor molecules continue to rise (Vingarzan, 2004, Meehl et al., 2007). Summer mean ozone concentrations across Europe are expected to reach 40 to 60 ppb by 2030 (Royal Society, 2008) and some predictions are for concentrations to reach 60 ppb across central and northwest Europe by 2100 (Andersson and Engardt, 2010). At current ozone concentrations, visible effects have been observed on plants growing in natural conditions across Europe (Mills et al., 2011a). Ozone exposure studies have demonstrated that ozone pollution can affect species of (semi-) natural vegetation communities in many ways including above-ground growth (Franzaring et al., 2000; Gimeno et al., 2004a; Hayes et al., 2010), root growth (Franzaring et al., 2000; Batty and Ashmore 2003), biomass partitioning (Cooley and Manning, 1987; Hayes et al., 2009), flowering (Rämö et al., 2007) and seed output (Gimeno et al., 2004b; Black et al., 2000) with differential responses by individual species.

Flowering is a critical stage in the life-cycle of a plant and alterations to this process could influence species survival within a plant community and reduce the important ecosystem services related to pollination and nectar sources. A recent meta-analysis of effects of ozone on plant reproductive growth and development revealed that current ambient ozone concentrations significantly reduced seed number, fruit number and fruit weight compared to charcoal-filtered air conditions (Leisner and Ainsworth, in press). There have been several studies that have shown changes in flower number or flower biomass in response to ozone, although these studies have mainly used individual plants/monocultures, relatively few ozone treatments (up to 4), and usually only occasional assessments of flower number even though this is a very dynamic process. An exposure study of a sown species mixture in Finland showed that the elevated ozone treatment corresponded with a significantly reduced number of flowers in *Campanula rotundifolia* compared to the non-filtered air control (Rämö et al., 2007). A reduction in flower biomass with increasing ozone exposure has been demonstrated for *Trifolium cherleri, Trifolium subterraneum* and *Trifolium striatum* grown as individual species (Gimeno et al., 2004b). Flower weights were also significantly reduced by ozone for *Eupatorium cannabinum* and *Plantago lanceolata*, also grown as individual species (Franzaring et al., 2000). There can be carry-over effects of ozone exposure which can influence subsequent flowering. For example, following exposure to ozone of *Trifolium striatum* when the plants were in a vegetative state there was reduced flowering that persisted for one month following cessation of the ozone exposure (Sanz et al., 2007). In a separate study *Carex echinata* exposed to elevated ozone showed a reduction in flower biomass of approximately 30% in spring following exposure to ozone in the previous summer (Hayes et al., 2006). In addition, for *Leontodon hispidus* there was acceleration in the progression from flowers to seed-heads in the year following ozone exposure (Hayes et al., 2011).

Despite evidence of alterations in flower numbers/biomass following ozone exposure, comparatively few studies have investigated the effect of ozone on the timing of flowering. Comparing only two ozone treatments, *Campanula rotundifolia* and *Vicia cracca* showed delayed flowering with increasing ozone exposure in the second year of exposure of simulated meadow communities (Rämö et al., 2007). These two species also showed reduced early season coverage in the high ozone treatment, suggesting that there was reduced resource availability following the first year of exposure. In a single-season study *Spartina alterniflora*, grown as individual plants, showed delayed flowering and a reduction in the number of flower spikes produced in the elevated ozone treatment compared to control (Taylor et al., 2002). These plants also showed reduced shoot and leaf number, again suggesting reduced resource availability. In contrast, for some species there are suggestions of earlier flowering with increasing ozone exposure. *Betula pendula* flowered earlier with elevated ozone compared to the ambient air control, with a non-significant increase in female flower formation (Darbah et al., 2007); the authors did not suggest a mechanism for this. Similarly for *Rubus cuneifolius* an initial acceleration in flowering occurred in the second year of ozone exposure for the highest ozone treatment compared to the lower treatments, with increased flower numbers and an earlier time of peak production (Chappelka, 2002).

There was no overall relationship between timing of flowering and ozone concentrations in this study as the flowering time of plants in charcoal-filtered air was intermediate to the 2x ambient air and non-filtered air treatments.

Future climate change scenarios predict changes in rainfall patterns (IPCC, 2007), with reduced rainfall across many temperate regions and an increase in the frequency and severity of summer droughts across much of Europe (Blenkinsop and Fowler, 2007; Lehner et al., 2006), therefore any interaction between effects of ozone pollution and reduced rainfall on plants is an important consideration when investigating effects of future ozone patterns. Although drought itself has been shown to reduce growth on grassland species (e.g. Bahrani et al., 2010), some studies have demonstrated that drought has a protective effect against ozone, for example reduced visible injury caused by ozone exposure (Loew et al., 2006). Effects of ozone pollution on vegetation have been shown to be more strongly related to flux of ozone into the plant, rather than to ozone concentrations in the surrounding air and a critical level approach using the modelled flux of ozone through the stomata has been developed (LRTAP Convention, 2010, Mills et al., 2011b). It has been proposed that drought can induce stomatal closure, therefore reducing ozone uptake and protecting against ozone damage (e.g. for *Populus spp.*, Silim et al., 2009). However, some recent results have shown that the response of the plant to ozone can interfere with the signalling process that induces stomatal closure in response to drought, reducing the ability to tolerate drought conditions (Mills et al., 2009; Wilkinson and Davies, 2010).

In this study we exposed mesocosms containing seven-species mixtures representing the Biodiversity Action Plan (BAP) priority community ‘Calcareous Grassland’ to eight simulated ozone regimes, with half of the mesocosms remaining well-watered and the other half receiving a reduced water regime during the ozone exposure, but remaining well-watered for the remainder of the experiment. The ozone regimes used were chosen to simulate previous, current and projected future ozone concentrations in remote rural areas and were applied in two successive growing seasons. Effects are reported for elevated ozone treatments using both the 24h mean ozone concentration and species-specific stomatal ozone flux (determined using the DO$_3$SE model, LRTAP Convention, 2010) as ozone metrics. We focus here on the flowering response and report the results of detailed flower counts made throughout the second exposure period. Thus, the overall aims of the study were to investigate whether ozone influenced the timing and number of flowers in this ecologically important community.

2. Materials and Methods

Ozone system and treatments

Plants were exposed to ozone in solardomes (hemispherical greenhouses 3m diameter, 2m tall). Ozone was generated from oxygen concentrated from air (Workhorse 8, Dryden Aqua, Edinburgh, UK) using an ozone generator (G11, Dryden Aqua, UK) and distributed to each solardome via PTFE tubing. Ozone was delivered to each solardome using mass flow controllers (Celerion, Dublin, Ireland) controlled by computer software (Labview version 7). Ozone concentrations were continuously monitored in one solardome using a dedicated ozone analyser (Thermo Electron, Waltham, MA, USA; Model 49C), allowing feedback to compensate for small variations in ozone production. In all solardomes, the ozone concentration was measured for 5 minutes in every 30 minutes using two additional ozone analysers (Envirotech, St Albans, UK; Model API 400A) of matched calibration.

Eight ozone treatments were randomly allocated to the solardomes, with one solardome used for each treatment. A weekly profile based on an ozone episode was used for each treatment. The treatments used were increments above and below a simulated ambient profile of peaks of +10 to +25 ppb on four days, followed by three days of low peaks (ca. 5 ppb) superimposed on a background of ca. 45 ppb, mimicking an ambient episode at Keenley, Northumberland, UK (20th-27th May 2008, Grid reference NY794562). The other seven treatments increased or decreased concentrations by -30, -20, -10, +10, +20, +30 and +40 ppb. The target weekly ozone profile is shown in Figure 1.

Mesocosm set-up

Mesocosms representing the BAP priority habitat 'Calcareous Grassland' were established in spring 2009 in 14 litre pots (33.3 cm diameter x 24.0 cm deep), lined with perforated plastic sheeting to deter roots from growing through the drainage holes in the base of the pot. Pots were filled with a mixture of topsoil (Humax, UK), sand and grit in the ratio 50:3:3 by volume. 200 g horticultural powdered lime (J. Arthur Bowers, UK) was added to each pot to increase the soil pH. Mesocosms were established using plug plants (British Wildflower Plants, UK) on 19th June 2009 and maintained in ventilated polytunnels until the experiment started. Each mesocosm contained *Briza media* (2 'plugs'), *Festuca ovina* (2 'plugs'), *Campanula rotundifolia*, *Sanguisorba minor*, *Scabiosa columbaria*, *Helianthemum nummularium* and *Lotus corniculatus* (1 'plug' of each), planted in an identical arrangement, and watered as required.

Mesocosms (10 replicates per solardome) were moved into the solardomes (at 20 ppb ozone) on 22nd July 2009, and the watering treatments were applied by hand from 17th July (before the ozone exposure started) until the end of the ozone exposure on 14th October, with five replicates of each watering regime per solardome. Soil moisture was continuously recorded in two well-watered mesocosms (WW) and two reduced water (RW) mesocosms using theta probes (ML2x, Delta-T, UK) attached to a datalogger (DL6, Delta-T, UK) and the mean soil moisture content was 32% and 21% for the WW and RW mesocosms respectively. All of the mesocosms were overwintered outdoors during 2009/10 and were exposed to a second 12-week ozone exposure in 2010 from 21st April to 15th July, with hand watering to provide the WW and RW regimes (mean soil moisture content 31% and 23% respectively).

Assessments of flowering

During the course of the ozone exposure in 2010 the numbers of flower buds and flowers were counted weekly for all species in each mesocosm, with the exception of the grass *Festuca ovina* (which had over 150 flowers per mesocosm and therefore could not be counted due to time constraints).

Stomatal conductance measurements and parameterisation of a flux-model

Stomatal conductance measurements were made on *L. corniculatus* (216 measurements), *C. rotundifolia* (205 measurements), *S. columbaria* (307 measurements), *B. media* (105 measurements) and *S. minor* (321 measurements) during the course of the two ozone exposures using a porometer (AP4, Delta-T), with corresponding measurements of soil moisture using a hand-held portable theta probe (ML2x probe attached to HH2 Moisture meter, Delta-T, UK), and using climatic measurements within the solardomes made using an on-site weather station. These measurements were made between 27th July and 8th October 2010, with some additional measurements made during June 2010, over a range of times and weather conditions, and were used to parameterise a stomatal flux-model for each of these species based on that described by Emberson et al., 2000 and LRTAP Convention (2010).

For the parameterisations for the modification of stomatal conductance by light, temperature, VPD and soil water potential (f_{light}, f_{temp}, f_{VPD} and f_{SWP}) respectively, the x-axis was subdivided into segments and for each segment the 90th centile for relative stomatal conductance was calculated. A physiologically relevant curve, as described in Emberson et al., (2000) was then fitted to these datapoints. The values of the constants calculated for these parameterisations are indicated in Table 1. The phenology function f_{phen} was considered to be 1 throughout the growing season and fO_3 (the modification of stomatal conductance due to the ozone concentration) was not included in the model as there was insufficient data to show a clear effect of ozone on stomatal aperture. G_{max} (the species-specific maximum stomatal conductance) was calculated for each species as the 95th centile of the stomatal conductance measurements. G_{min} (the minimum stomatal conductance) was considered to be $0.1*g_{max}$. These parameterisations were applied to the DO_3SE model (LRTAP Convention, 2010; Emberson et al., 2000) to calculate stomatal conductance (g_{sto}):

$$g_{sto} = g_{max} * [\min (f_{phen,} fO3)] * f_{light} * \max[f_{min}, (f_{Temp} * f_{VPD} * f_{SWP})] \qquad \text{[Eq.1]}$$

The stomatal flux of ozone ($F_{st}O_3$) was calculated according to the equation of Emberson et al. (2000), using a conversion factor of 0.663 to account for the ratio of the molecular diffusivity of ozone compared to that of water vapour (LRTAP Convention, 2010):

$$F_{st}O_3 = [O_3] * 0.663 * g_{sto} \qquad \text{[Eq.2]}$$

Calculations of stomatal fluxes were made using hourly averages of the variables needed for the model. It was assumed that for each species, light, VPD, and temperature were the same for each ozone and watering treatment. The hourly ozone fluxes were accumulated over a threshold of 1 nmol for daylight hours (POD_1, the Phytotoxic Ozone Dose) and were summed over the duration of both the first ozone exposure and the second exposure. This threshold was used as it was selected by ‘expert judgement’ in the determination of flux-based critical levels of ozone for trees and semi-natural vegetation within the LRTAP Convention, and agreed at a LRTAP Convention workshop held in 2009 (Mills et al., 2011b), and represents the detoxification capacity of the vegetation. POD_1 varied by 10-20% between the two seasons (depending on the species), and effects are presented against the mean POD_1 for the two exposure seasons.

Data analysis and statistics

Scatter plots of the number of flowers on each assessment date were used to determine the Julian date of peak flowering. All datasets were analysed using the solardome (O_3 treatment) mean values for each watering regime. Linear responses in the data were analysed using the General Linear Model (Minitab, version 14), using 24h mean ozone concentration or POD_1 and watering regime as inputs to the model, or by linear regression.

3. Results

Ozone exposure

In 2009 the ozone exposure ranged from a seasonal 24h mean of 15.6 ppb to 73.0 ppb whilst in 2010 the seasonal 24h mean ranged from 19.0 ppb to 73.3 ppb (Table 2). Mean temperature within the solardomes during the ozone exposure was 20.6°C in 2009 and 20.4°C in 2010, and mean humidity was 76.5% in 2009 and 68.6% in 2010.

Lotus corniculatus

Early season formation of flowers was accelerated with increasing ozone concentration for *L. corniculatus* during the second ozone exposure period. Increasing ozone concentration corresponded with a significantly earlier date on which 20% of the maximum number of flowers (used as a surrogate for the start of flowering) was reached ($p=0.017$; Figure 2a). The difference in the time taken to reach 20% of the maximum number of flowers varied across the range of ozone exposures by nine days in the WW treatment and by seven days in the RW treatment. In the early weeks of flowering for *L. corniculatus* this resulted in increased numbers of flowers in the higher ozone treatments. For example, on 27th May, after exposure to the ozone regime for five weeks in 2010, there was a linear increase in flower number with increasing ozone exposure for the WW treatment ($r^2=0.67$, $p=0.013$) and a non-significant increase for the RW treatment ($r^2=0.32$, $p=0.145$; Figure 2b). Despite the differences in flower number between treatments in the early weeks of flowering, there were no differences in the maximum number of flowers between ozone treatments for this species (Figure 4a). However, as there were no significant differences in the time taken to increase from 20% to either 50% or 90% of the maximum flower number with either the ozone or the watering regime (data not presented), the date on which the maximum number of flowers occurred during the second exposure season was increasingly earlier for *L. corniculatus* with increasing ozone exposure ($p=0.009$; Figure 3a), with the total range for the date of maximum flowering between treatments being 14 days. An increase in the mean ozone concentration from 30 ppb to 70 ppb corresponded with maximum flowering occurring six days earlier in both the WW and RW treatments. Flower numbers decreased to approximately 50% of the maximum number by the final assessment, after exposure for 11 weeks (6th July, data not presented).

For all ozone treatments, flowering was slightly later in the RW treatment compared to the WW treatment (Figure 3a), but this difference was not significant and there was no significant interaction between ozone and watering regime. The relationship between the date of maximum flowering and ozone concentration was linear for both watering regimes, with the correlation coefficient having an r^2 of 0.46 for WW plants and 0.45 for RW plants (Figure 3a). The difference in peak flowering date for plants in the WW compared to the RW treatment can be explained by ozone flux as there was a linear relationship between ozone flux and the date of peak flowering when the data were plotted together (r^2=0.49, p=0.002; Figure 3b). Based on 95% confidence intervals for this relationship, the POD_1 needed to give a significant change in flowering date was 2.5 mmol m^{-2}.

There was a decrease in the total number of flowers of approximately 50% in response in the RW treatment compared to WW (p=0.01; Figure 4a). However, there was no relationship between total flower number and ozone flux using either the actual flower number (data not presented) or the relative flower number, normalised to account for the influence of watering regime (Figure 4b).

Campanula rotundifolia

The maximum number of flowers of *C. rotundifolia* was significantly reduced with increasing ozone concentration (p=0.029; Figure 5a). Although there was no significant effect of watering regime, and no significant interaction between ozone and watering regime, the relationship between ozone concentration and maximum flower number was much stronger for plants of the WW treatment (r^2=0.63) compared to those of the RW treatment (r^2=0.20), although due to the low numbers of flowers, these statistics should be interpreted with caution. For the WW treatment, an increase in mean ozone concentration from 30 ppb to 70 ppb corresponded to a 40% decline in flower number. Combining both watering treatments, the decline in maximum flower number for *C. rotundifolia* showed a significant linear relationship with POD_1 (r^2=0.33, p=0.02; Figure 5b). Based on 95% confidence intervals for this relationship, the POD_1 needed to give a significant change in flower number was 12.2 mmol m^{-2}.

Scabiosa columbaria

For *S. columbaria* the total numbers of buds were used for analysis as this species flowers later and the end of the ozone exposure was before the maximum number of flowers was reached. Overall, the maximum number of buds showed a large decline with increasing ozone exposure (p=0.043; Figure 6a) in the WW treatment (r^2=0.65) but not in the RW treatment (r^2=0.04), although the statistics should be treated with caution due to the low numbers of buds per mesocosm. An increase in ozone concentration from 30 ppb to 70 ppb corresponded to a 20% decline in flower number in the WW treatment only. There was a reduction in maximum bud number in the RW compared to WW treatment that showed a strong trend (p=0.058) but there was no significant interaction between ozone and watering regime. When the number of buds was related to the calculated ozone flux there was a strong trend for a reduction in maximum bud number with increasing POD_1 (r^2= 0.19, p=0.096; Figure 6b), and no improvement to the relationship when the numbers of buds were normalised to account for differences due to the influence of the watering regime (data not presented). There were no significant effects of either ozone or watering regime on either the onset of flowering (using the date when 20% of the maximum number of buds recorded was reached) or the timing of peak bud number (data not presented).

Briza media, Sanguisorba minor* and *Helianthemum nummularium

The maximum number of flowers of *B. media* and *S. minor* showed no significant response to either watering regime or ozone (data not presented). There was also no effect of either ozone or drought on the timing of flowering for these species. *H. nummularium* flowered sporadically throughout the exposure season, but the low numbers of flowers meant that it was not possible to determine whether or not there were responses to ozone or watering regime. A summary table showing F-values and significance for the relationships between the maximum number of flowers for *L. corniculatus, C rotundifolia, S. columbaria, S. minor* and *B. media* in response to ozone, watering regime, the interaction between ozone and watering regime, and the relationship with ozone flux (POD_1) is shown in Table 3.

4. Discussion

The detailed flowering assessment regime of this study has revealed effects that may have been overlooked in previous studies where flower numbers have usually been counted on a single occasion. For example, counts of *L. corniculatus* flowers early in the exposure period indicated that there was a large effect of ozone on flower number, whereas subsequent counts revealed that rather than affecting the maximum flower number, the effect of ozone was to alter the timing of flowering in this species. Therefore, single assessments at different times in the growing season would have indicated different results.

This study has revealed species-specific effects of both drought and ozone which could potentially change the dynamics of calcareous grassland ecosystems. Of the six species that had flowering assessed during this study, *L. corniculatus, C. rotundifolia* and *S. columbaria* showed significant effects of increasing ozone on flower number or phenology. *H. nummularium* did not have sufficient flowers to show any trends. Only *B. media* and *S. minor* showed no effects of ozone. The high proportion of species from this community responding to ozone is of concern for the viability of this habitat in future ozone conditions. In addition, this study has shown that some species show an interaction between ozone and watering regime, whereas others do not. The combination of reduced water and increased ozone, as predicted in future ozone and climate scenarios, could therefore have a large effect on the numbers, composition and timing of flowering of plant communities such as calcareous grassland due to the species-specific responses. The linear relationships between the timing of flowering and numbers of flowers in response to ozone shown in this study, and evidence from a recent study indicating that in the UK 72% of lowland calcareous grassland occurred in regions where the AOT40 was greater than 6.5 ppm h (averaged over 1999 – 2003; Morrissey *et al.*, 2007) implies that changes in flowering number and phenology of species from native calcareous grassland habitats may already be occurring at current ambient compared to pre-industrial ozone concentrations.

The consequence of earlier flowering of a species in a community as a result of ozone exposure could be a lack of synchronicity with pollinating species. In a recent review of plant and pollinator phenology in response to climate change, Hegland et al. (2009) emphasised that, in many cases, both plant and insect phenology appear to be governed by temperature, so that they remain synchronized. When synchronization is not maintained, there can be severe consequences. For example, Kudo et al. (2004) found a mismatch between early flowering plants in Japan, which advanced their flowering time in a warm spring, and bumble bee emergence, which did not advance, resulting in a decreased seed-set in bumble bee pollinated plants. It has also been shown that for some species the abundance of other flowers before or during its own flowering can influence reproductive success due to competition for pollination (Brown et al., 2002). In addition to effects on the plant species, when plants and pollinators do not move in parallel, then it is predicted that a large proportion of pollinators may suffer population declines from a reduced diet breadth (Memmott et al., 2007). Studies on interactions between pollinators and plant phenology in response to ozone have not so far been carried out, but it is possible that these mismatches in synchronicity normally associated with climate change may also occur, with possible detrimental effects on both the plants and their associated pollinators as a consequence.

The current study has also shown large reductions in flower number in response to increased ozone exposure for *C. rotundifolia* and *S. columbaria*. This is in agreement with other studies that have shown reductions in flower numbers or flower biomass (e.g. Rämö et al., 2007). In addition to the response to increased ozone concentrations, the current study has also highlighted the differential response to drought of the component species of this community. A reduction in watering of 30% corresponded with reductions in flower number of 50% for *L. corniculatus* and 16% for *S. columbaria.* Reduced flower numbers would result in reduced chances of pollination for these species and could therefore reduce the reproductive success, thereby decreasing the long-term viability of these species within the plant community. Although these reductions in flowering could be a result of reduced resource availability, responses in reproductive structures do not always correspond to reductions in growth, but may be a result of reduced resource allocation. This is in contrast to the hypothesis of Saikkonen et al., 1998, who suggested that under stress conditions there would be increased allocation to reproductive structures. These large effects of drought on flower number also indicate that although it could be argued that reduced watering protects some species from the effects of ozone, for others e.g. *L corniculatus* and *S. columbaria* the severe effect of the drought itself far outweighs any benefit of a reduction in ozone flux.

For *L. corniculatus, C. rotundifolia* and *S. columbaria* there were significant (or nearly significant) relationships between POD_1 and the timing or number of flowers (p=0.002, 0.010 and 0.096 respectively), with the calculated fluxes incorporating the reduction in stomatal conductance due to drought. As there were also no significant interactions between ozone concentration and drought for any of the response parameters, the differences in flower numbers and timing reported can be attributed to ozone uptake in these species. Flowering of plants is controlled by complex and highly regulated signalling pathways. It is thought that for one of the pathways abscisic acid (ABA) affects hormone signalling processes in plants including the transition from the vegetative to reproductive phase (see review by Barth et al., 2006). Recent studies have shown that ozone reduces the responsiveness of plants to ABA (Mills et al., 2009; Wilkinson and Davies 2009, 2010), and this could potentially be happening in the flowering response. Effects of ozone crosstalk with the flowering signalling pathways are thus worthy of further investigation.

In this study the acceleration of flowering in *L. corniculatus* with an increase in mean ozone concentration from 30 to 70 ppb was six days. This suggests that the increases in ozone concentration expected over the next few decades may accelerate flowering in this, and possibly other species. In comparison, in Europe a comprehensive analysis of a large systematic phenological dataset has shown that the phenological response to climate change, based on temperature, shows an advance in spring/summer of 2.5 days per decade (Menzel et al., 2006). Although slightly smaller than the changes associated with predicted increases in temperature, the potential acceleration in timing of flowering in response to increases in ozone concentration could result in significant ecological impacts on plant communities, and should be studied further.

5. Conclusions

Increased ozone concentrations affected flower numbers and timing in calcareous grassland species. Decreased flower numbers for *C. rotundifolia* and *S. columbaria* may have resulted from decreased resource availability; however, an observed acceleration in the timing of maximum flowering for *L. corniculatus* may have been a consequence of crosstalk to one of the flowering signalling pathways. These effects on flowering were observed in the second consecutive ozone exposure, demonstrating the importance of longer-term studies to investigate responses. The results found suggest that increases in tropospheric ozone concentrations could have indirect effects on plant pollinators, although further studies would be needed to confirm this.

Acknowledgements

This work was supported by the UK Department of Environment, Food and Rural Affairs (Project Reference number CPEA 33). We would like to thank Aled Williams Mechatronics for engineering support of the solardome system.

References

Andersson C, Enghardt M (2010) European ozone in a future climate: Importance of changes in dry deposition and isoprene emissions. Journal of Geophysical Research 115: D02303.

Bahrani MJ, Bahrami H, Haghighi AAK (2010) Effect of water stress on ten forage grasses native or introduced to Iran. Grassland Science 56, 1-5.

Barth C, De Tullio M, Conklin PL (2006) The role of ascorbic acid in the control of flowering time and the onset of senescence. Journal of Experimental Botany 57(8): 1657-1665.

Batty K, Ashmore MR. (2003) Lower threshold ozone exposures for effects on roots than shoots in wetland species. In: Karlsson et al. (Eds), Establishing ozone critical levels II, 2003 UNECE Workshop Report. IVL report B1523 IVL Swedish Environmental Research Institute, Gothenburg, Sweden. pp. 199–203.

Black VJ, Black CR, Roberts JA, Stewart CA (2000) Impact of ozone on the reproductive development of plants. New Phytologist 147, 421-427

Blenkinsop S and Fowler HJ (2007) Changes in drought frequency, severity and duration for the British Isles projected by the PRUDENCE regional climate models. Journal of Hydrology 342(1-2): 50-71.

Brown BJ, Mitchell RJ, Graham SA (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. Ecology 83(8): 2328-2336

Chappelka AH (2002) Reproductive development of blackberry (*Rubus cuneifolius*), as influenced by ozone. New Phytologist 155: 249-255

Cooley DR and Manning WJ (1987) The impact of ozone on assimilate partitioning in plants – a review. Environmental Pollution 47, 95-113.

Darbah JNT, Kubiske ME, Nelson N, Oksanen E, Vaapavuori E and Karnosky DF (2007) Impacts of elevated atmospheric CO_2 and O_3 on paper birch (*Betula papyrifera*): reproductive fitness. Scientific World Journal 7 Supplement 1: 240-6

Derwent RG, Simmonds PG, Manning AJ, Spain TG (2007) Trends over a 20-year period from 1987 to 2007 in surface ozone at the atmospheric research station, Mace Head, Ireland. Atmospheric Environment 41, 9091-9098.

Emberson LD, Ashmore MR, Cambridge HM, Simpson D, Tuovinen JP (2000) Modelling stomatal ozone flux across Europe. Environmental Pollution 109, 403-413

Franzaring J, Tonneijck AEG, Kooijman AWN and Dueck TA (2000) Growth responses to ozone in plant species from wetlands. Environmental and Experimental Botany 44: 39-48

Gimeno BS, Bermejo V, Sanz J, de la Torre D and Elvira S (2004a) Growth response to ozone of annual species from Mediterranean pastures. Environmental Pollution 132: 297-306

Gimeno BS, Bermejo V, Sanz J, de la Torre D and Gil JM (2004b) Assessment of the effects of ozone exposure and plant competition on the reproductive ability of three therophytic clover species from Iberian pastures. Atmospheric Environment 38, 2295-2303

Hayes F, Mills G, Williams P, Harmens H and Büker P (2006) Impacts of summer ozone exposure on the growth and overwintering of UK upland vegetation. Atmospheric Environment 40: 4088-4097

Hayes F, Mills G and Ashmore M. (2009) Effects of ozone on inter- and intra-species competition and photosynthesis in mesocosms of *Lolium perenne* and *Trifolium repens.* Environmental Pollution 157(1): 208-214.

Hayes, F, Mills G, Jones MLM and Ashmore M (2010) Does a simulated upland community respond to increasing background, peak or accumulated exposure of ozone? Atmospheric Environment 44(34): 4155-4164.

Hayes F, Mills G, Harmens H, Wyness K (2011) Within season and carry-over effects following exposure of grassland species mixtures to increasing background ozone. Environmental Pollution 159: 2420-2426.

Hegland SJ, Nielsen A, Lazaro A, Bjerknes AL, Totland O (2009) How does climate warming affect plant-pollinator interactions? Ecology Letters 12(2): 184-195

IPCC 2007. Climate Change 2007: The physical science basis. In Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor.

Kudo G, Nishikawa Y, Kasagi T, Kosuge S (2004) Does seeds production of spring ephemerals decrease when spring comes early? Ecological Research 19(2): 255-259.

Lehner B, Doll P, Alcamo J, Henrichs T, Kaspar F (2006) Estimating the impact of global change on flood and drought risks in Europe: A continental, integrated analysis. Climatic Change 75(3): 273-299

Leisner P, Ainsworth EA (in press). Quantifying the effects of ozone on plant reproductive growth and development. Global Change Biology doi: 10.1111/j.1365-2486.2011.02535.x.

Loew M, Herbinger K, Nunn AJ, Haberle KH, Leuchner M, Heerdt C, Werner H, Wipfler P, Pretzsch H, Tausz M, Matyssek R. (2006) Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*). Trees-Structure and Function 20(5): 539-548

LTRAP Convention (2010) Manual on methodologies and criteria for modelling and mapping Critical Loads and Levels and air pollution effects, risks and trends. Chapter 3: Mapping critical levels for vegetation. 2010 revision of 2004 document. http://www.icpmapping.org.

Meehl GA, Stocker TF, Collins WD et al. (2007) In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Eds: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL), pp. 747–846. Cambridge University Press, Cambridge, UK/New York, NY, USA.

Memmott J, Craze PG, Waser NM, Price MV (2007) Global warming and the disruption of plant-pollinator interactions. Ecology Letters 10(8): 710-717.

Menzel A, Sparks TH, Estrella N, Koch E, Asa A, Ahas R, Alm-Kubler K, Bissolli P et al., (2006) European phenological response to climate change matches the warming pattern. Global Change Biology 12(10): 1969-1976

Mills G, Hayes F, Simpson D, Emberson L, Norris D, Harmens H and Büker P (2011a) Evidence of widespread effects of ozone on crops and (semi-)natural vegetation in Europe (1990 – 2006) in relation to AOT40– and flux-based risk maps. Global Change Biology, 17, 592-613

Mills G, Hayes F, Wilkinson S and Davies WJ (2009) Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. Global Change Biology 15: 1522-1533

Mills G, Pleijel H, Braun S, Büker P, Bermejo V, Calvo E, Danielsson H, Emberson L, González Fernández I, Grünhage L, Harmens H, Hayes F, Karlsson PE, Simpson D (2011b) New stomatal flux-based critical levels for ozone effects on vegetation. Atmospheric Environment 45**,** 5064-5068.

Morrissey T, Ashmore MR, Emberson LD, Cinderby S, Büker P (2007). The impacts of ozone on nature conservation. JNCC Report 403, Joint Nature Conservation Committee, Peterborough.

Rämö K, Kanerva T, Ojanperä K and Manninen S (2007) Growth onset, senescence, and reproductive development of meadow species in mesocosms exposed to elevated O_3 and CO_2. Environmental Pollution 145: 850-860

Royal Society (2008) Ground-level ozone in the 21st century: future trends, impacts and policy implications. Science Policy Report 15/08. The Royal Society, London, UK.

Saikkonen K, Koivunen S, Vuorisalo T, Mutikainen P. (1998) Interactive effects of pollination and heavy metals on resource allocation in *Potentilla anserina* L. Ecology 79(5): 1620-1629

Sanz J, Bermejo V, Gimeno BS, Elvira S and Alonso R (2007) Ozone sensitivity of the Mediterranean terophyte *Trifolium striatum* is modulated by soil nitrogen content. Atmospheric Environment 41: 8952-8962

Silim S, Nash R, Reynard D, White B, Schroeder W. (2009) Leaf gas exchange and water potential responses to drought in nine poplar (*Populus* spp.) clones with contrasting drought tolerance. Trees – Structure and Function 23(5): 959-969

Solberg S, Bergström R, Langner J, Laurila T and Lindskog A (2005) Changes in Nordic surface ozone episodes due to European emission reductions in the 1990s. Atmospheric Environment 39: 179-192

Taylor MD, Sinn JP, Davis DD and Pell EJ (2002) The impact of ozone on salt marsh cordgrass (*Spartina alterniflora*). Environmental Pollution 120: 701-705

Tripathi OP, Jennings SG, O'Dowd CD, Coleman L, Leinert S, O'Leary B, Moran E, O'Doherty SJ, Spain TG (2010) Statistical analysis of eight surface ozone measurement series for various sites in Ireland. Journal of Geophysical Research-Atmospheres 115, article number D19302.

Vingarzan R (2004) A review of surface ozone background levels and trends. Atmospheric Environment 38: 3431-3442

Volz A and Kley D (1988) Evaluation of the Montsouris series of ozone measurements made in the nineteenth century. Nature 332: 240-242

Wilkinson S and Davies WJ (2009) Ozone suppresses soil drying- and abscisic acid (ABA)-induced stomatal closure via an ethylene-dependent mechanism. Plant Cell and Environment 32: 949-959

Wilkinson S, Davies WJ (2010) Drought, ozone, ABA and ethylene: new insights from cell to plant to community. Plant Cell and Environment 33(4): 510-525

Table 1: Values of the constants used for parameterisation of the stomatal flux model for *L. corniculatus, C. rotundifolia*, *S. columbaria*, *B. media* and *S. minor*.

Parameter	Units	*L. corniculatus*	*C. rotundifolia*	*S. columbaria*	*B. media*	*S. minor*
g_{max}	mmol m^{-2} (H$_2$0)	246	550	660	210	900
T_{min}	°C	12	15	11	1	11
T_{opt}	°C	22	26	26	23	26
T_{max}[1]	°C	32	35	35	30	35
VPD_{max}	kPa	2.1	2.1	2.1	2.1	2.1
VPD_{min}	kPa	3.5	3.5	3.5	3.5	3.5
L	Constant	-0.007	-0.007	-0.007	-0.007	-0.007
SWP_{max}	MPa	0	0	0	0	0
SWP_{min}	MPa	-0.03	-0.60	-0.45	-0.25	-0.45

[1] Outside the range of temperature measurements made and interpolated from available data.

Table 2: Season 24h mean ozone concentrations for each ozone treatment in 2009 and 2010,

Ozone treatment	2009 Season 24h mean ozone, ppb	2010 Season 24h mean ozone, ppb
AA-30	15.6	19.0
AA-20	23.2	25.5
AA-10	31.7	34.8
AA	40.3	40.8
AA+10	50.1	51.2
AA+20	57.4	60.3
AA+30	68.8	66.2
AA+40	73.0	73.3

Table 3: F-values for the maximum number of flowers of selected species, using General Linear Model, in response to ozone, watering regime and the interaction between ozone and watering regime, and relationship between maximum number of flowers and time integrated ozone flux (POD_1) using regression analysis **, * and (*) indicate significant differences at $p<0.01$, $p<0.05$ and $p<0.1$ respectively.

Species	Ozone	Watering regime	Interaction between ozone and watering	Ozone flux, POD_1
L. corniculatus	0.15	10.19 **	0	2.93
C. rotundifolia	6.17 *	0.27	0.08	6.89 *
S. columbaria	5.12 *	4.38 (*)	2.10	3.19 (*)
S. minor	0.12	1.95	0.02	0
B. media	2.18	0.20	0.53	2.40

Figure 1: The target weekly ozone regime used in the solardomes in 2009 and 2010.

Figure 2: (a) The Julian date when flower number reached 20% of maximum in *L. corniculatus* in response to ozone concentration in both the WW and RW treatments and (b) Mean number of flowers per mesocosm on 27th May (after 5 weeks of exposure in 2010).

Figure 3: Julian date of maximum flower number for *L. corniculatus* in response to (a) ozone concentration and (b) ozone flux in both the WW and RW treatments.

Figure 4: Maximum flower number in the WW and RW treatments for *L. corniculatus* in response to (a) ozone concentration and (b) in relation to ozone flux, normalised for the effect of watering regime.

Figure 5: Maximum flower number for *C. rotundifolia* in the WW and RW treatments in relation to (a) ozone concentration and (b) ozone flux.

Figure 6: Maximum flower number in the WW and RW treatments for *S. columbaria* in relation to (a) ozone concentration and (b) ozone flux.

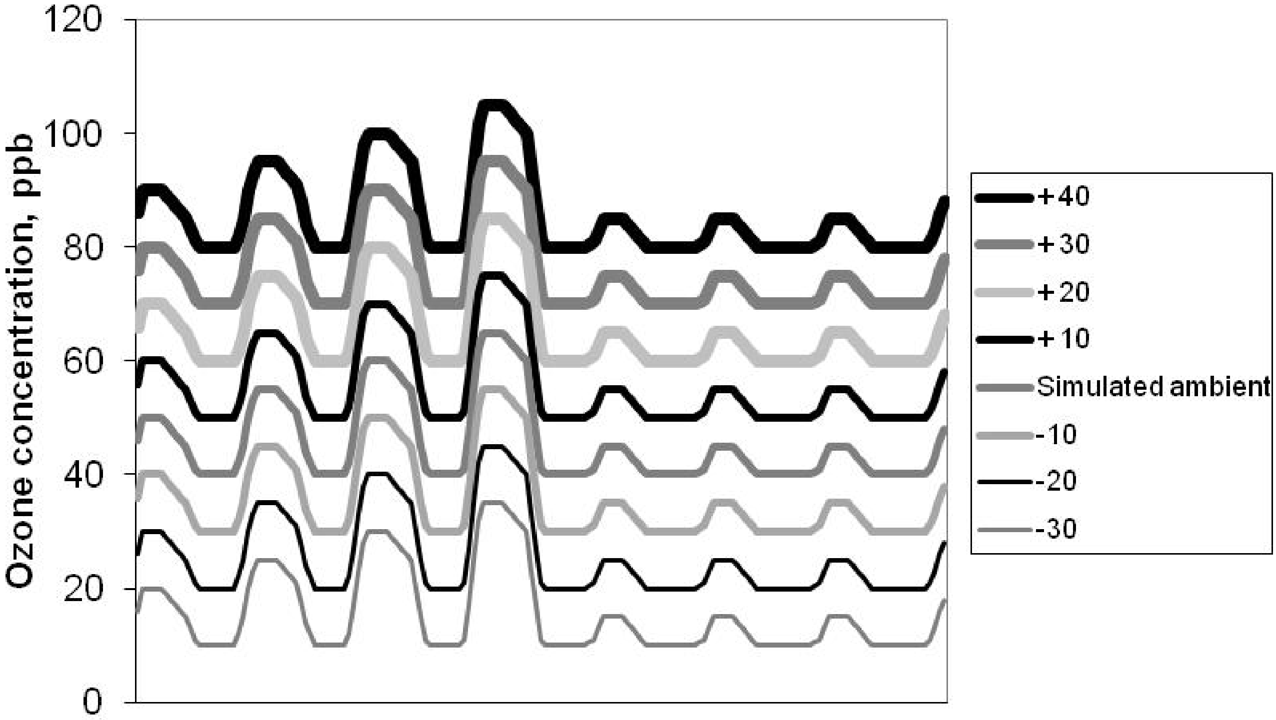
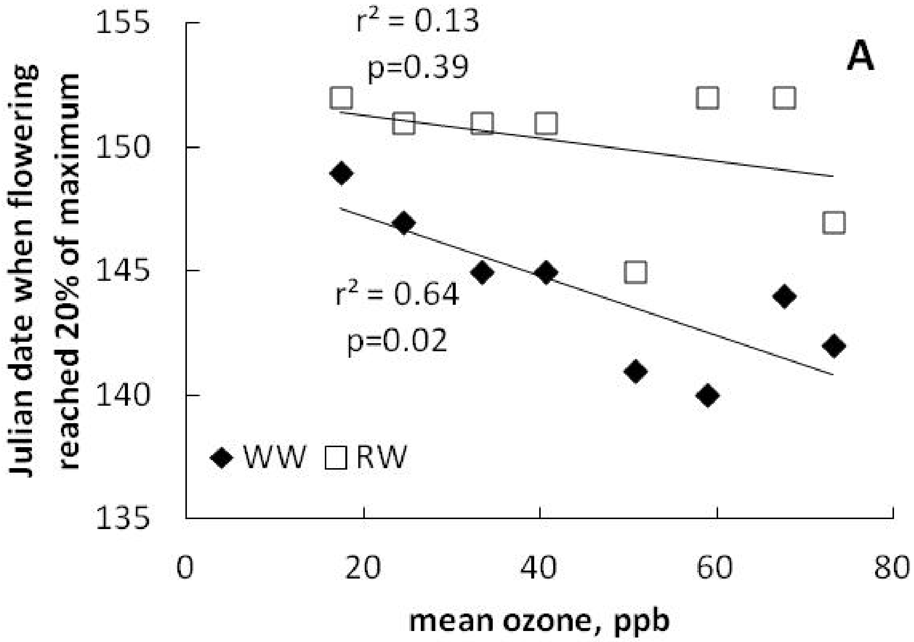


Figure 1: The target weekly ozone regime used in the solardomes in 2009 and 2010.



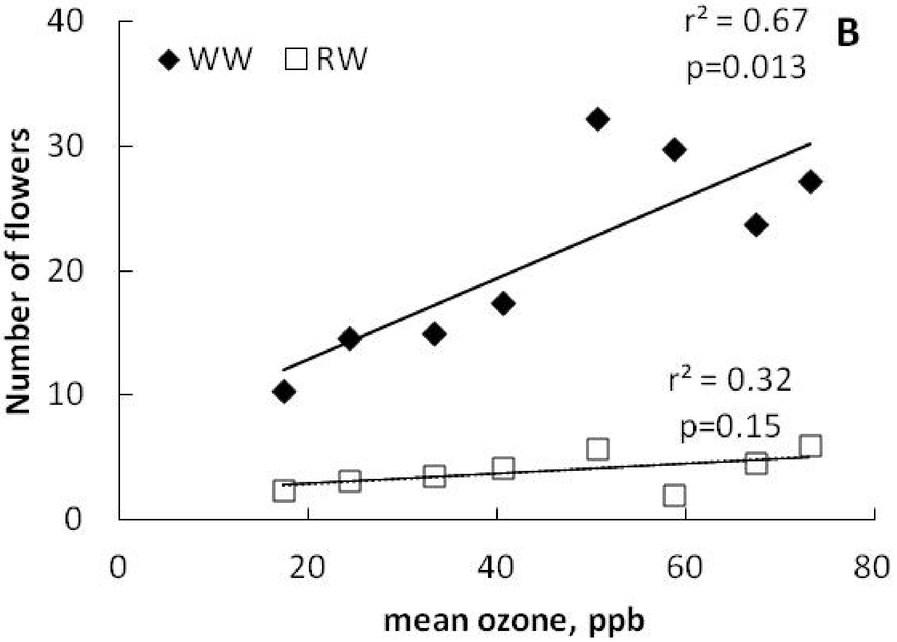


Figure 2: (a) The Julian date when flower number reached 20% of maximum in *L. corniculatus* in response to ozone concentration in both the WW and RW treatments and (b) Mean number of flowers per mesocosm on 27th May (after 5 weeks of exposure in 2010).

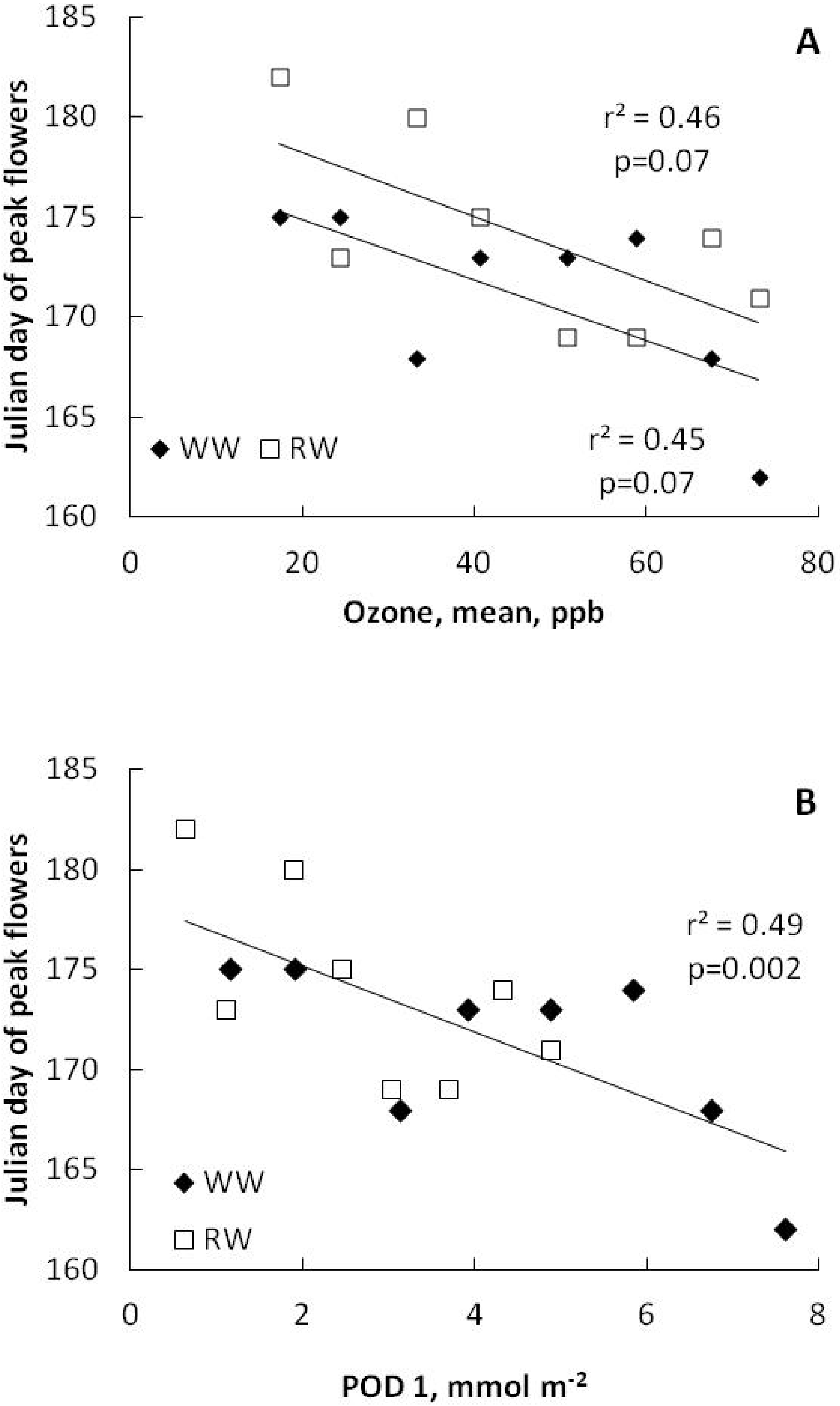


Figure 3: Julian date of maximum flower number for *L. corniculatus* in response to (a) ozone concentration and (b) ozone flux in both the WW and RW treatments.

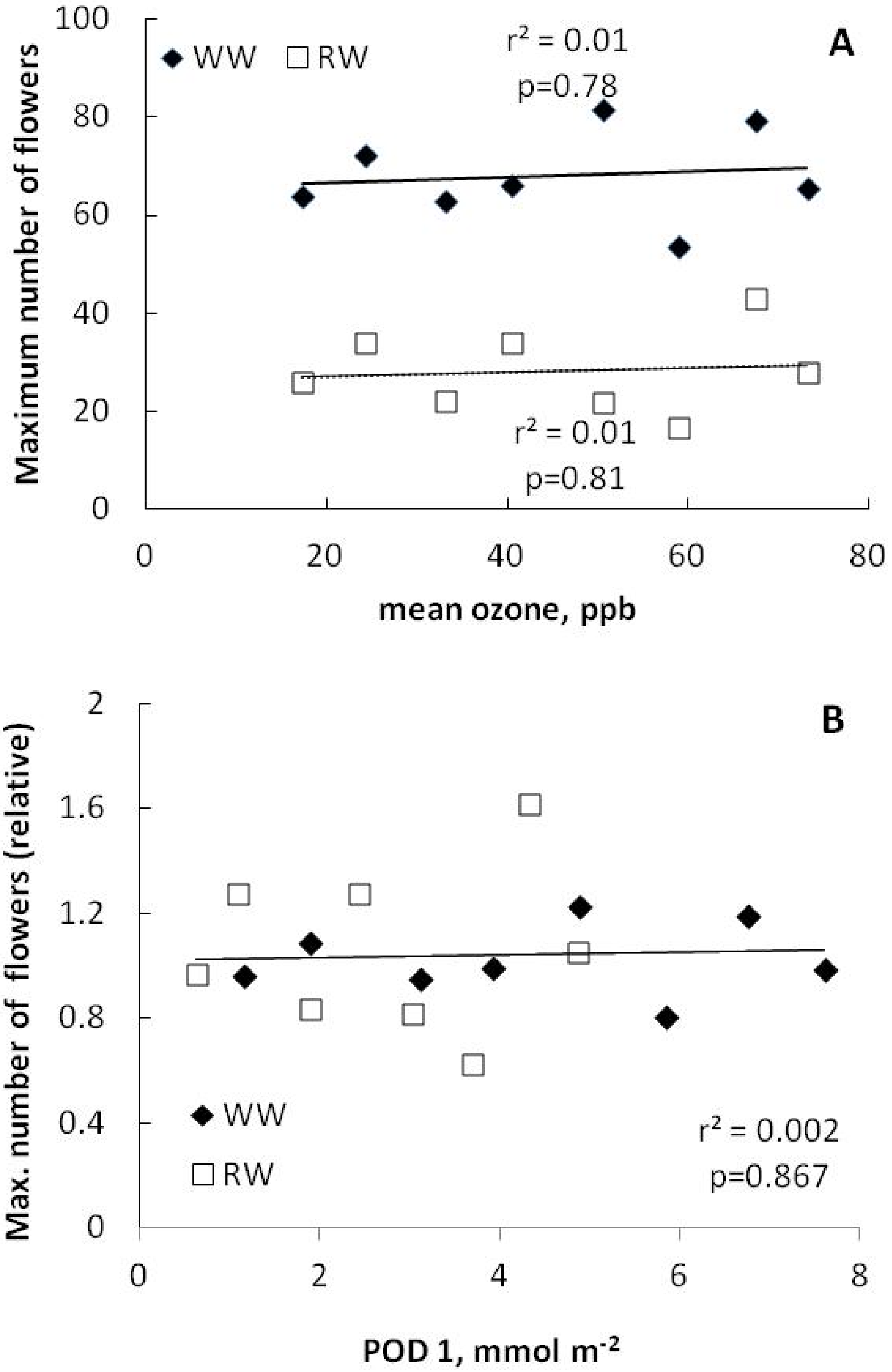


Figure 4: Maximum flower number in the WW and RW treatments for *L. corniculatus* in response to ozone concentration and (b) in relation to ozone flux, normalised for the effect of watering regime.

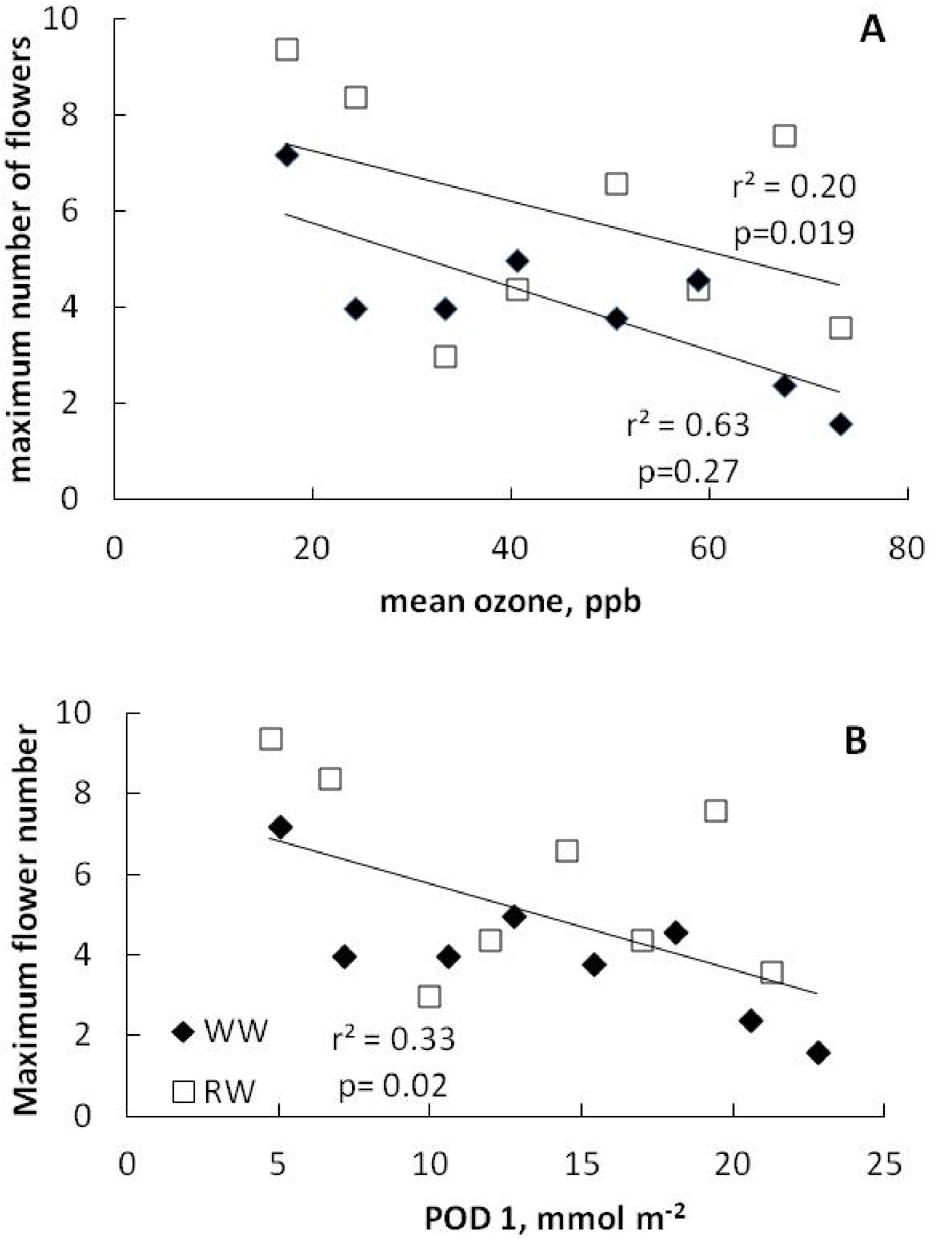


Figure 5: Maximum flower number for *C. rotundifolia* in the WW and RW treatments in relation to (a) ozone concentration and (b) ozone flux.

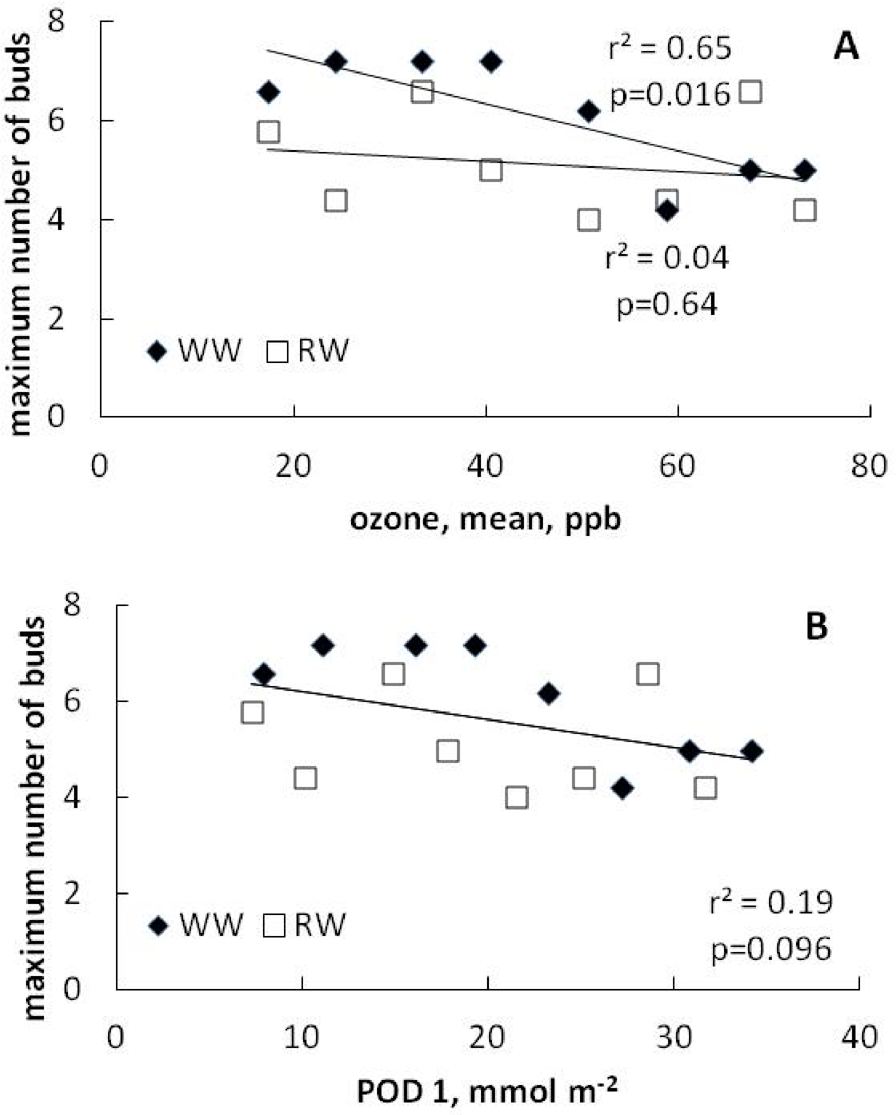


Figure 6: Maximum flower number in the WW and RW treatments for *S. columbaria* in relation to (a) ozone concentration and (b) ozone flux.