OZONE EFFECTS ON THE BIOMASS, NODULATION AND NITROGENASE ACTIVITY OF CLOVER CULTIVARS

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How do you make a cow?

Amongst other things, 1kg of beef needs roughly:

• 460 L drinking water
• 43kg grain/hay
• 46,000 L water to make the feed
• 25g of N per kg of meat
• 700kg cow ~ 17.5kg N

This N must be obtained from the pasture forage
A temperate pasture may require >100kg/ha⁻¹ yr⁻¹ of N input to meet the demands of grazing livestock.

• Some will be deposited (UK total N deposits <10kg - >21kg/ha yr⁻¹)

• Applied in fertilisers

• Obtained via clover (Trifolium spp.)
• Bare symbioses with N-fixing rhizobia in organs called root nodules in N-limited conditions.

• Legumes are the primary source of protein for a substantial proportion of human population.

• Importance of legumes in agriculture known for millennia
Ozone concentrations have increased since pre-industrial era

- Ground level ozone most damaging air pollutant
- Causes multi-billion $ losses annually in a range of important arable crops.
- Further increases predicted during cause of this century
• Numerous studies show ozone may affect reductions in composition yield, & forage quality in temperate pasture

• Ozone shown to reduce nodulation/fixation in number of legumes

• Modern cultivars of clover
Solardome study

• Studies based at the CEH solardome facility (nr Bangor)

• Test of modern clover cultivars recommended for general use in grazed pasture (British Grassland Society, 2012).

• White clover (*Trifolium repens* cv. Crusader)
• Red Clover (*Trifolium pratense* cv. Merviot)

At start of experiment

Crusader

Merviot
Broad aim to quantify physiological impacts on the cultivars.

- Plants exposed in to 7 ozone scenarios (with a unique scenario per dome).
- Scenarios based on a profile recorded at Aston Hill monitoring station (Flintshire, UK).
- 6 replicates per scenario; exposed for 12 weeks
Ozone injury and stomatal conductance (gs)

Ozone injury

- 3 week AOT40 ppm h⁻¹
  - R² = 0.49
  - P = 0.01
  - R² = 0.87
  - P < 0.001

- 3 Month AOT40ppm h⁻¹
  - Ozone injury and stomatal conductance (gs)
  - ● = Merviot
  - ○ = Crusader

- R² = 0.2108
  - P = 0.09
- R² = 0.0004
  - P = 0.54
Shoot biomass

\[ R^2 = 0.01 \quad P = 0.34 \]

\[ R^2 = 0.2728 \quad P = 0.13 \]

Root biomass

\[ R^2 = 0.96 \quad P < 0.01 \]

Root:shoot

\[ R^2 = 0.98 \quad P < 0.01 \]

● = Merviot
○ = Crusader
Nodule biomass

Nodules per pot

Mass per nodule

Nodule mass per pot

● = Merviot
○ = Crusader
Nodule size

Crusader

Merviot

* \( P < 0.05 \)

Nodules
- 0.7-1.5mm
- <0.1mm-0.7mm

AOT40 ppm h\(^{-1}\)

Node number

Nodule number

0.5
5.2
18.6

0
10
20
30
40
50
60
70
80
90
In-situ nitrogenase activity (Acetylene reduction assays)

**Week 10**

Low ozone

High ozone

*P* < 0.1

**Week 11**

*P* < 0.05
What does this mean for the UK?

Using 10 x 10km grid square and AOT40 values for pasture-growing areas of the UK*, in a high ozone year (2006), there was potentially:

April – June: a mean 12% reduction in nodule mass
July – September: a mean 8% reduction in nodule mass

* Data from Mhairi Coyle, in Mills et al., 2011, O3 and food security in the UK report for Defra
Summary and conclusions

• Systemic reductions in below ground biomass and nodulation, and reduction in nitrogenase activity.

• Perhaps arising from a reduction in the translocation of assimilate.

• Continuing increases in the level of ozone may cause lasting declines in pasture fertility and productivity.
This growing season......

- Mixed community of high sugar Ryegrass (‘AberMagic’) and Crusader
- 6 O$_3$ treatments +/- Intermittent drought
- Sequential harvests and nitrogen fixation assays (= 220 pots!).
- Paper close to submission
Special thanks to Aled Williams, Anni Gibeif, Steve Hughes, Harry Harmens, Chris Evans, Peter Wieland, Vicent Calatayud, Serena Wagg and Chiara Premoli for their advice and assistance. This is a NERC funded project (project code: NEC04456).
Nodules g$^{-1}$

- $R^2 = 0.99$, $P = 0.01$
- $R^2 = 0.54$, $P = 0.07$

Nodule mass g$^{-1}$

- $R^2 = 0.95$, $P = 0.01$
- $R^2 = 0.17$, $P = 0.43$