

# Smoke-Stimulated Germination in Heathland Plants

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University  
of Exeter

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# Background

Arrested succession – heathlands require management.

Unmanaged heath reverts to woodland over time.  
Burning, grazing, and mowing can halt this succession.

Vegetative regrowth does not add genetic diversity, but germination does.

Management strategies that encourage germination are beneficial.

- Post-fire conditions can be beneficial to plants.
- Small periodic fires may reduce the danger posed by future fires.
- Heathlands are UK's most flammable ecosystems.
- Both smoke and heat can impact germination:
  - Extreme heat can rupture seed cases
  - Plant smoke contains karrikins, water-soluble compounds that aid germination



## This project:

- Looking for fire adaptations in UK heathland plants.
- Investigating if smoke stimulates germination.
- Comparing seeds from multiple sites with a range of burning histories.
- Exploring the impact of controlled burning.

# Summary of Work to Date

## Trials on agar in petri dishes

- Mixed seed trials
  - 2 trials
  - 4 species per plate
  - Control and 5 different concentrations of smoke solution
  - Sterilised vs non-sterilised seeds
  - *Calluna vulgaris*, *Molinia caerulea*, *Deschampsia flexuosa*, *Eriophorum angustifolium*
- Single species trials
  - *Calluna vulgaris*, 2 trials
  - *Erica cinerea*, 1 trial
  - 22 seeds per plate
  - Control and 5 different concentrations of smoke solution

## Trials on soil

- *Molinia caerulea*
  - 1 trial
  - 5 seeds per cell, 24 cells per treatment
  - Control and 0.06...% smoke solution

## Trials on filter paper in glass vials

- *Calluna vulgaris*
  - 1 trial
  - 5 seeds per vial, 34 vials per treatment
  - Control and 0.06...% smoke solution

## Smoke solution creation

- Solutions created by passing plant smoke through water
  - *Calluna vulgaris*
  - *Erica tetralix*
  - *Erica cinerea*
  - *Molinia caerulea*
  - *Ulex europaeus* and *Ulex gallii*
  - *Pteridium aquilinum*

## Fieldwork and collections

- Seed collection
  - New Forest
  - Wareham Forest
  - Dartmoor
- Woodbury Common (Plant material)



# Smoke Solutions

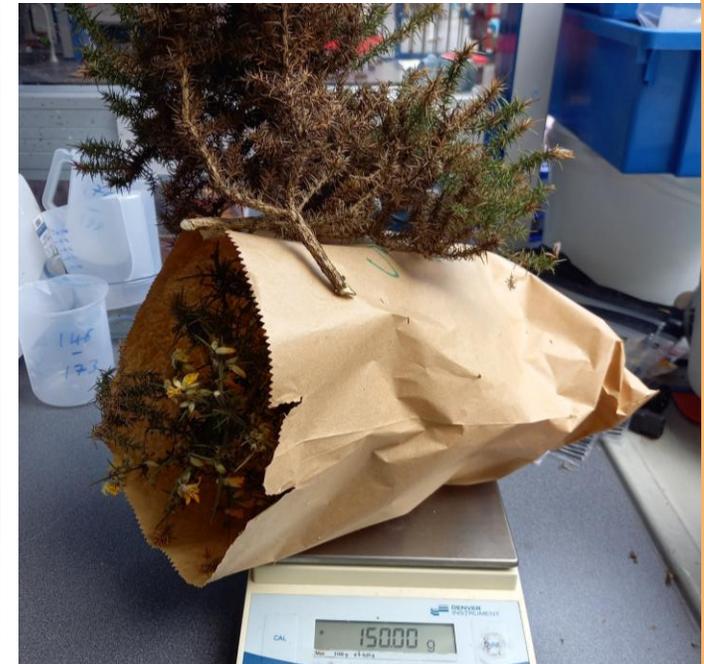
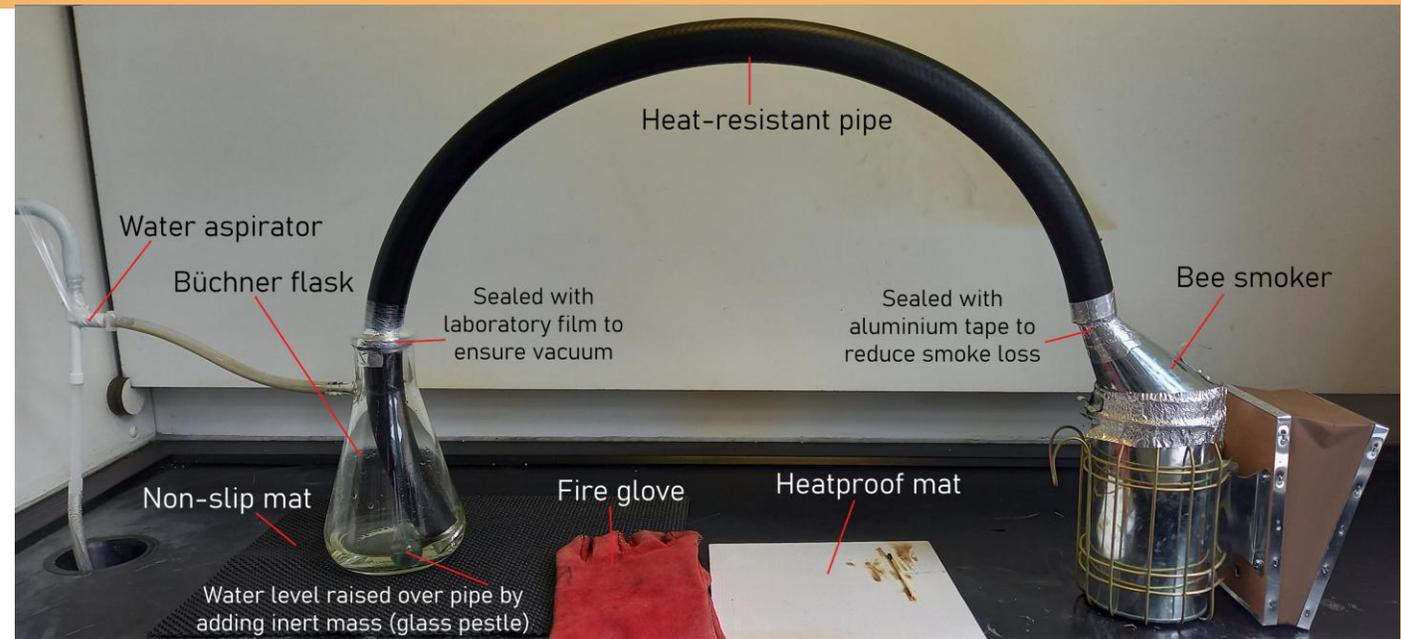
To simulate the effects of smoke and eliminate the effects of heat, aqueous smoke solutions were made using a method similar to Coons et al., 2014.

The heathland plant material burnt was gathered on Woodbury Common, just outside of Exeter, Devon.

Species collected were separated and made into the following solutions:

- *Calluna vulgaris*
- *Erica tetralix*
- *Erica cinerea*
- *Molinia caerulea*
- *Ulex europaeus* and *Ulex gallii*
- *Pteridium aquilinum*

The solution used in most of the experiments was an equal parts mix of *Calluna*, *Erica tetralix*, and *Ulex sp.*





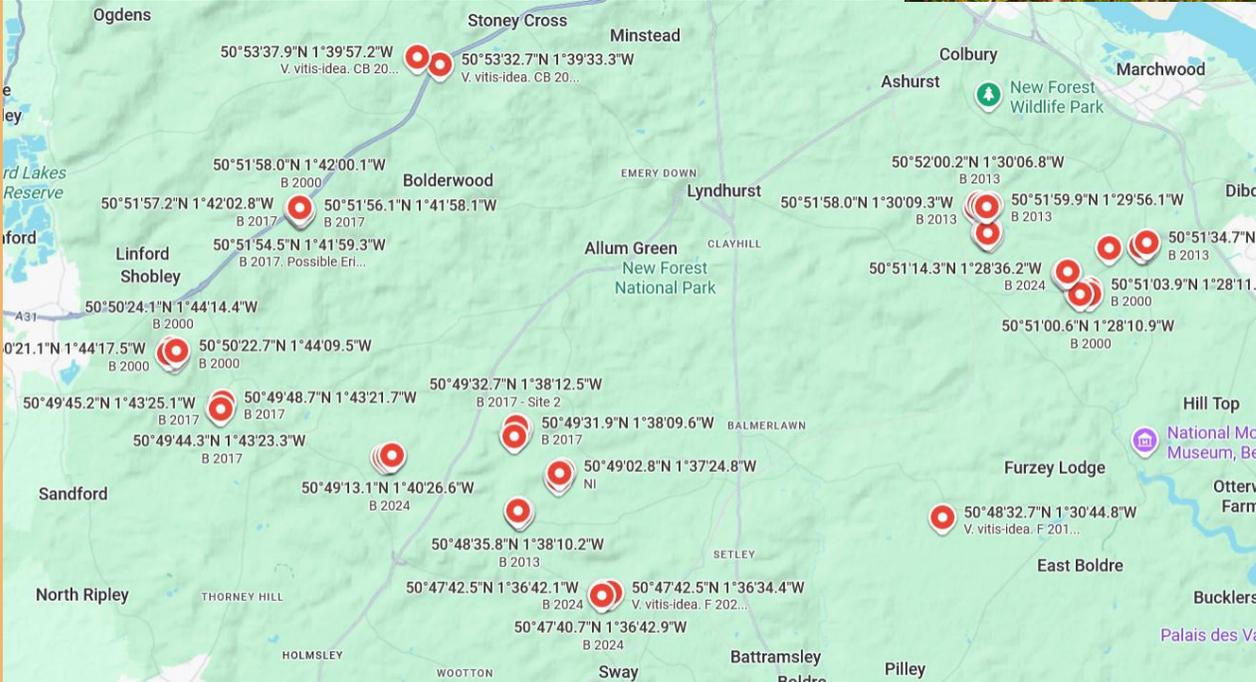
*Calluna vulgaris*



*Erica tetralix*



*Erica cinerea*



*Molinia caerulea*



*Cytisus scoparius*



*Vaccinium myrtillus*

# New Forest Fieldwork

- Seeds collected from sites chosen for burn history
- Paired sites, one site with and another without burning
- Species collected:
  - *Calluna vulgaris*
  - *Erica tetralix*
  - *Erica cinerea*
  - *Molinia caerulea*
  - *Cytisus scoparius* (Turbury Common)
  - *Vaccinium myrtillus* (outside sites)
- Could not find any *Vaccinium vitis-idaea* in the New Forest
- Dried in drying oven then stored protected from light at room temperature.



# Mixed Seed Trials

- Two 8-week mixed seed experiments on agar
- Seeds from Peak District
- Grew
  - *Calluna vulgaris*
  - *Eriophorum angustifolium*
  - *Molinia caerulea*
  - *Deschampsia flexuosa*in marked sections on agar plates
- Treated the plates with five concentrations of smoke solution and a control (water)
- The grasses, *Deschampsia* and *Molinia*, germinated quicker and more reliably than the heather *Calluna*
- No signs of germination from *Eriophorum*



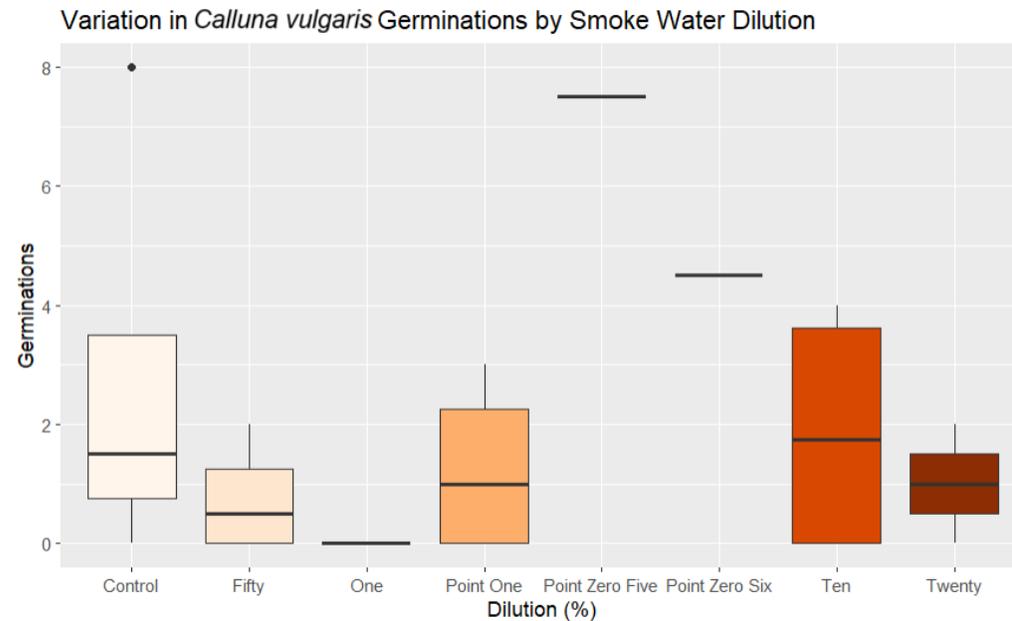
Seen growing: *Molinia caerulea*



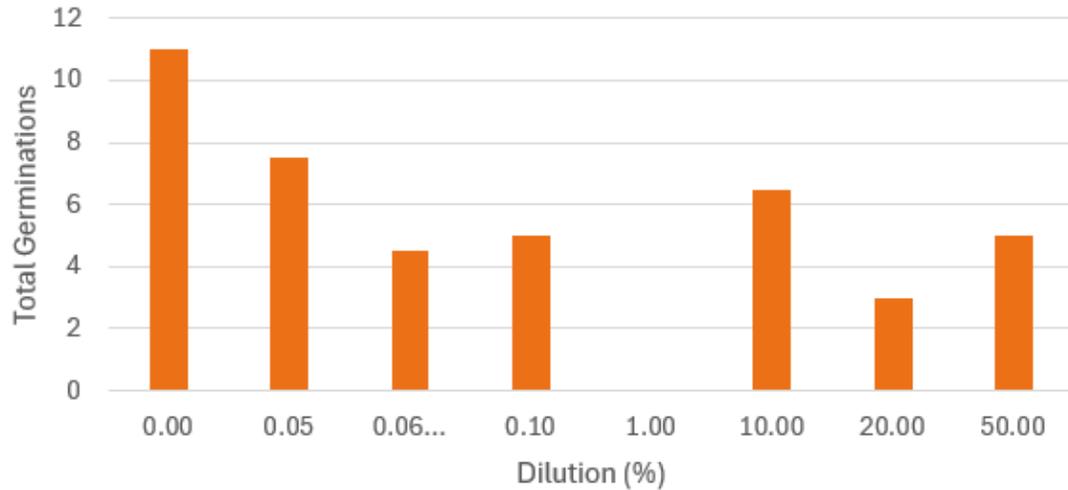


# *Calluna vulgaris*

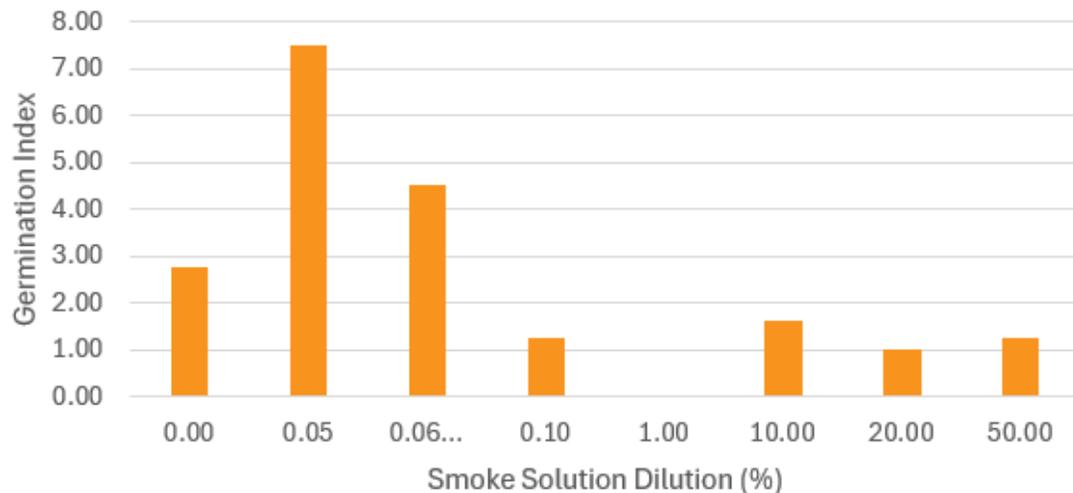
- Seeds from Peak District
- Included in the two 8-week mixed seed experiments on agar
- Two more 8-week experiments with only *Calluna* on agar
- One 8-week experiment with only *Calluna* on filter paper in vials
- Started by testing different dilutions of smoke solution to find the most effective, moved on to using 1:1500 dilution vs water



Total Germinations of *Calluna vulgaris* at Different Dilutions of Smoke Solution



Germination of *Calluna vulgaris* at Different Dilutions of Smoke Solution



# Calluna Data

- Germination Index =  $\frac{\text{Number of germinations}}{\text{Tests at that dilution}}$
- Some dilutions have been used in more tests than others.
- When accounting for the number of tests a dilution has been used in, the 0.05% dilution is more effective than the control.
- Actual number of germinations (all treatments) over four separate experiments was 43.5/912, or a ~4.8% germination rate overall.
- There was more variance in some dilutions than others.
- The 1% dilution never produced any germinations.
- Number of final day germinations per experiment was used to produce this box plot.
- More tests are needed at 0.05% and 0.06...% to properly compare them.



## *Erica cinerea*

- Seeds from New Forest
- One extended experiment with only *Erica cinerea*
- No germinations at the normal 8-week end point
- Mostly no germinations
- Only heather experiment to produce full seedlings
- Two of 396 seeds germinated:
  - Control condition
  - 1:2000 dilution

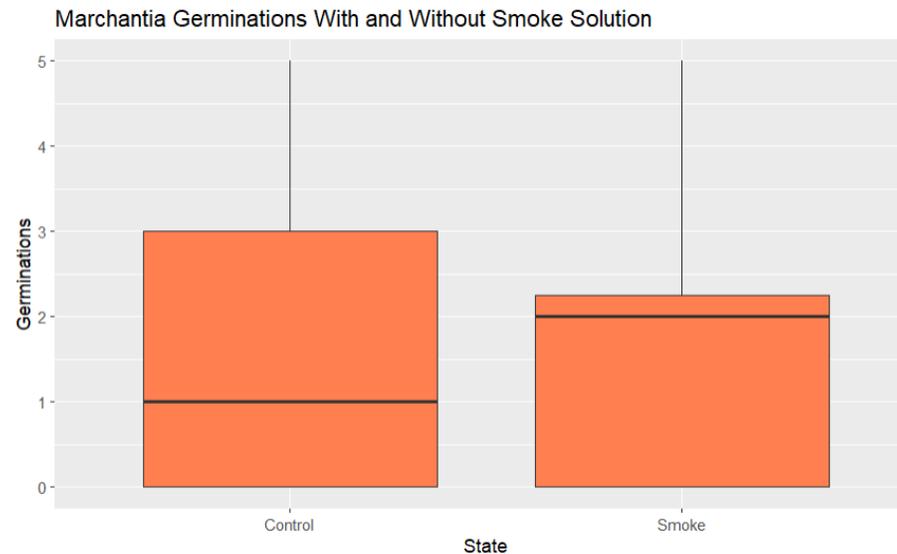


# *Molinia caerulea*

- Seeds from Peak District (on agar) and New Forest (on soil)
- Two 8-week mixed seed experiments on agar plates
- One soil-based experiment Four trays, 12 cells per tray, 5 seeds per cell
- Data recorded at 8 weeks, 9 weeks, 10 weeks
- Two trays watered with purified water (Milli-Q), two trays watered with dilute smoke solution (0.06...%)
- *Molinia* did not grow in soil, but *Marchantia* (liverwort) did



Above: *Molinia* growing on agar,  
Below: *Marchantia* growing on soil



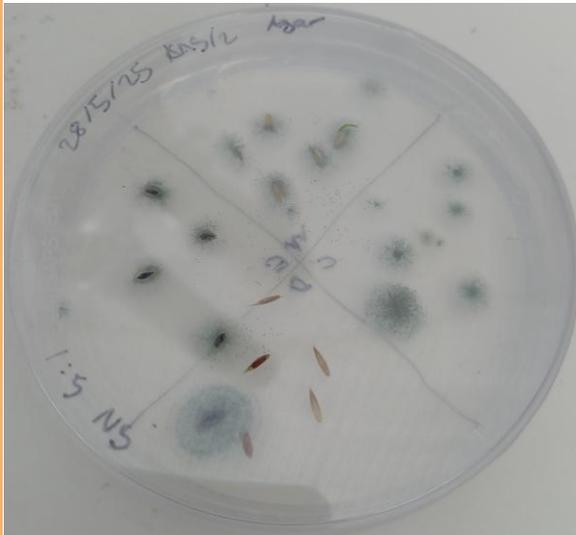
# Challenges

## Contamination and Media

- Moulds
- Bacterial colonies
- *Marchantia* (liverworts)
- Overwatering (soil)
- Dehydration (agar)

## Failure to Germinate

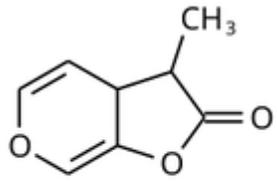
- Low germination rates overall
- Some experiments yielded zero germinations
- *Calluna* and *Erica* Life strategies
- Stratification and scarification
- Seed identification and storage



# Next Steps and Solutions

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- Smoke solution analysis
- Gorse trials
- Repeat *Molinia* soil trial with less watering
- Growing seeds from New Forest soil samples



KAR<sub>1</sub>



# Smoke Solution Analysis

Aiming to quantify and compare karrikinolide content of the smoke solutions using High Performance Liquid Chromatography (HPLC), as performed by Hrdlička et al., 2019.

Karrikins are...

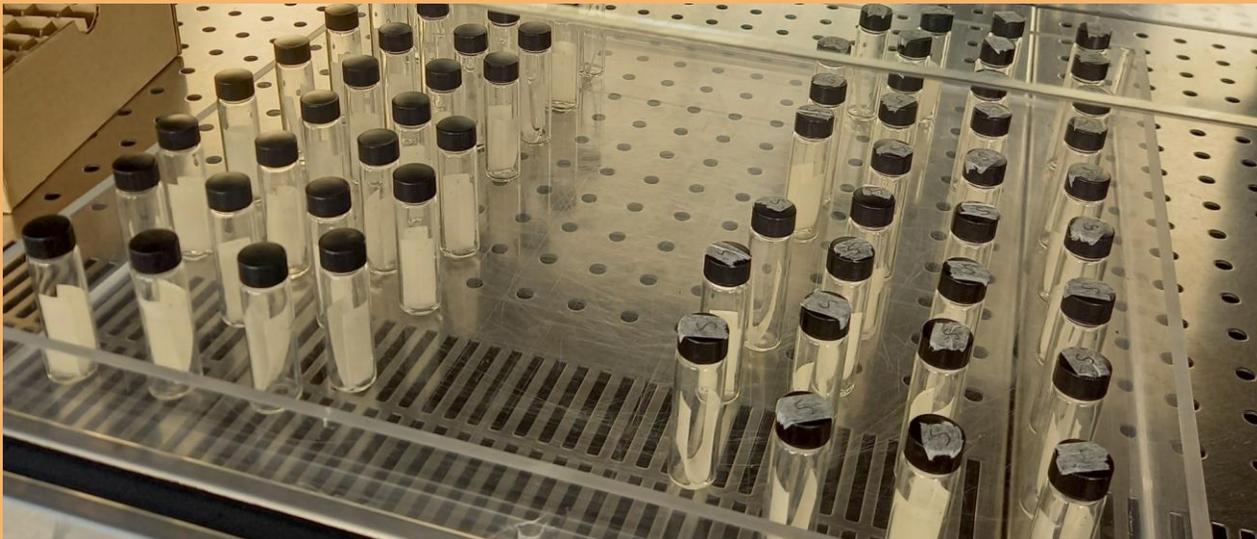
- A group of water-soluble compounds produced by burning plant material, one of the active compounds in smoke. (Flematti et al., 2004)
- Known to increase germination rate and % in wide range of plants, even outside of fire-prone landscapes. (Chiwocha et al., 2009)
- Possibly acting through similarity to strigalactone (plant hormone). (Taiz et al., 2023)

Questions

- What species has the highest karrikin concentration?
- Does this vary by plant functional group?
- What were the actual concentrations applied to the seeds?
- How does the smoke solutions' concentrations compare to those in the literature?

It would be a shock to find no karrikins at all, as they are a combustion product of cellulose. (Flematti et al., 2004)





# Ulex europaeus Trials

Gorse seeds proved difficult to harvest. Despite having a long flowering period, most plants did not have more than a few usable seed pods at any of the times we attempted to collect them. Pods would often have spilt open and already ejected their seeds or have insect damage.

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
						Vaccinium vitis-idaea			Calluna vulgaris		
									Erica cinerea		
									Erica tetralix		
							Deschampsia flexuosa				
					Eriophorum angustifolium						
									Molinia caerulea		
							Rumex acetosella				
							Cytisus scoparius				
									Ulex galii		
										Ulex europaeus	



- Seeds obtained from Chiltern Seeds
- Plan to germinate seeds on filter paper or cotton wool in glass vials
- Scarification
  - Chemical method
  - Physical method





## Repeat Trial - *Molinia caerulea*

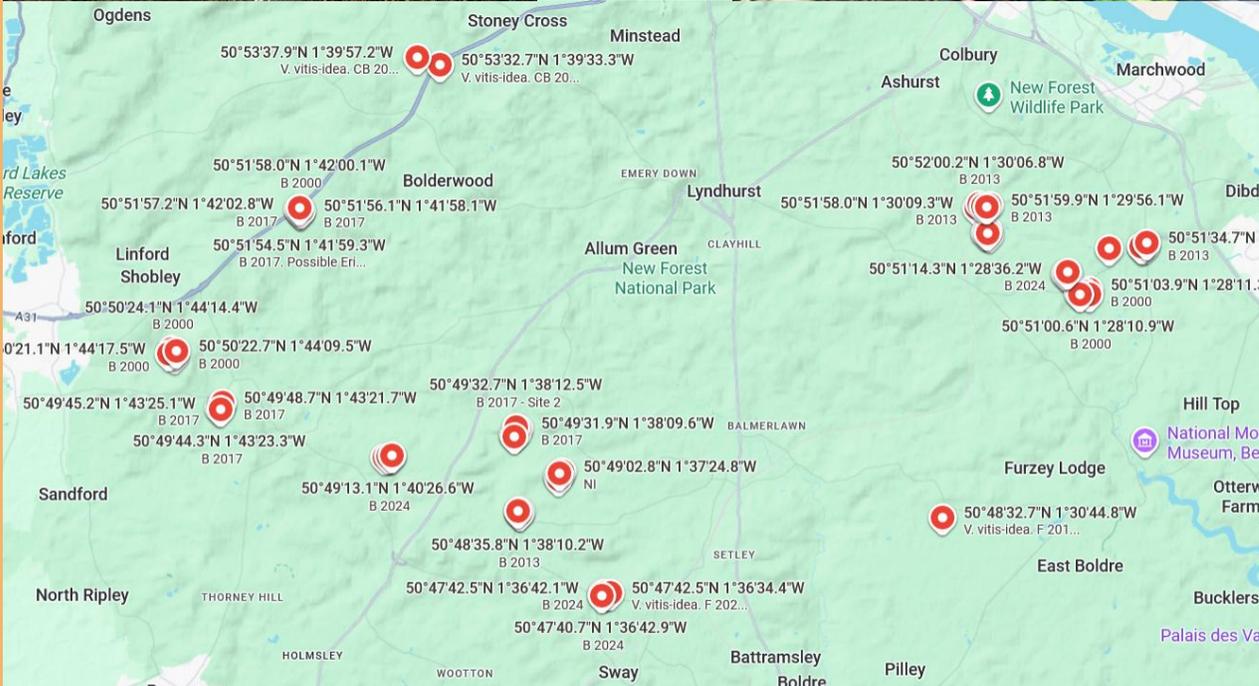
- Plan to repeat the soil-based experiment
- Four trays, 12 cells per tray, 5 seeds per cell
- Two trays watered with purified water (Milli-Q), two trays watered with dilute smoke solution (0.06...%)
- Less water will be provided, thought that overwatering may have killed the *Molinia* seeds and encouraged *Marchantia* growth
- *Molinia* seeds germinated on agar in 2-3 weeks, if this is replicated on soil this should be a quick turnover trial





# Wild Soil Testing

- Returning to seed collection sites
- Collecting small soil cores
- Attempting to grow seeds from the seedbank
- Seeds may germinate more readily than those collected from plants
- Half will be watered with smoke solution at 1:2000 or 1:15000 dilution, control will receive just water
- Eliminates some time-consuming and error-prone parts of previous methods:
  - Growing medium is collected rather than made
  - Seeds do not need to be individually sterilised or sown
  - Expect fewer mould issues



# References and Image sources

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- Vial trial, Green Smith.
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- Turbury Common post-fire, Green Smith.
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# Questions

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**Thank you for listening!**