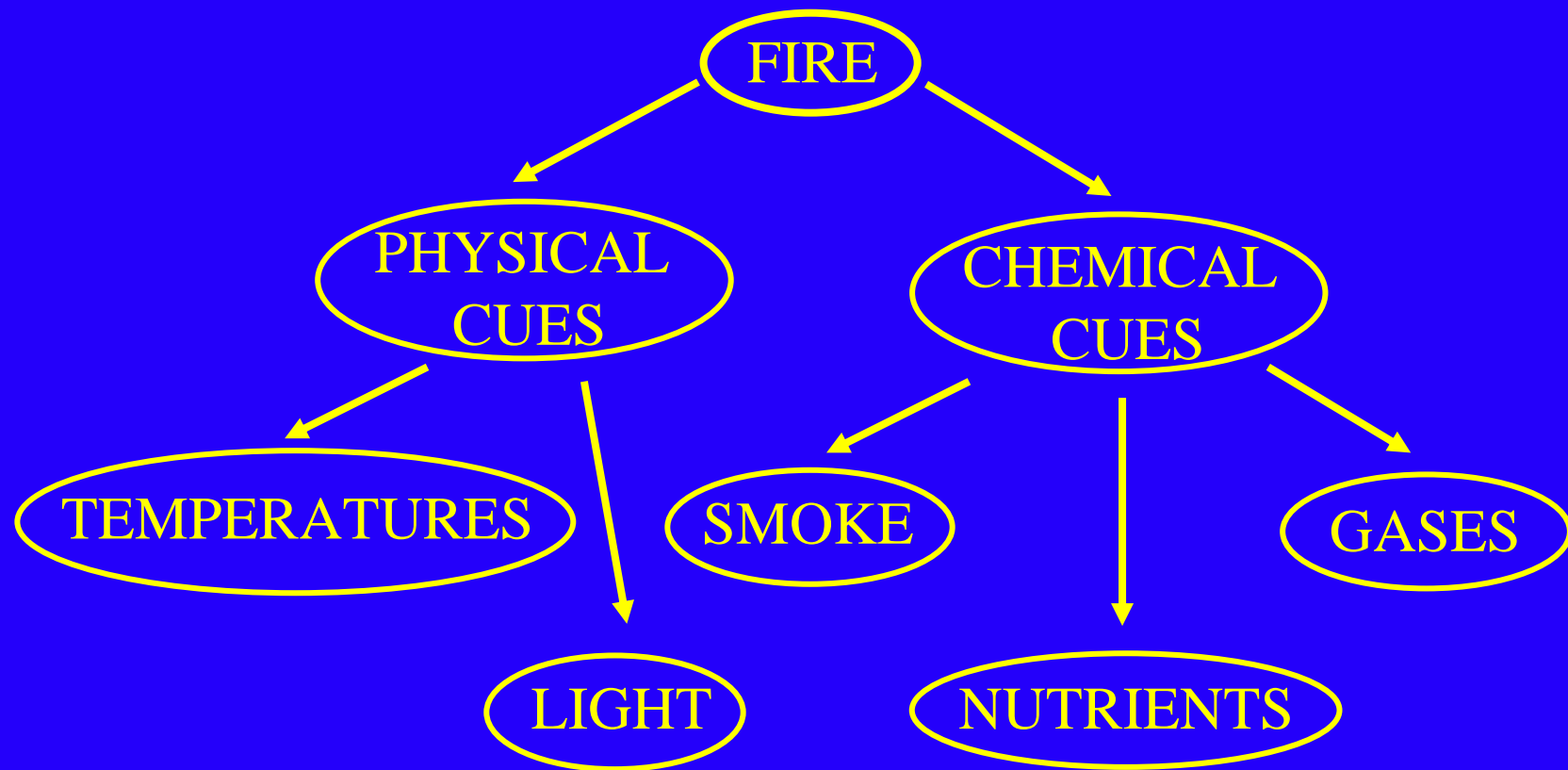


# CONTROL OF WEED SEED GERMINATION BY SMOKE- WATER AND SMOKE-DERIVED BUTENOLIDE

JAN KĘPCZYŃSKI

Department of Plant Physiology, University of Szczecin, ul. Wąska 13,  
71-415 Szczecin



**De Lange and Boucher (1990) were the first to demonstrate that smoke, and aqueous extracts of smoke, was responsible for the stimulation of seed germination**

South Africa- seeds of 157 species from 22 families  
'Kirstenbosch Instant Smoke Plus Seed  
Primer'

Australia- seeds of 170 species from 37 families  
'Seed Starter'

## The effect of smoke-water (10%) on germination of weed seeds at 15°C in the dark (Adkins and Peters 2001)

Species	Smoke-water concentration (%)	
	0	10
Monocots		
<i>Phalaris paradoxa</i>	50 ± 5	100 ± 0
<i>Alopecurus myosuroides</i>	45 ± 9	77 ± 8
<i>Avena sterilis</i>	12 ± 6	93 ± 3
Dicots		
<i>Malva neglecta</i>	28 ± 4	73 ± 12
<i>Polygonum pennsylvanicum</i>	9 ± 1	21 ± 1
<i>Veronica persica</i>	79 ± 3	100 ± 0

Smoke contains possibly up to several thousand compounds

Baldwin et al. 1994 identified 71 compounds by GC-MS and tested 233 compounds

Chemical(s) present in smoke was:

- water soluble

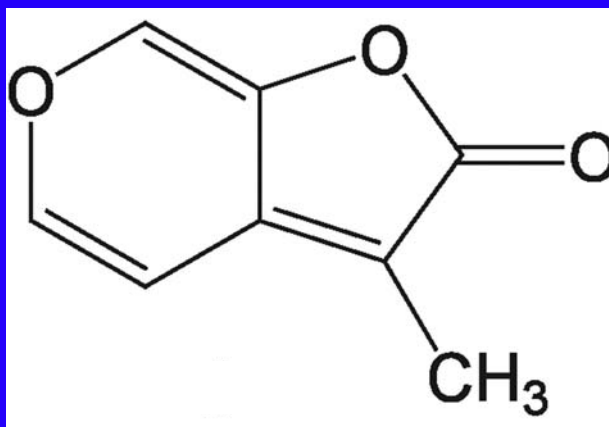
- thermostable

- long-lasting in solution

- highly active at low concentration

## DISCOVERY OF BUTENOLIDE

- Van Staden J, Jäger AK, Light ME, Burger BV 2004
- Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD 2004

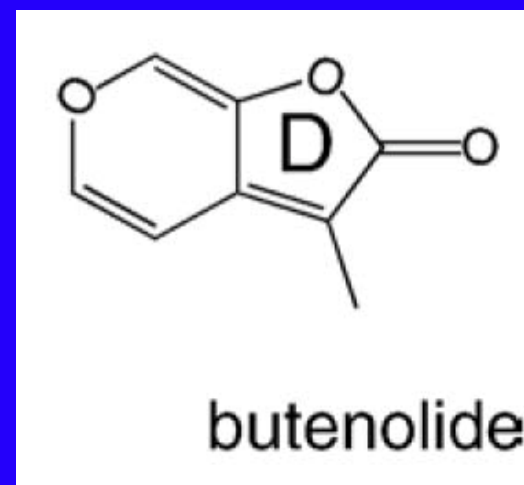
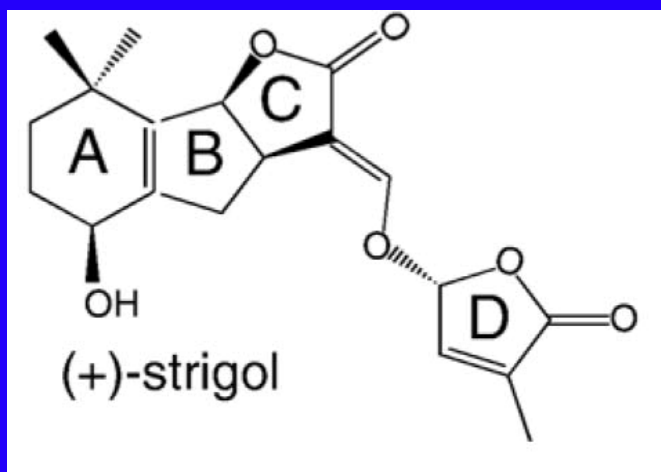


3-methyl-2*H*-furo[2,3-*c*]pyran-2-one

Activity from  $10^{-9}$  to  $10^{-4}$  M

Neither toxic nor genotoxic at  $3 \times 10^{-10}$ - $10^{-4}$  M

## THE CHEMICAL STRUCTURE OF STRIGOL AND BUTENOLIDE



## Synthesis of butenolide

**Flematti** et al. 2005. Synthesis of the seed germination stimulant 3-methyl-2H-furo[2,3-c]pyran-2-one. Tetrahedron Letters 46(34):5719-5721

**Goddard-Borger** et al. 2007. Synthesis of the germination stimulant 3-methyl-2H-furo[2,3-c]pyran-2-one and analogous compounds from carbohydrates. 2007. European J Organic Chemistry 3925-3934

**Nagase** et al. 2008. Synthesis of the seed germination stimulant 3-methyl-2H-furo[2,3-c]pyran-2-one utilizing direct and regioselective Ti-crossed aldol addition. Tetrahedron Letters 49:4509-4512

**Sun** et al. 2008. Synthesis of butenolides as seed germination stimulants. Tetrahedron Letters 49(18):2922-2925

## SEEDS RESPONDING TO BUTENOLIDE

- Fire -prone species
- Arable weeds
- Hemi- and holo-parasitic weeds
- Australian *Solanum* spp.
- Crop plants: *Lactuca sativa* L., *Lycopersicon esculentum* L.,  
*Abelmoschus esculentus* L., *Phaseolus vulgaris* L., *Zea mays* L.,  
*Oryza sativa* L.

# **BUTENOLIDE ENHANCES**

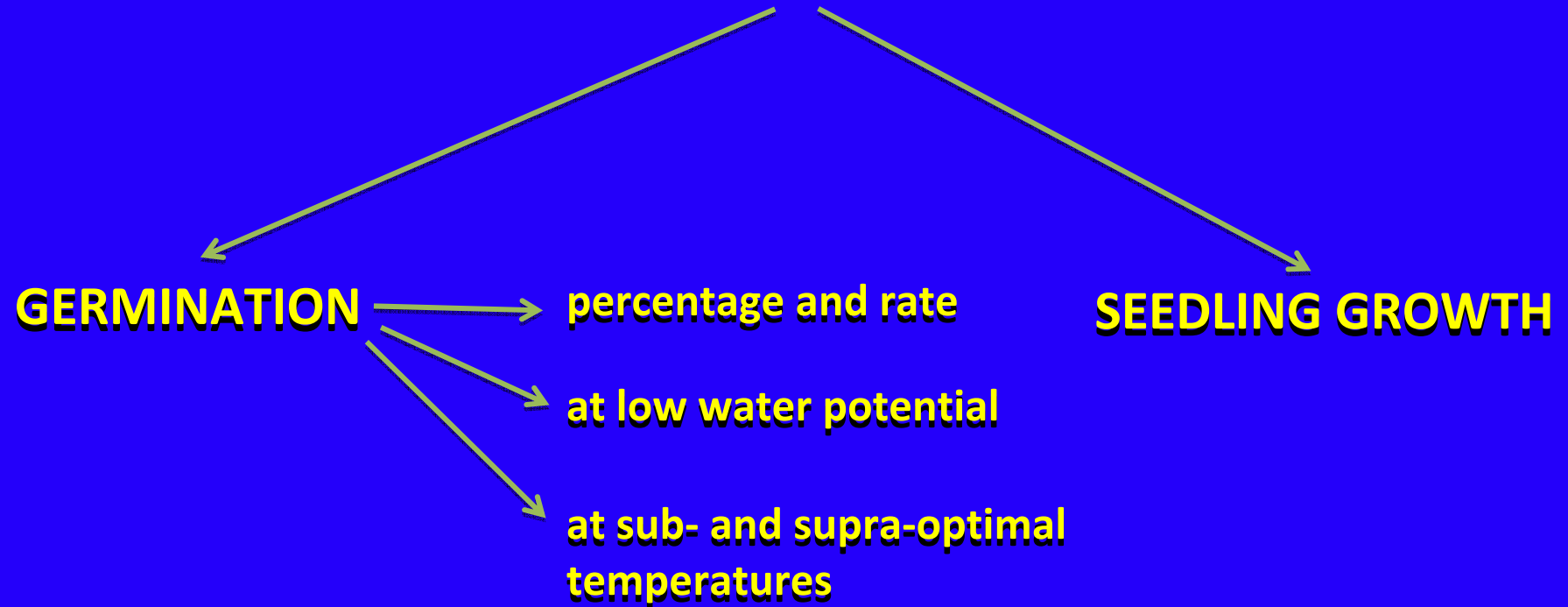
## **GERMINATION**

**percentage and rate**

**at low water potential**

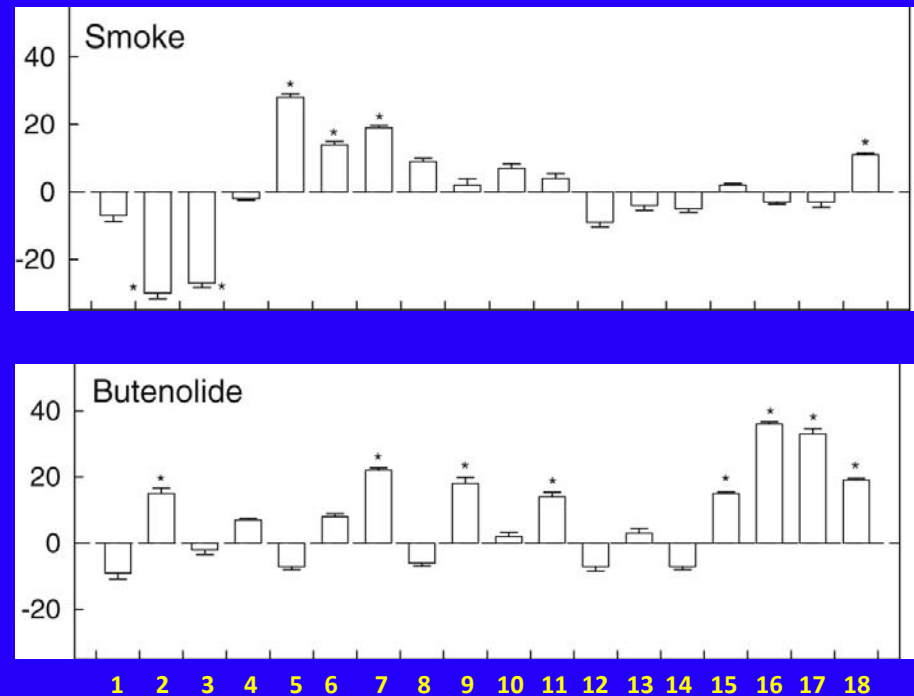
**at sub- and supra-optimal  
temperatures**

## **SEEDLING GROWTH**



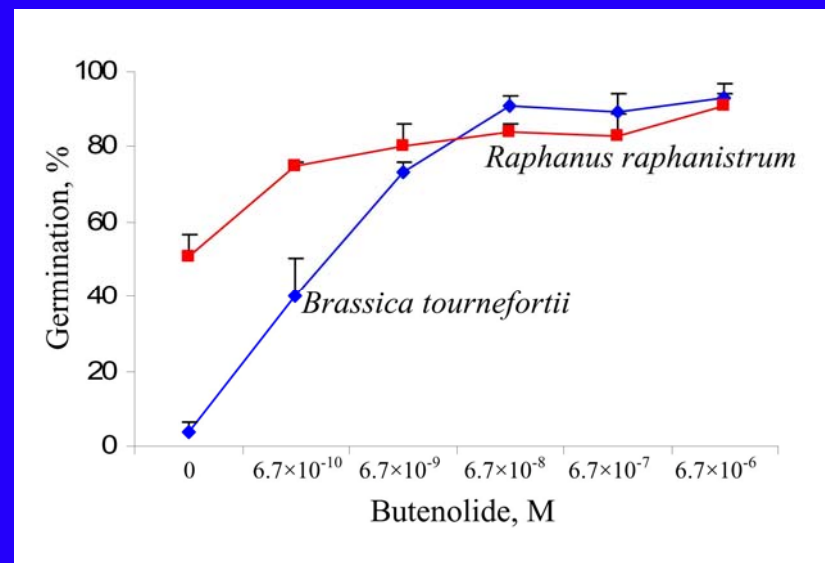
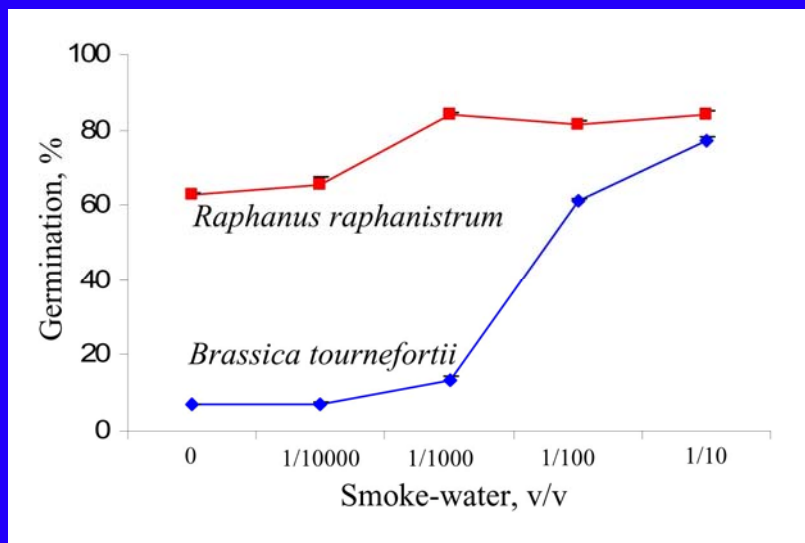
**The percentage difference in germination level between smoke-water, butenolide and the control germination level assessed at a constant 15°C for the 18 weed species (Davs et al. 2007)**

- 1- *Alopecurus myosuroides*
- 2- *Avena fatua*
- 3- *Bromus sterilis*
- 4- *Bromus tectorum*
- 5- *Capsella bursa-pastoris*
- 6- *Chenopodium album*
- 7- *Chrysanthemum segetum*

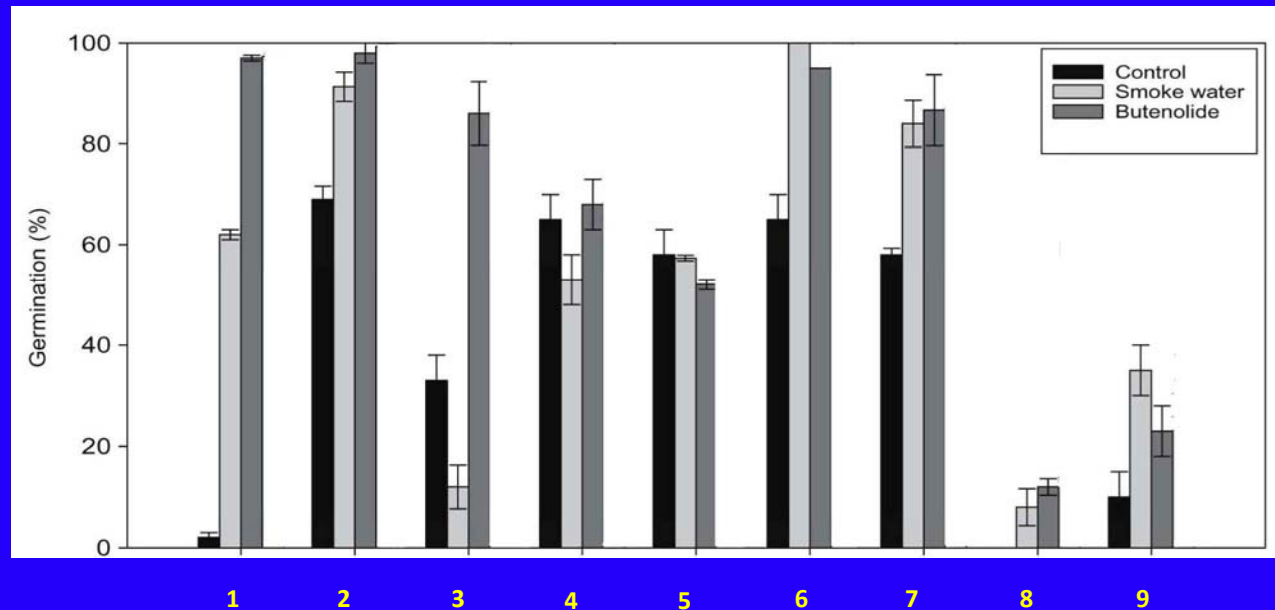


- 8- *Galium aparine*
- 9- *Malva neglecta*
- 10- *Matricaria matricoides*
- 11- *Papaver rhoeas*
- 12- *Phalaris paradoxa*
- 13- *Polygonum ariculare*
- 14- *Rumex obtusifolius*
- 15- *Senecio jacobinae*
- 16- *Sinapis alba*
- 17- *Sorghum halepense*
- 18- *Stellaria media*

## The effect of smoke-water and butenolide on germination of freshly collected seeds of *Brassica tournefortii* and *Raphanus raphanistrum* (Stevens et al. 2007)



# The effect of smoke-water (1/10 v/v) and butenolide ( $6.7 \times 10^{-7}$ M) on seed germination of weed species (Stevens et al. 2007)



1- *Brassica tournefortii*

2- *Raphanus raphanistrum*

3- *Sisymbrium orientale*

4- *Bromus diandrus*

5- *Lolium rigidum*

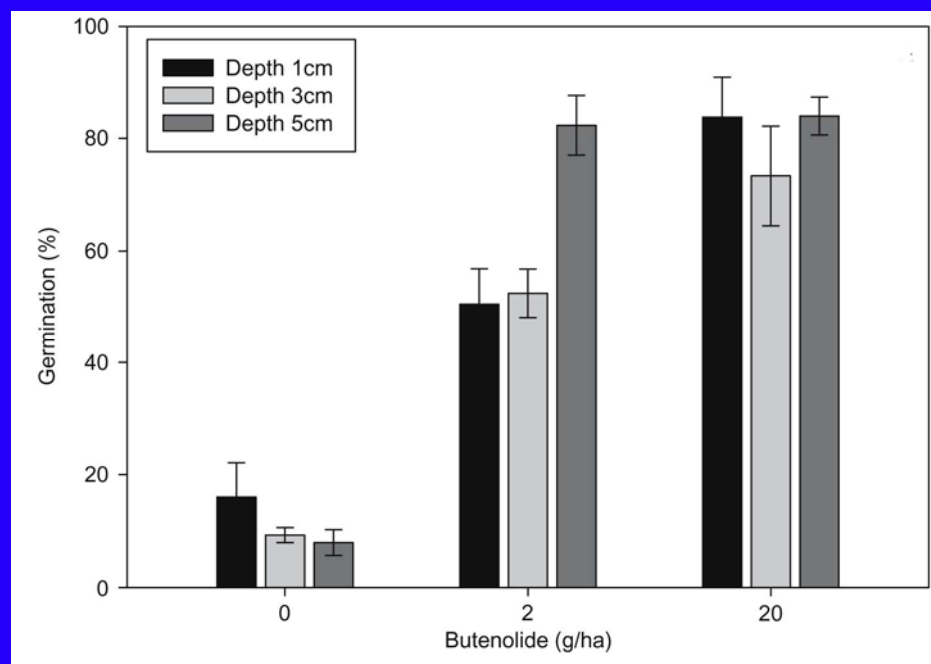
6- *Hordeum leporinum*

7- *Avena fatua*

8- *Arctotheca calendula*

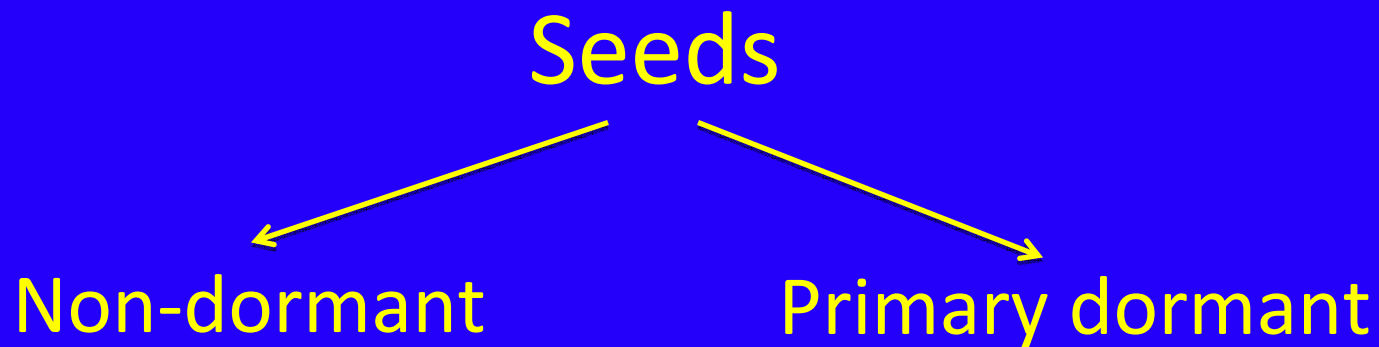
9- *Echium plantagineum*

**The effect of different rates of spray application of butenolide on germination of *Brassica tournefortii* seeds sachets buried in 2005 at different depths throughout the soil profile (Stevens et al. 2007)**



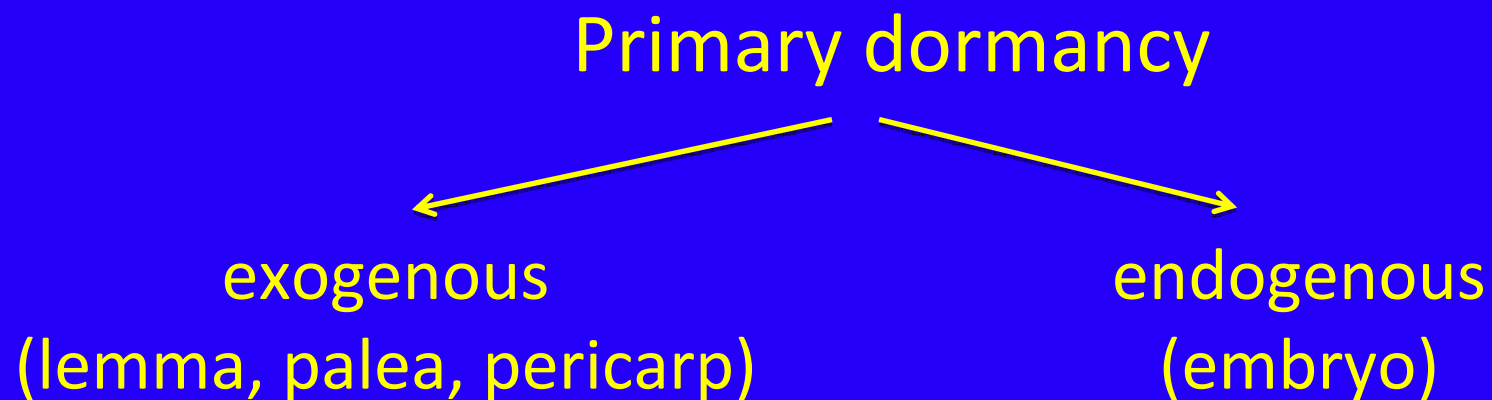
# *Avena fatua* L.

important economic weed that infests most major cereal producing regions of the world



# Primary dormancy

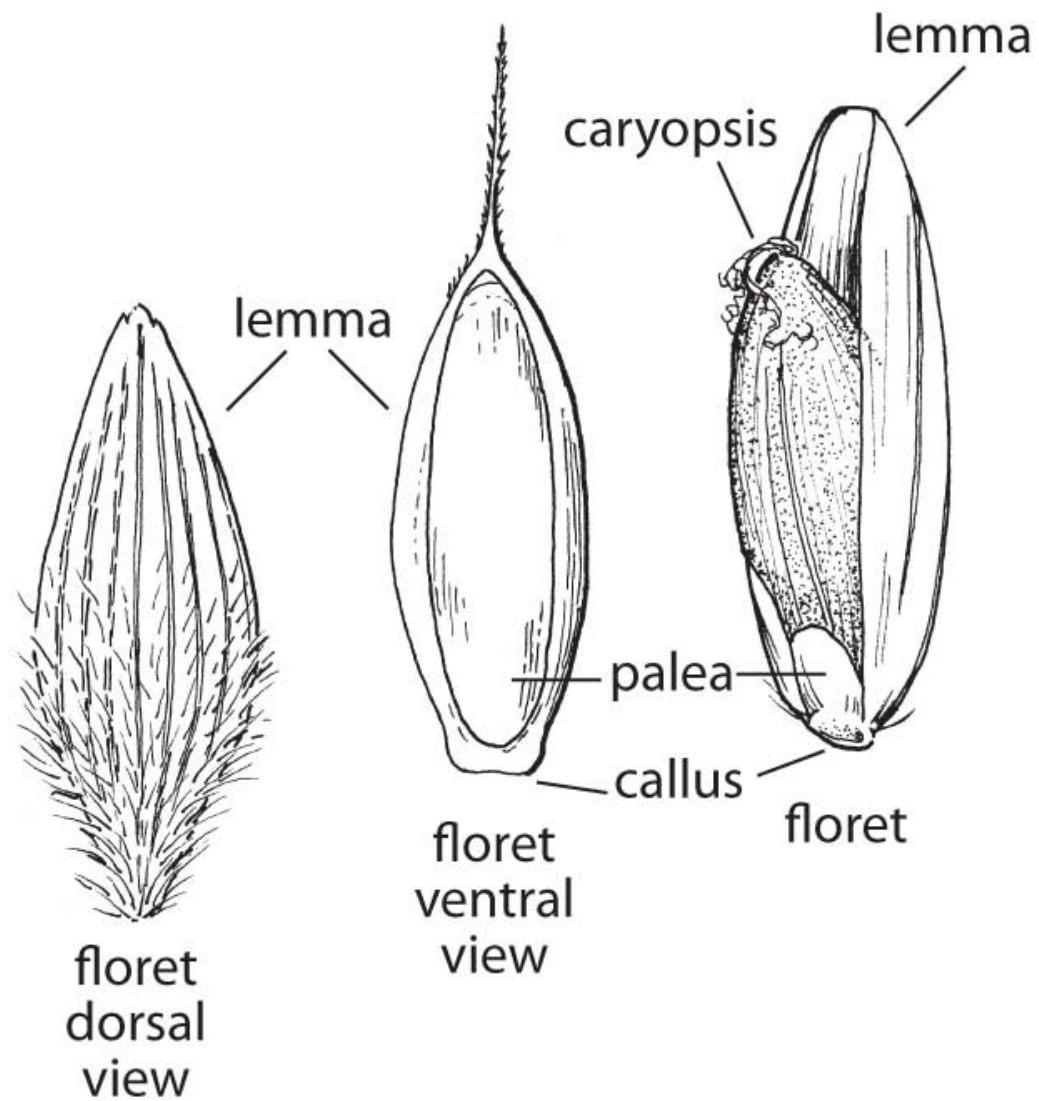
Seed dormancy is absence of germination of viable seed under conditions that are favourable to germination. Seed dormancy is common in wild plants, where it may ensure the ability of species to survive natural catastrophes, decrease competition between individuals of the same species or prevent germination out of season



## Non-dormant seeds

Non-dormant seeds are able to germinate only under suitable germination conditions (water, oxygen, temperature, light, dark)

Germination begins with water uptake by the seed (imbibition) and ends with the start of elongation by the embryonic axis usually the radicle. Germination does not include seedling growth

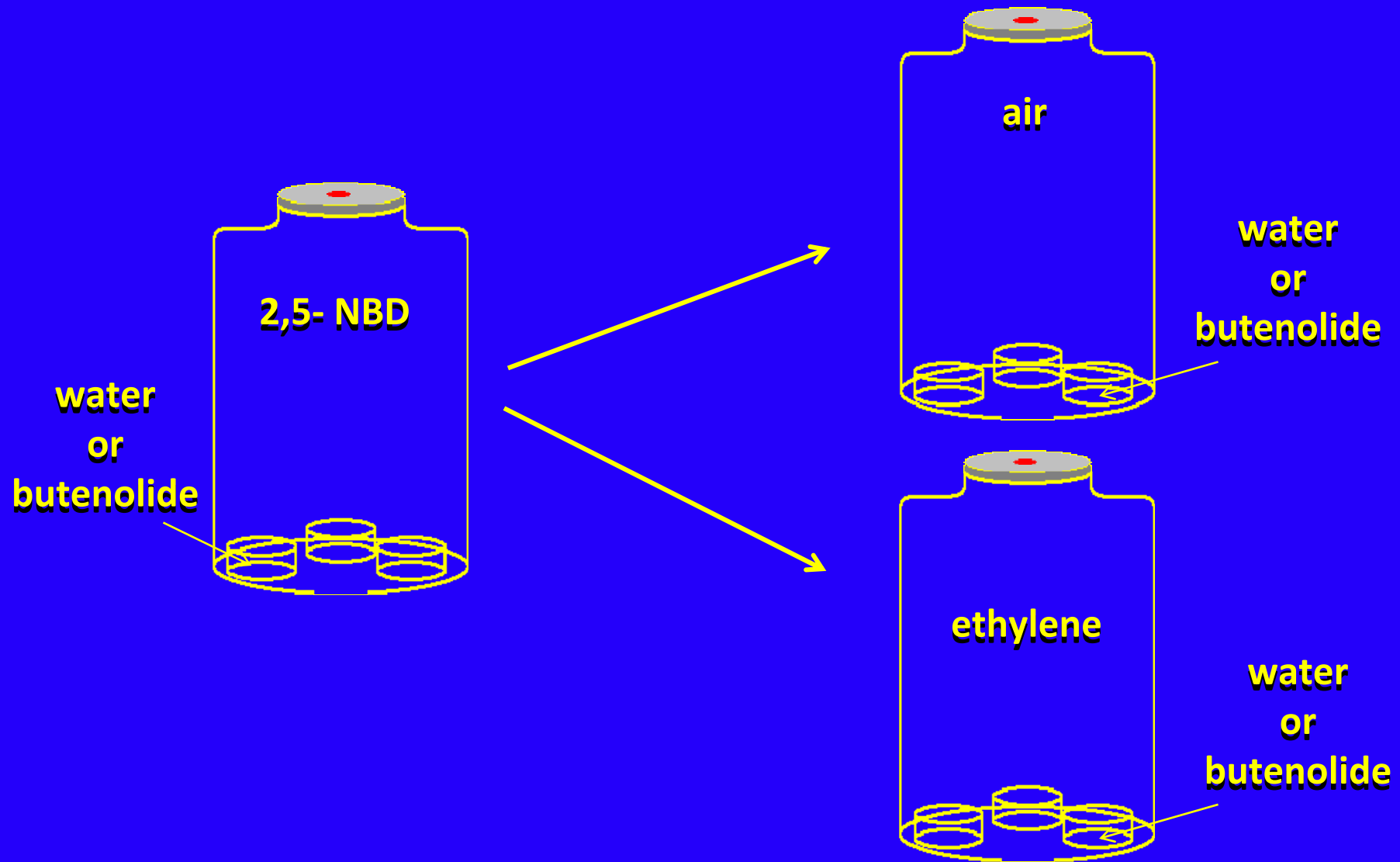


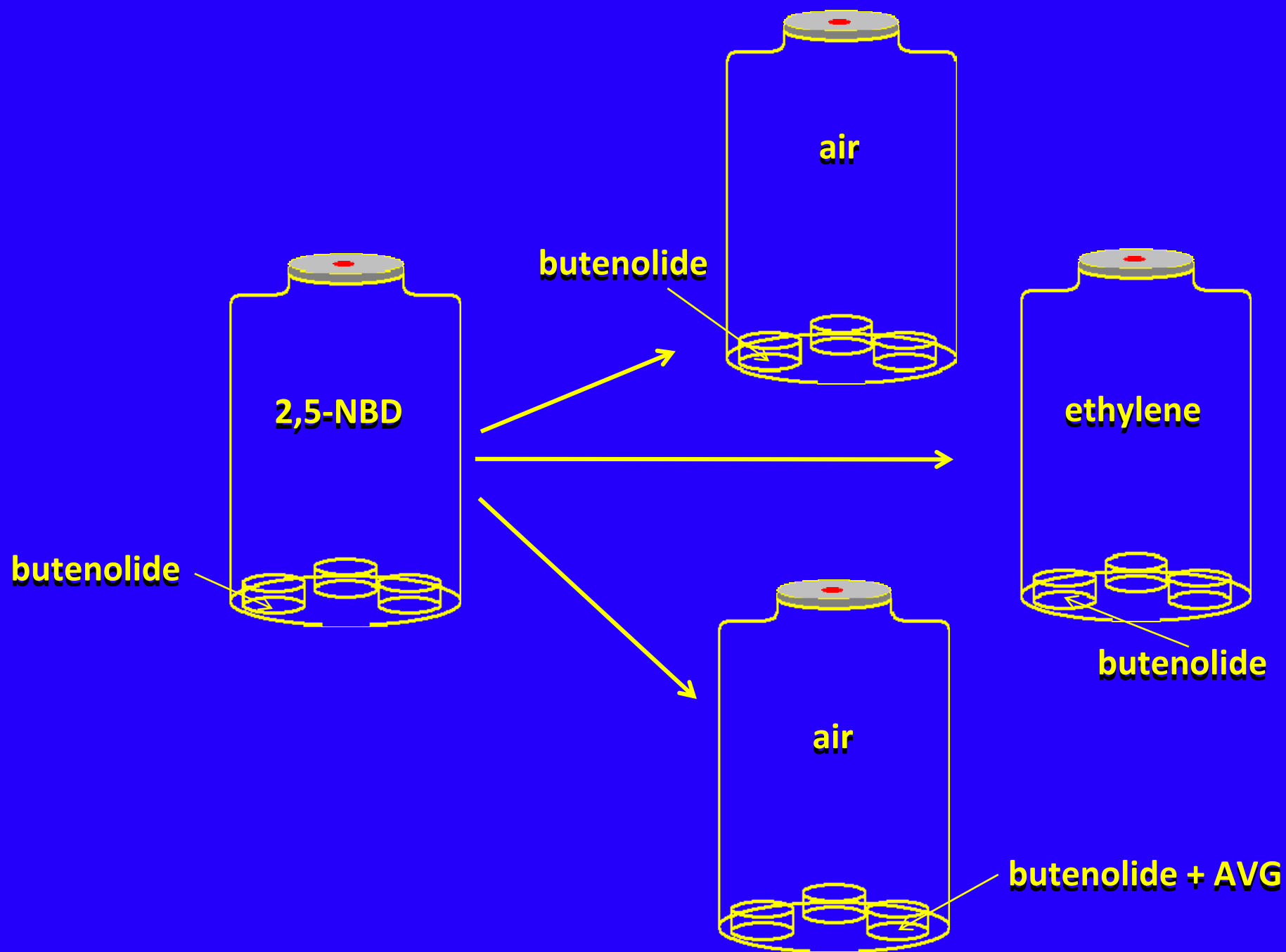
# **We determined:**

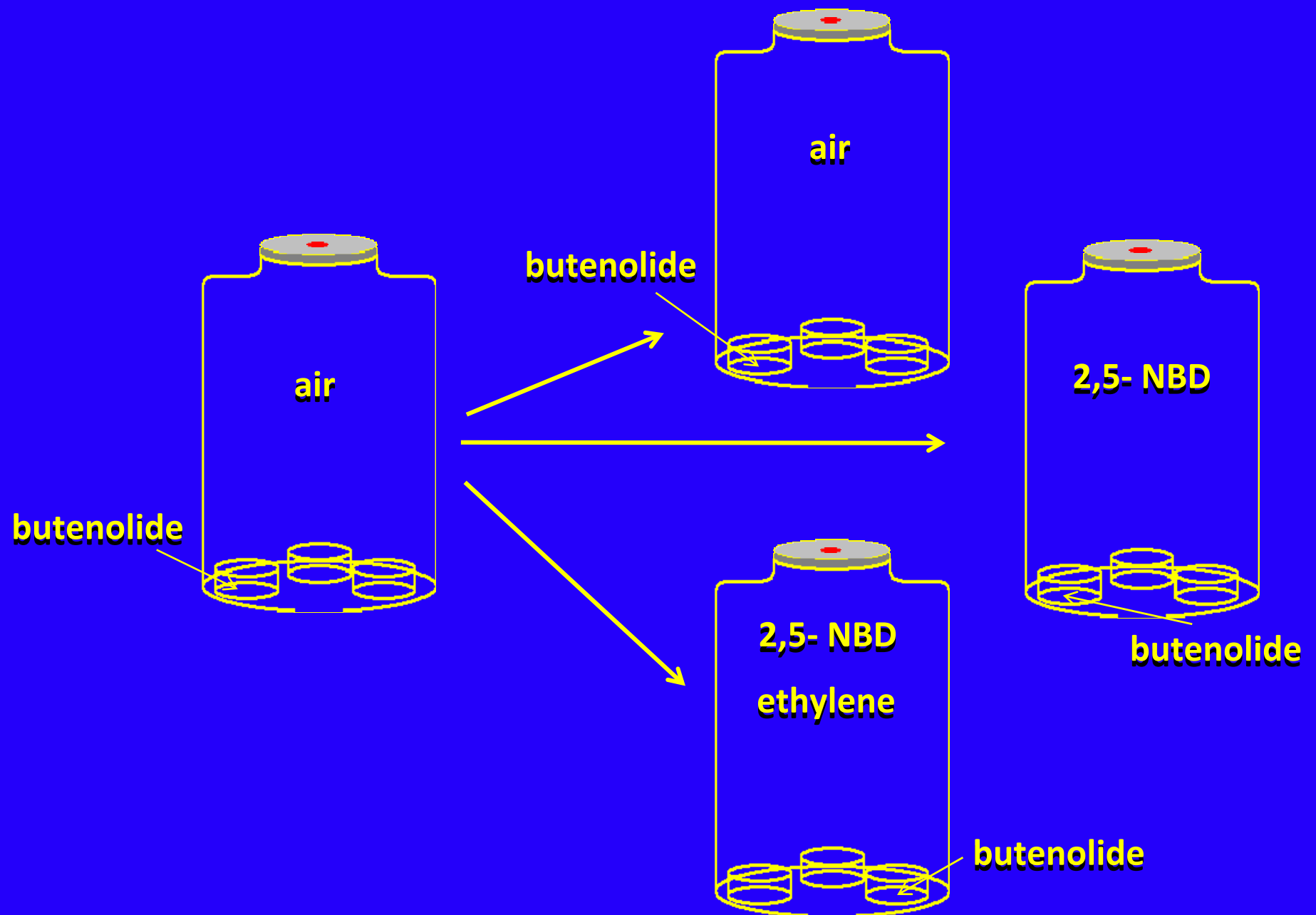
## **➤ Germination of caryopses in the presence of:**

- smoke-water**
- butenolide**
- butenolide + ACC**
- butenolide + AVG**
- butenolide + 2,5-norbornadiene**
- butenolide + 2,5-norbornadiene + ethylene, ethephon or ACC**
- butenolide + 1-MCP**

➤ **Germination of caryopses after preincubation:**







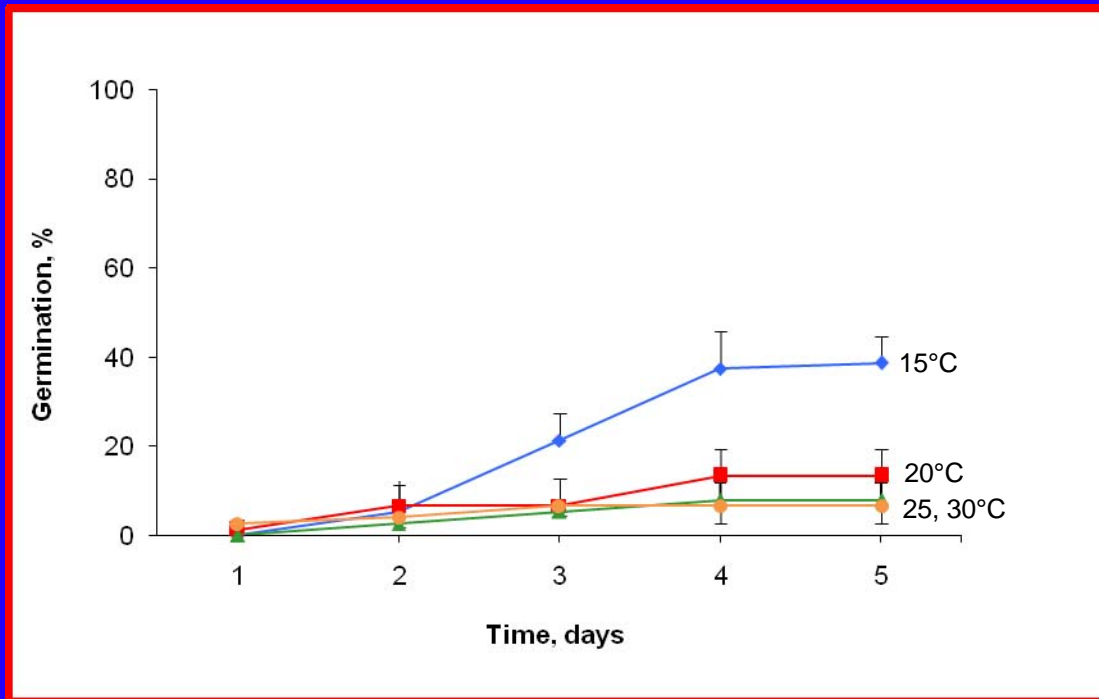
## ➤ Effect of butenolide on :

- ethylene production
- ACC oxidase activity
- nuclear DNA content
- $\alpha$ -amylase activity

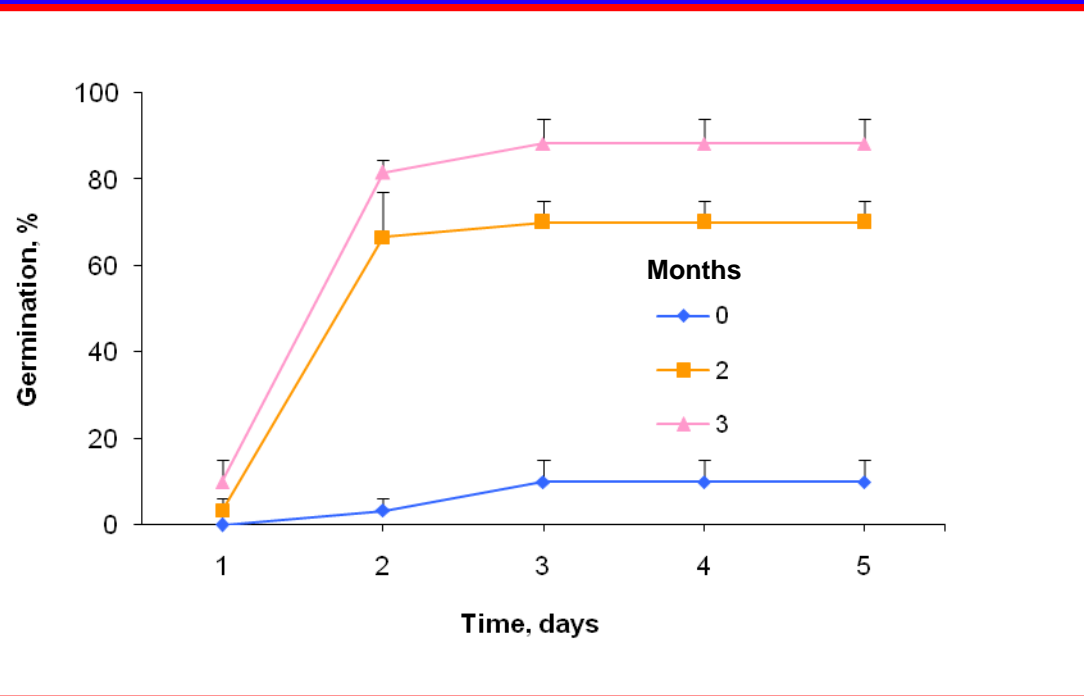


**All germination tests were conducted in darkness**

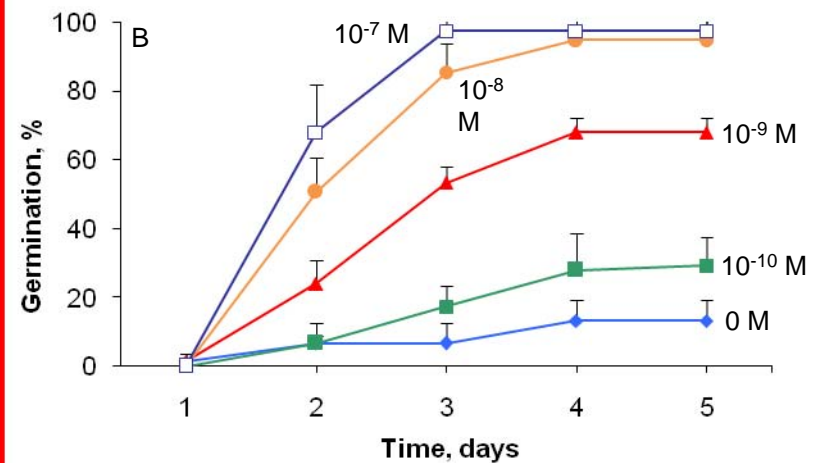
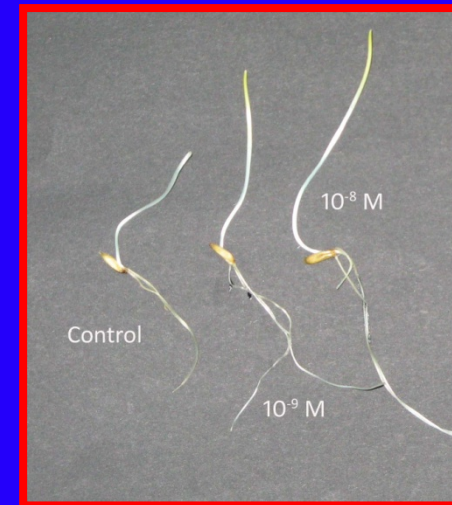
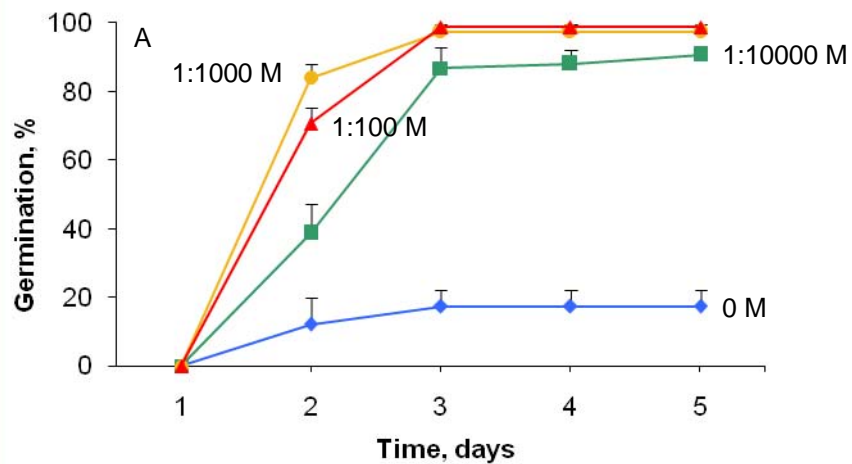
## The effect of temperature on the germination of *Avena fatua* L. caryopses in the dark



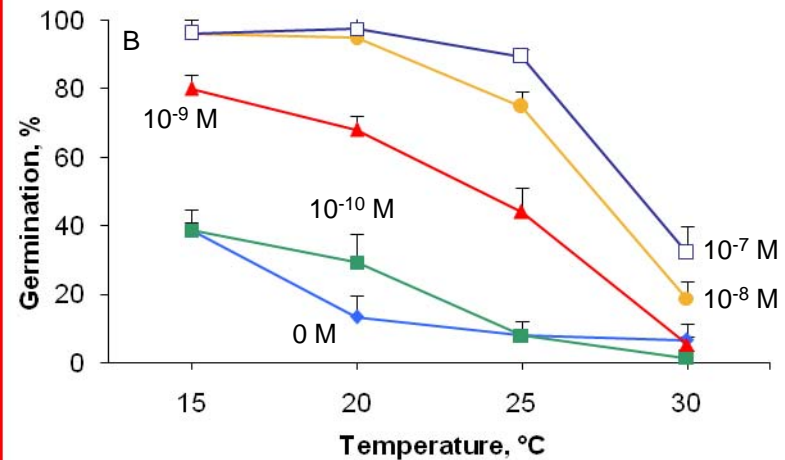
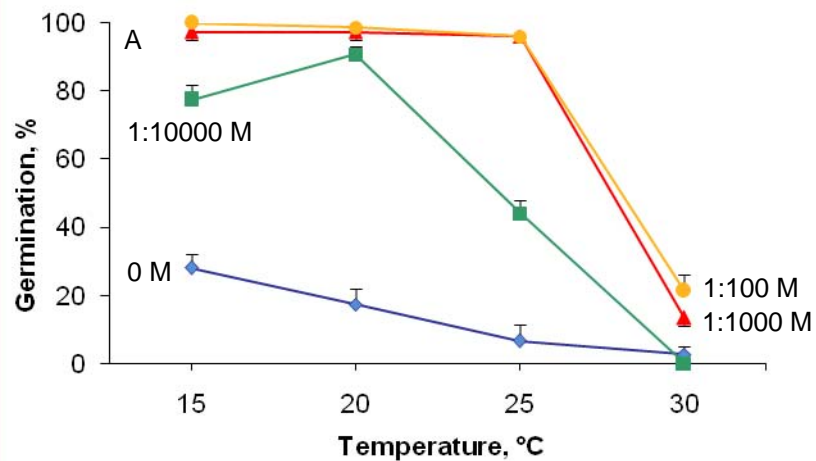
## The effect of after-ripening for 2 and 3 months at 25°C on the germination of *A. fatua* L. caryopses at 20°C



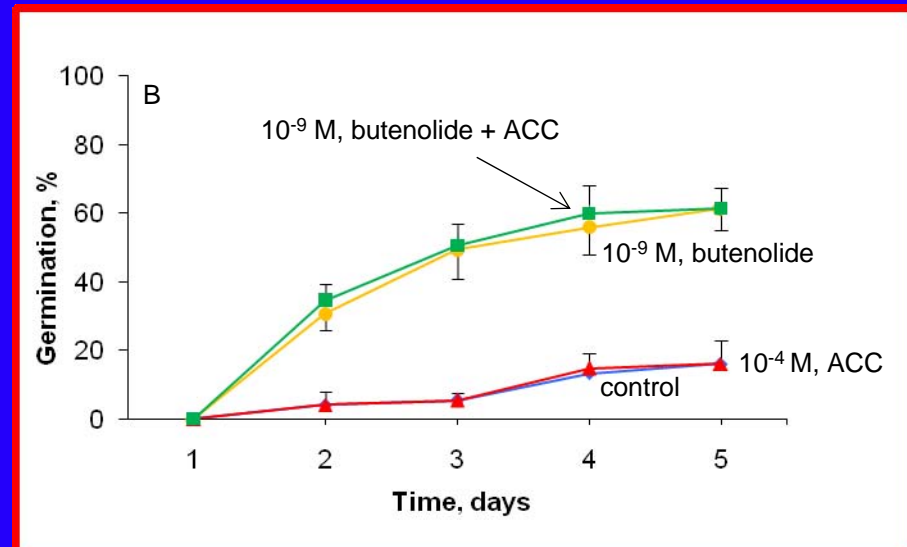
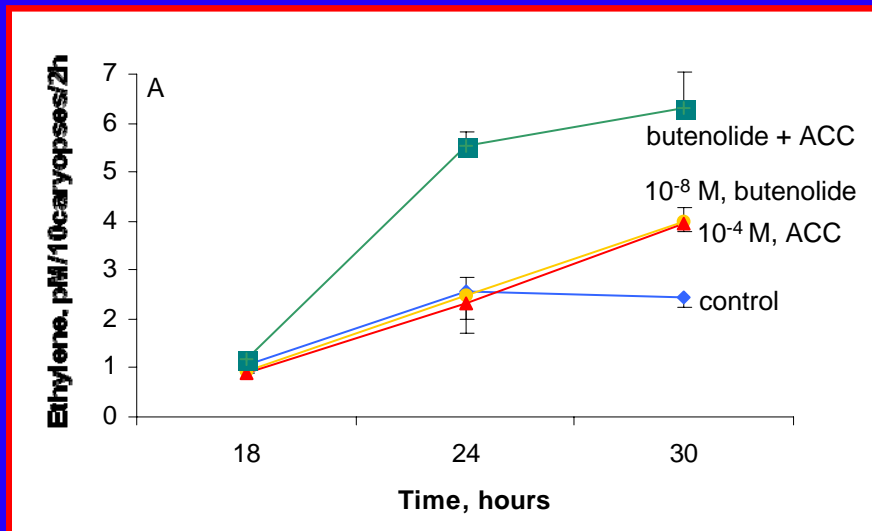
## The effect of smoke-water (A) and butenolide (B) on the time course of germination of *A. fatua* L. caryopses at 20°C



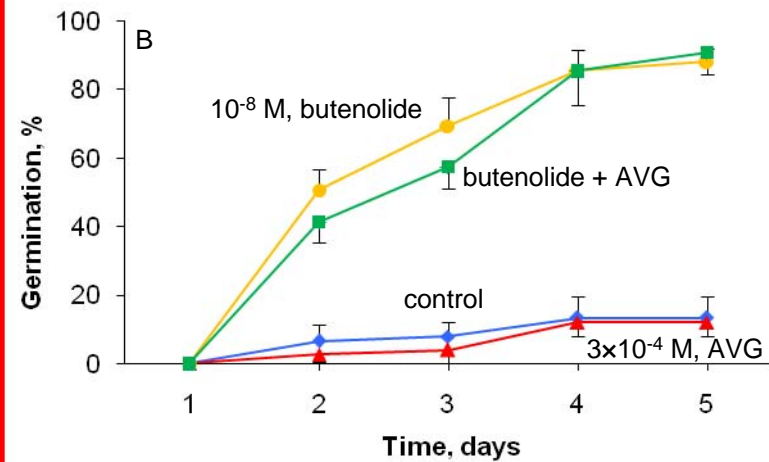
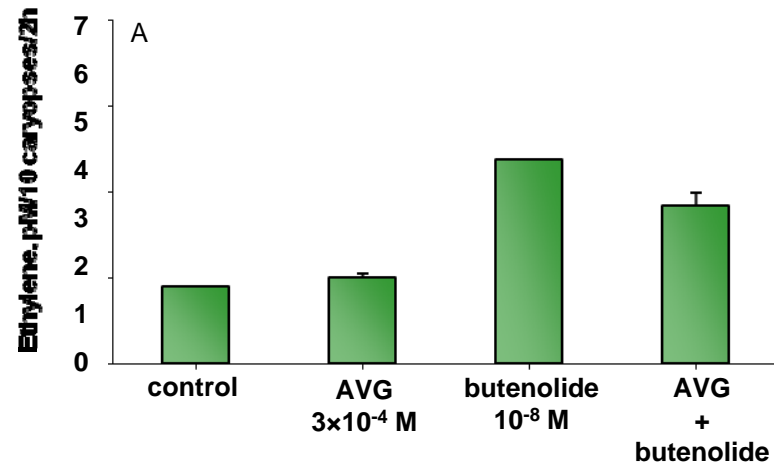
**The effect of smoke-water (A) or butenolide (B)  
on the germination of *A. fatua* L. caryopses after 5 days  
of incubation at 15, 20, 25 and 30°C**



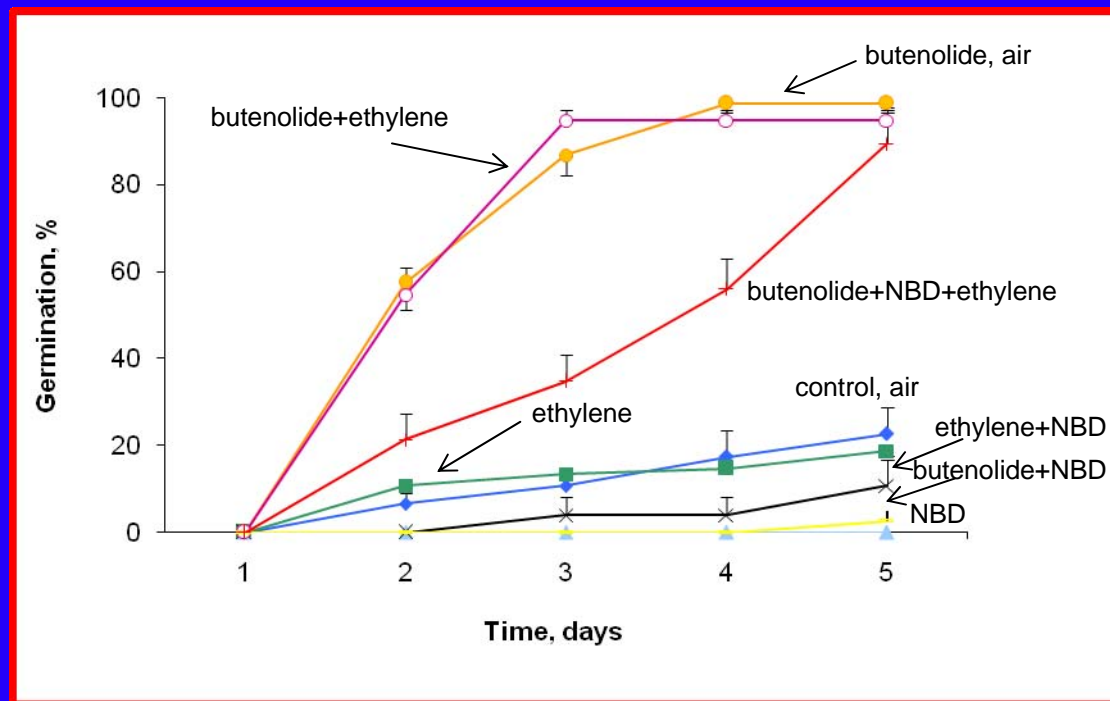
# The effect of butenolide and ACC on the ethylene production (A) and on the time course of germination (B) of *A. fatua* L. caryopses



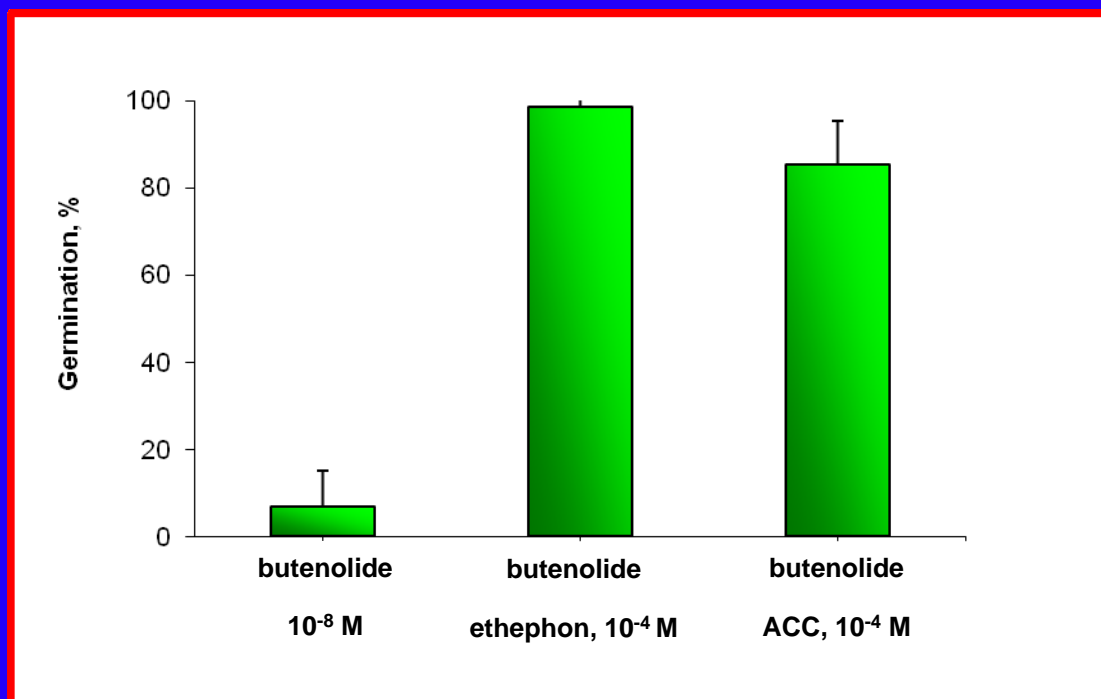
# The effect of butenolide in the presence of AVG on the ethylene production after 30 hours (A) and on the time course of germination (B) of *A. fatua* L. caryopses



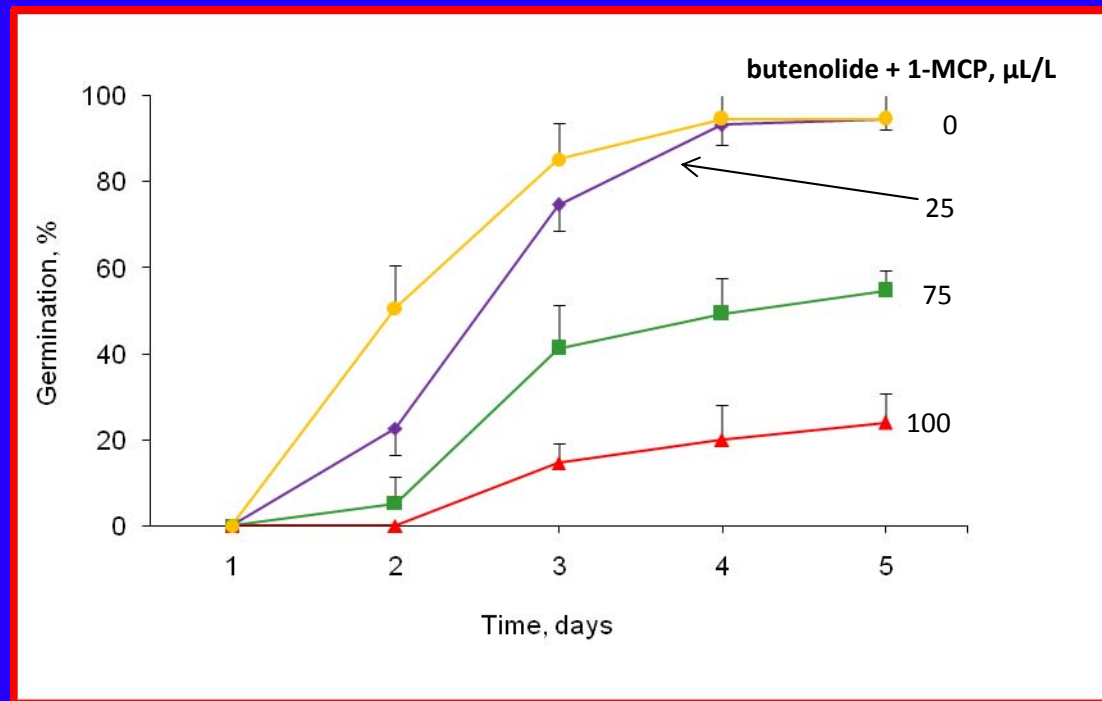
**The effect of butenolide on the germination of *A. fatua* L. caryopses in air, in the presence of ethylene, in an atmosphere containing 2,5-norbornadiene (NBD) or ethylene + NBD. Butenolide,  $10^{-8}$  M; NBD,  $5 \times 10^{-5}$  M; ethylene,  $4.5 \times 10^{-7}$  M.**



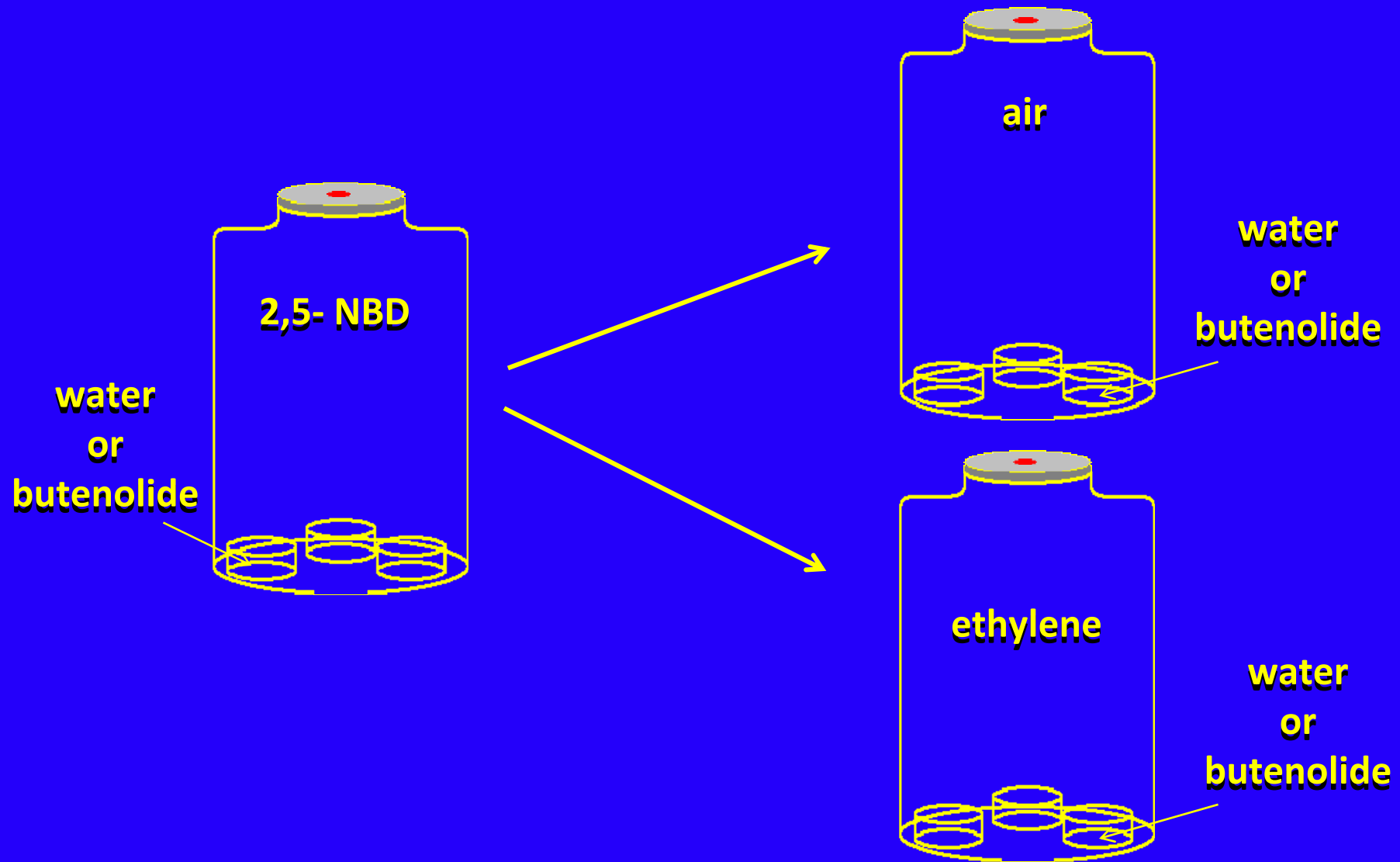
**The effect of butenolide on the germination of *A. fatua* L.  
caryopses in an atmosphere containing  $5 \times 10^{-5}$  M  
2,5-norbornadiene and in the presence of ethephon or ACC  
after 5 days of incubation**



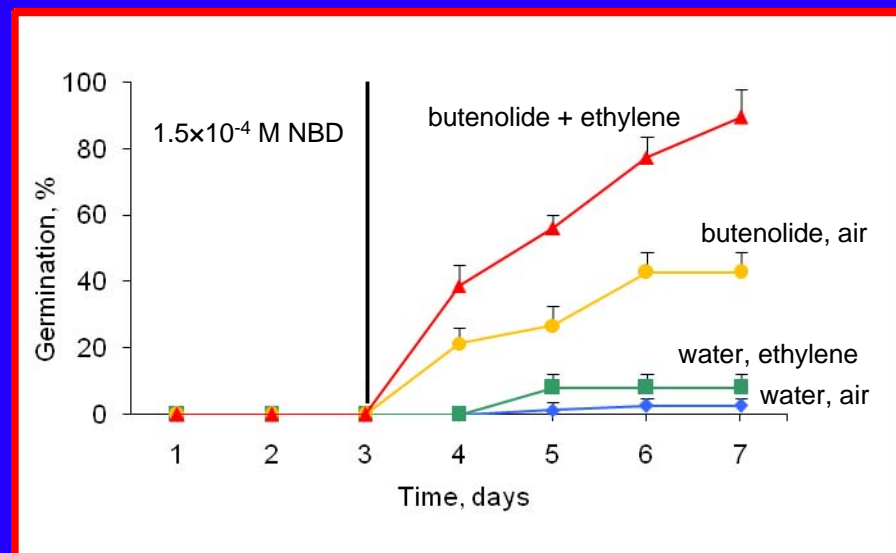
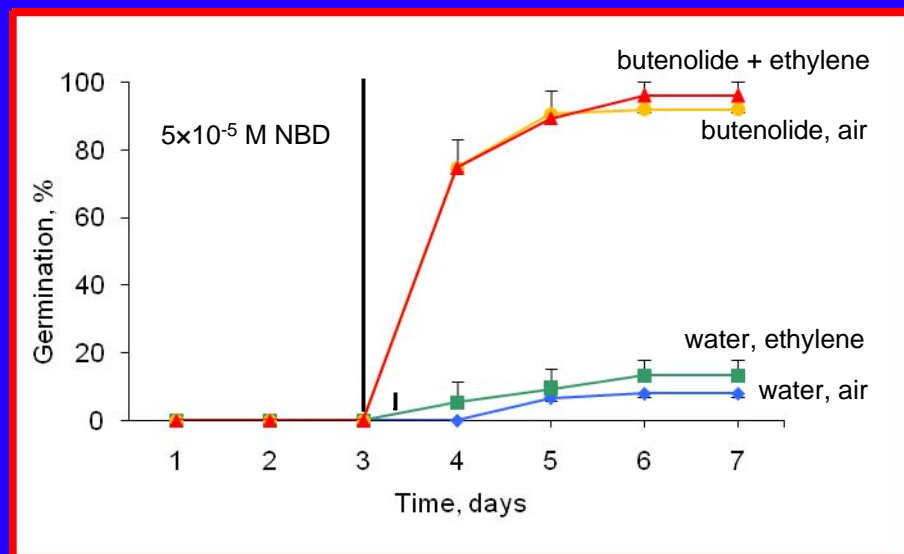
## The effect of butenolide on the germination of *A. fatua* L. caryopses in the presence of 1-MCP



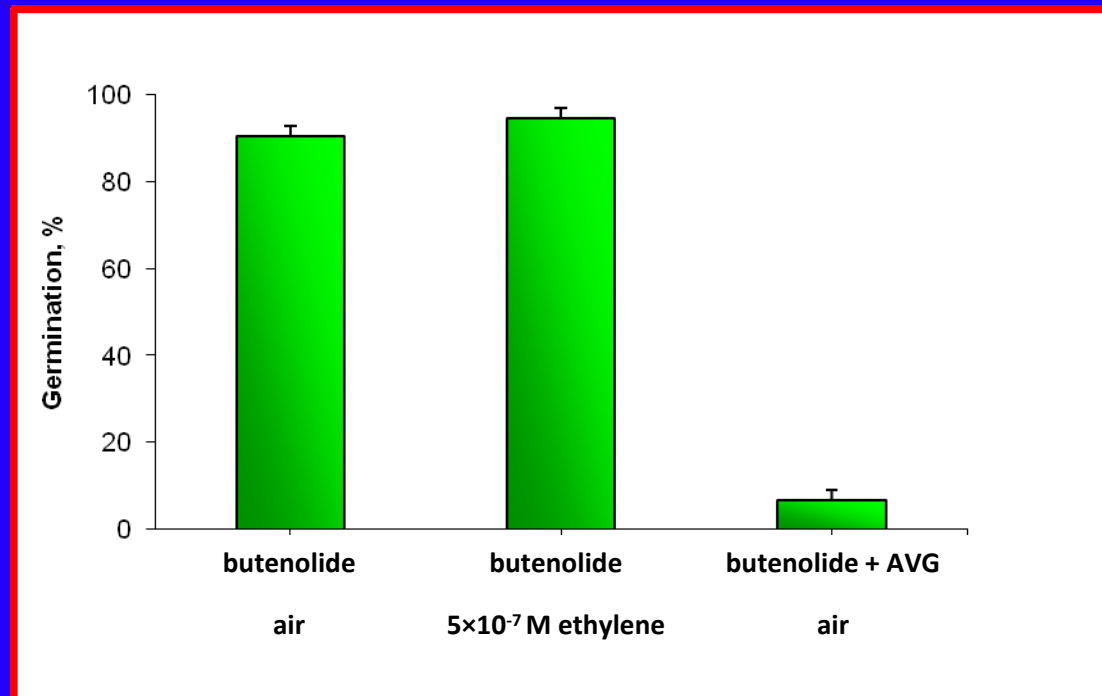
➤ **Germination of caryopses after preincubation:**

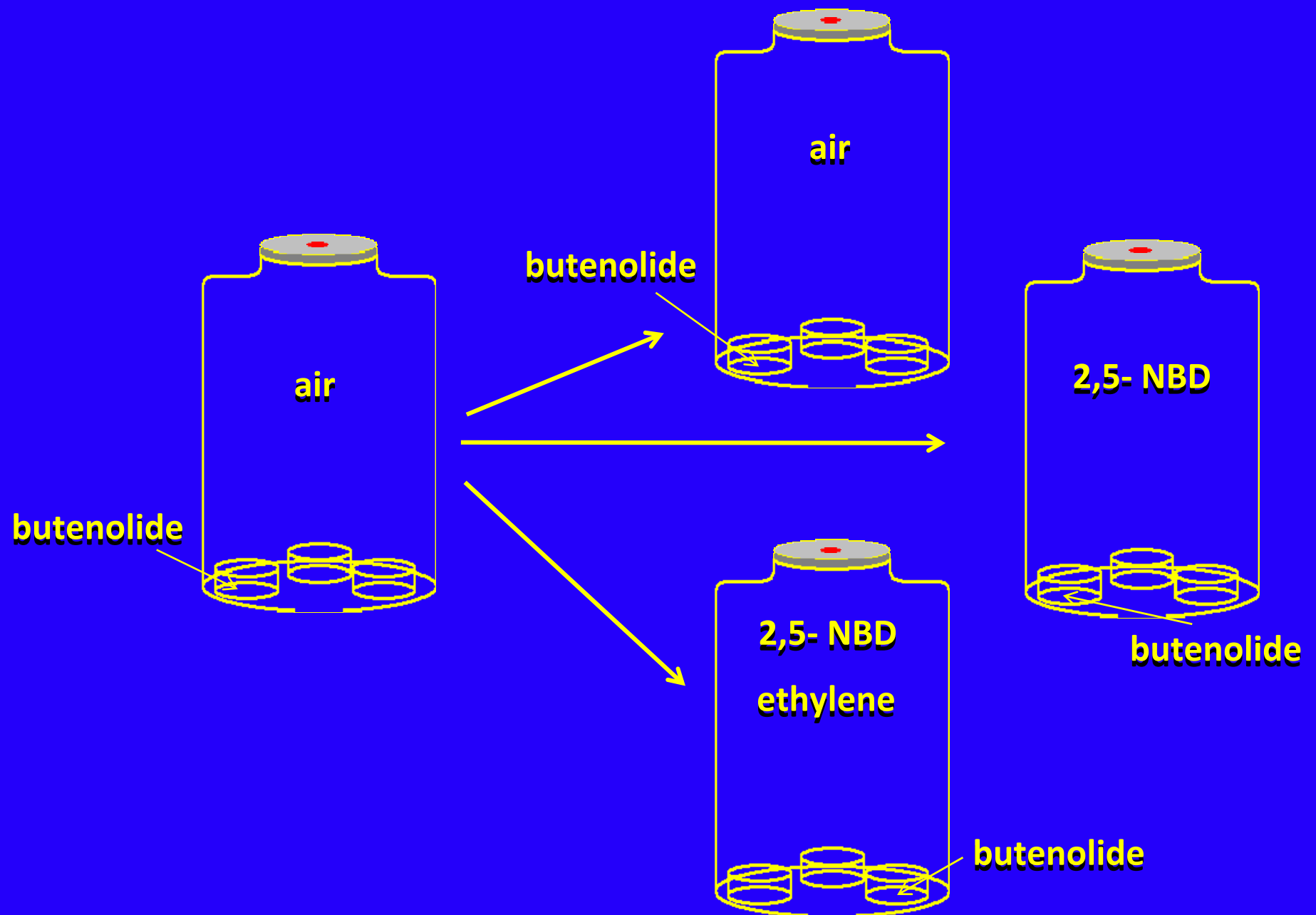


**The effect of a transfer from 2,5-norbornadiene (NBD) to air or ethylene on the germination of *A. fatua* L. caryopses in the absence or presence of butenolide. The caryopses were incubated during 3 days in  $5 \times 10^{-5}$  M or  $1.5 \times 10^{-4}$  M NBD and from 3 to 7 days in air or  $5 \times 10^{-7}$  M ethylene. The caryopses were incubated constantly in water or in the presence of  $10^{-8}$  M butenolide.**

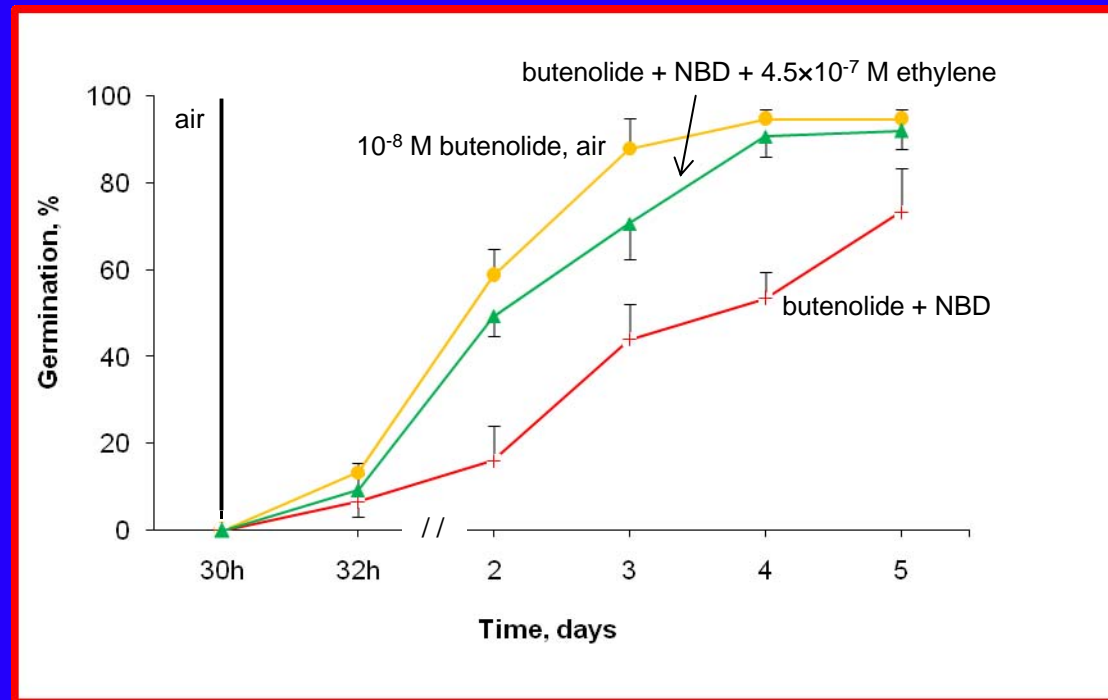


**The effect of a transfer from 2,5-norbornadiene (NBD) to air or ethylene on the germination of *A. fatua* L. caryopses in the presence of butenolide or butenolide + AVG. The caryopses were incubated during 3 days in  $8 \times 10^{-5}$  M NBD and 4 days after transfer in air or  $5 \times 10^{-7}$  M ethylene. The caryopses were incubated constantly in the presence of  $10^{-8}$  M butenolide.**

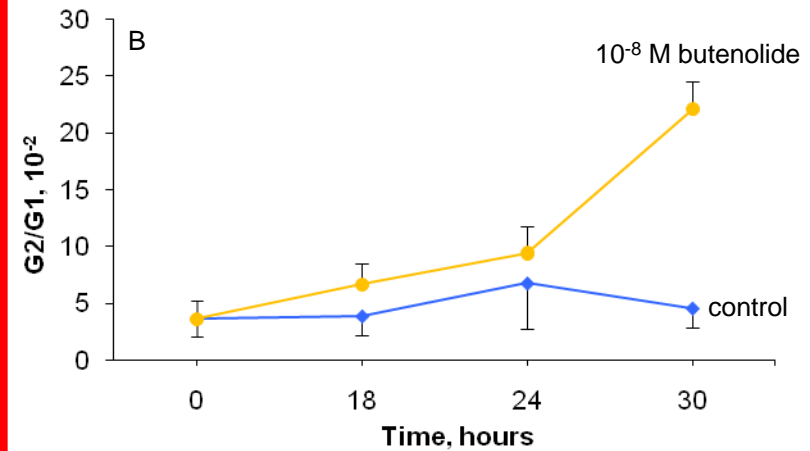
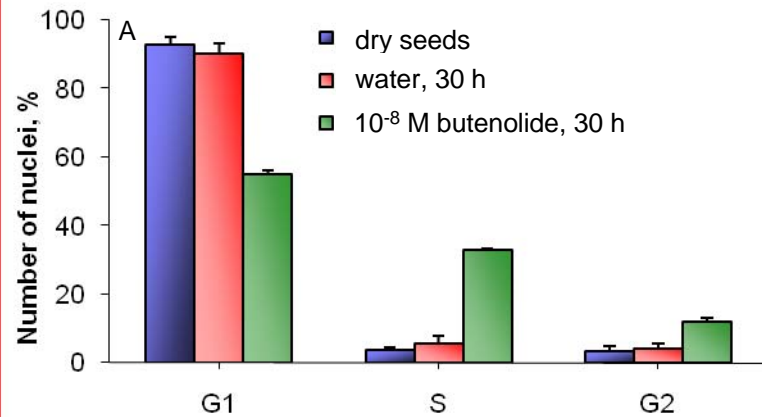




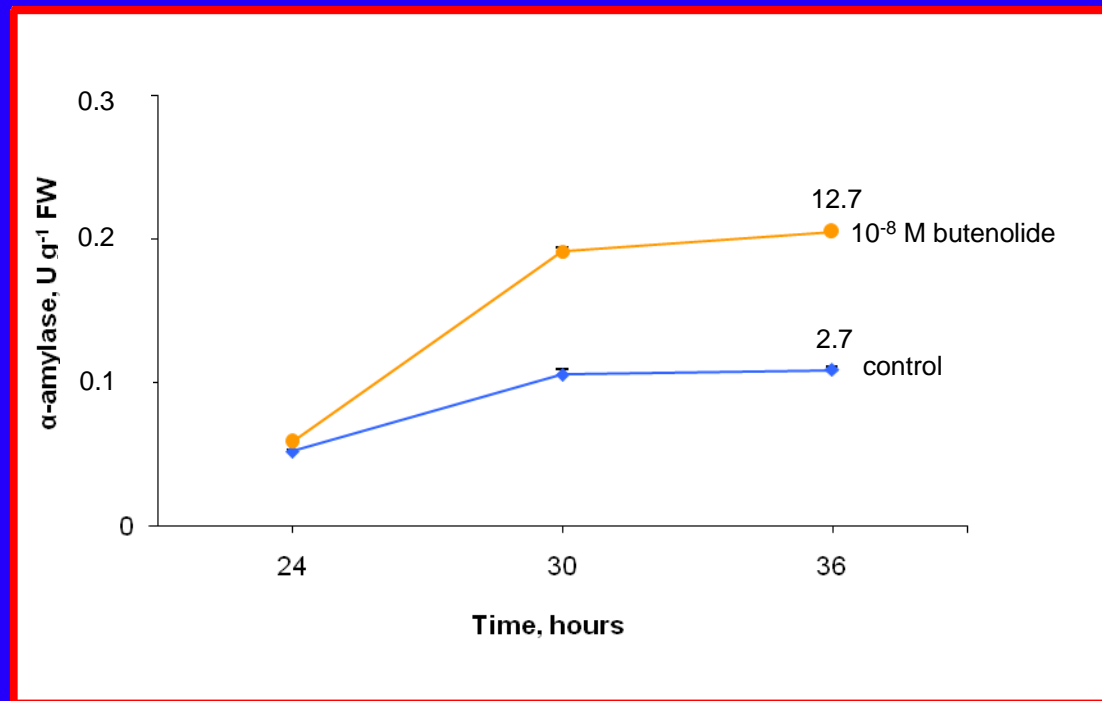
**The effect of butenolide on the germination in the presence of 2,5-norbornadiene (NBD) or NBD + ethylene of *A. fatua* L. caryopses preincubated for 30 h in air. The caryopses were incubated for 30 h in the presence of butenolide in air and from 30 h to 5 days in the presence of butenolide in air, in an atmosphere containing  $5 \times 10^{-5}$  M NBD or NBD+ethylene.**



## The effect of butenolide on the nuclear DNA content (A) and G2/G1 ratio (B) of *A. fatua* L. radicle tip cells



## The effect of butenolide on the $\alpha$ -amylase activity in *A. fatua* L. caryopses after 24, 30 and 36 h of incubation



# Conclusions

- Smoke and butenolide are very active stimulators of seed germination of weed and caryopses of *Avena fatua* L.
- The release of dormancy in *Avena fatua* L. caryopses by butenolide appears to involve endogenous ethylene
- Breaking dormancy is related to increase in G2 DNA content, ratio G2/G1 and increasing  $\alpha$ -amylase activity

# **Acknowledgement**

**The author would like to thank Giovanni Regiroli,  
AgroFresh Inc, Italy, for 1-MCP**



Professor  
Johannes Van Staden  
Research Centre for Plant Growth and Development , School of  
Biological and Conservation Sciences, University of KwaZulu-Natal  
Pietermaritzburg , South Africa



PhD Student  
Danuta Cembrowska  
The Faculty of Natural Sciences , Department of Plant Physiology,  
University of Szczecin

**THANKS FOR YOUR ATTENTION**

