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biodetection

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June 14th, 2005
Standing Comittee Meetings

Carsten Meier
Biologist, researcher
cm@aresa.dk

Thank you Designated co-chairmen, for giving me the opportunity to address this assembly.

I represent the danish research entity, funded by the ministry of science, called Aresa biodetection.

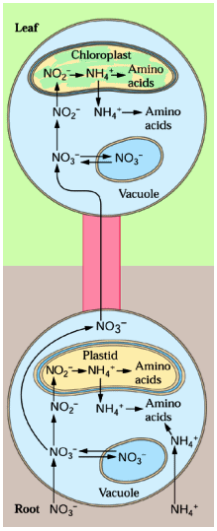
Aresa has engineered a plant that detects UXOs and landmines by changing color from green to red when the plant grows in the vicinity of explosives.

After successful development and tests in a laboratory environment, Aresa has now begun testing in the field in cooperation with the danish government.

I would like to present this project to you.

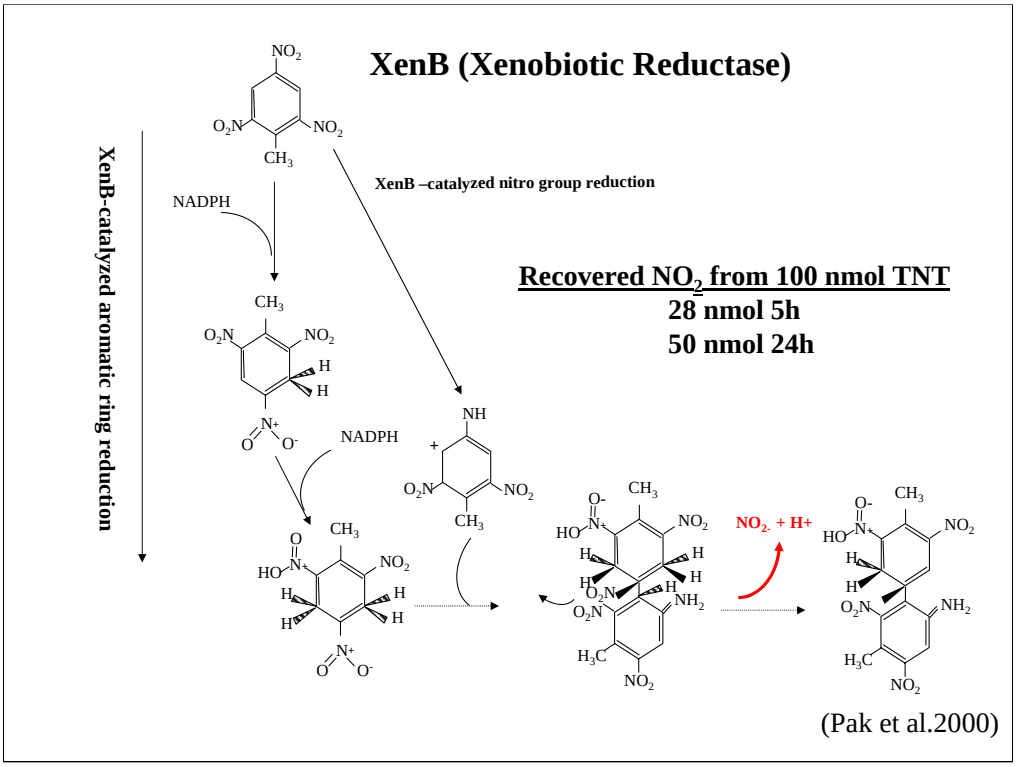
Why plants ?

4 weeks old Arabidopsis root system



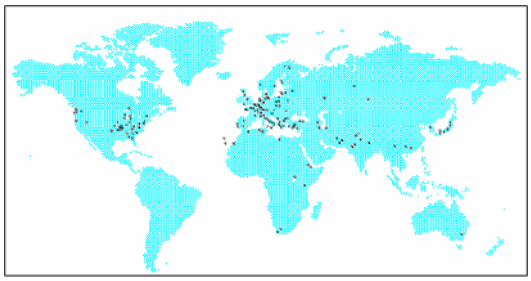
N metabolism In plants

Plants ar able to adapt and therefor have a biochemical reaction path f.x. For NO2. NO2 is part of explosives.

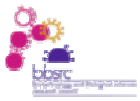


Genes are well described. Able to convert explosives in the soil.

NASC
The European Arabidopsis Stock Centre
NASC provides seed and information resources to the International Arabidopsis Genome Programme and the wider research community. (Old NASC home page)
About NASC | Address & Staff | Ask a Question | Background lines | Bioinformatics at NASC | Feedback | Growing Arabidopsis | Links | PLANET | Plant Science Division | UKPGRG | University of Nottingha



(Jonathan Clarke 1993)

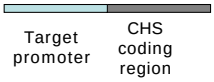


Thale cress is wide spread all over the world, also the palnt for research, whole genome is sequenced.

The technology platform of Aresa

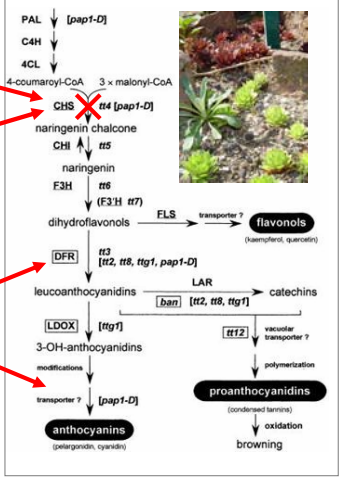
The system regulating red pigment production:

- *tt4* mutant in chalcone synthetase (CHS)
- Introduction of CHS controlled from any promoter of interest



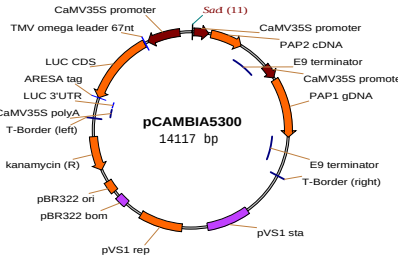
- Overexpression of PAP1/PAP2 to boost anthocyanin production

Biosynthetic route of red pigment production:

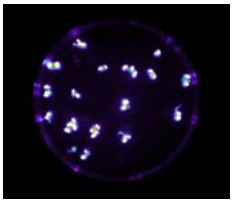


Look out the window, autumn, pigments of the

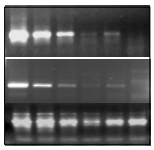
Development of the system



Transformation into tt4-0

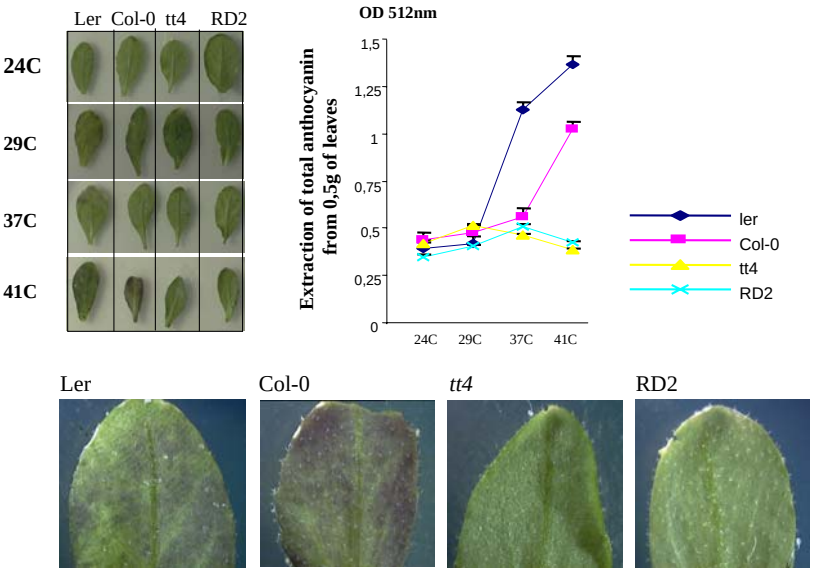


RT-PCR (30 cycles)
on T1 plants line 1-5 and Col-0



T1 Line 1 2 3 4 5 con

Heat and light stress of background line



Can it become red by accident?

Positive control for RD2 T1 35S-CHS-E9

- Introduction of CHS controlled from any promoter of interest

Completely red plant

NO_2 regulated promotors

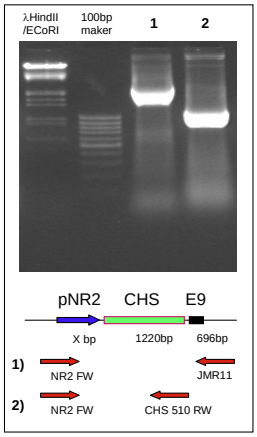
Nii (At nitrite reductase) At2g15620
predicted function:
Nitrite reductase (NO-forming) activity

Ntr12 (High-affinity nitrate transporter ACH2) At1g08100
predicted function:
Nitrate transporter activity

NR1 (Nitrate reductase 1) At1g77760
predicted function:
Nitrate assimilation and nitrate reductase activty

NR2 (Nitrate reductase 2) At1g37130
predicted function:
Nitrate assimilation, nitrate reductase activty
And nitrate reductase (NADH) activity

pNR2-CHS-E9



Explosives NO2 degraded in soil,

Experiment: TNT inducible promoters

Experiment Summary | Samples | Slides & Datasets | Array Design | View All

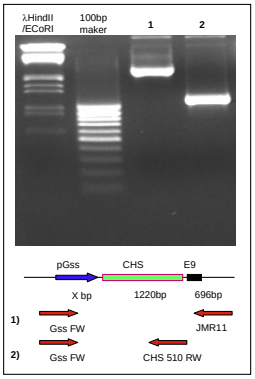
Submission Number	235
TAIR Accession	ExpressionSet:1005823601
Experimenter(s)	James Stewart
Experimental Variables	TNT

Ribo (small nuclear ribonucleoprotein) At2g47580
predicted function: increased tolerance to salt stress when expressed in the yeast.

GSS (glutamine-dependent asparagine synthetase) At3g47340
predicted function: amino acid catabolism.

GTP (glucose transporter) At1g11260
predicted function: carbohydrate transporter activity.

pGSS-CHS-E9



Experiments done, how does plants break down explosives.

SAGE (Serial analysis of gene expression)

Arabidopsis Transcriptome Responses to 2,4,6-Trinitrotoluene

Table 1. *SAGE tags induced at least 10-fold by exposure to TNT*

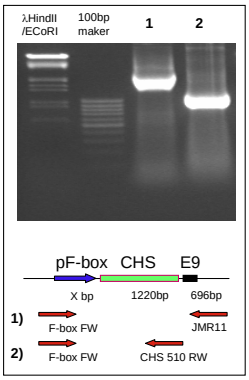
Tag Sequence	Tag Abundance		-Fold Increase[a]	P-Chance[b]	Locus	Annotation
	TNT	Control	TNT/ Control			
GTCAGTTTGA	30	0	>30.0	0	At2g18915[c]	F-box protein LKP2/ADO2
CCAAATTCTG	55	2	27.5	0	At1g17170	GST, putative

(Ekman et, al. 2003)

F-Box F-box family protein / LOV kelch protein 2 (LKP2) At2g18915.1
predicted function: signal transducer activity

GST glutathione S-transferase, putative At1g17170
predicted function: toxin catabolism

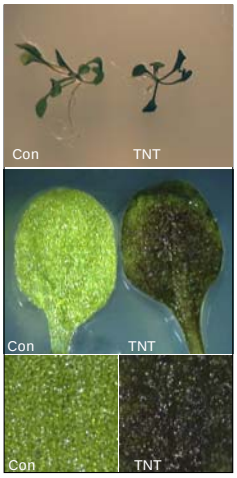
pF-BOX-CHS-E9 PCR

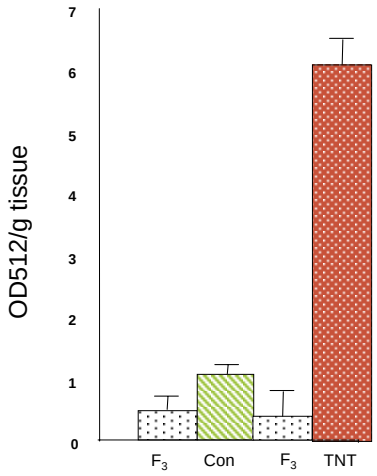


5 days of treatment with explosives RDX and TNT

Put the switch in and see results.

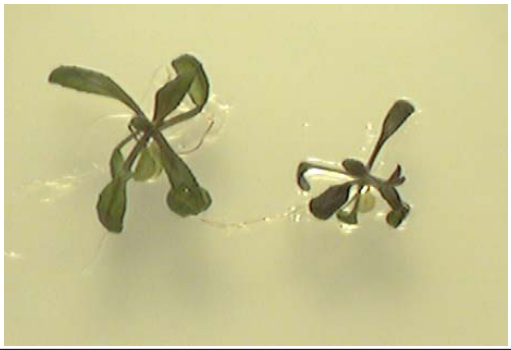
Line 67 Nii-CHS-E9 from plates -/+ 12,5 mg/l TNT

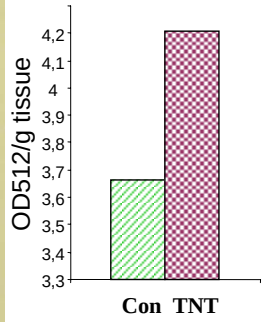




Sample	g Tissue	Factor	OD 512nm	g/OD
Con-67-rød-2	0,4871	2,052967	0,523	1,073701
TNT-67-rød-2	0,1449	6,901311	0,884	6,100759

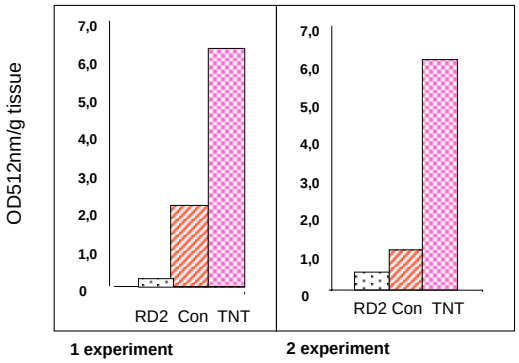
Line 58 RIBO-CHS-E9 from plates -/+ 12,5 mg/l TNT





sample	g Tissue	factor	OD 512nm	
con-85-rød	0,3049	3,279764	1,118	3,666776
tnt-85-rød	0,2231	4,482295	0,939	4,208875

Line 67 Nii-CHS-E9 cultivated in soil mixed with TNT 12mg/L^{1-}

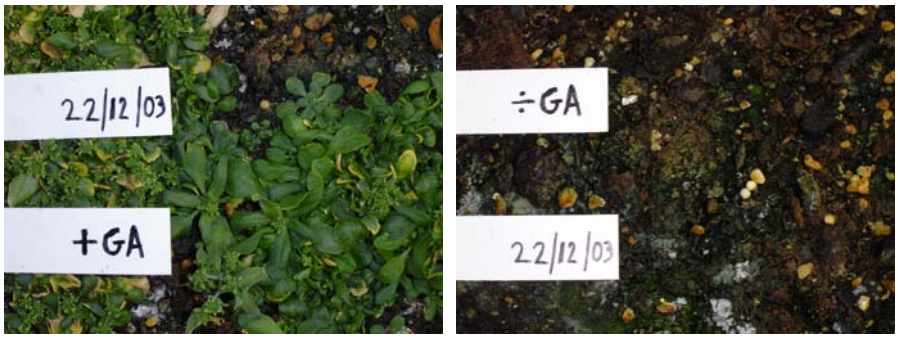


Plants from the 2 experiment

Flow in experiment, does it change cloo

Spread of GMO plants to the environment

ga1-3 gibberllin deficient mutant +/- GA3 treatment



EC GMO debate

Gibberellins in agriculture and horticulture

Dwarf Tall

High yielding semi-dwarf rice has reduced endogenous gibberellin

-GA

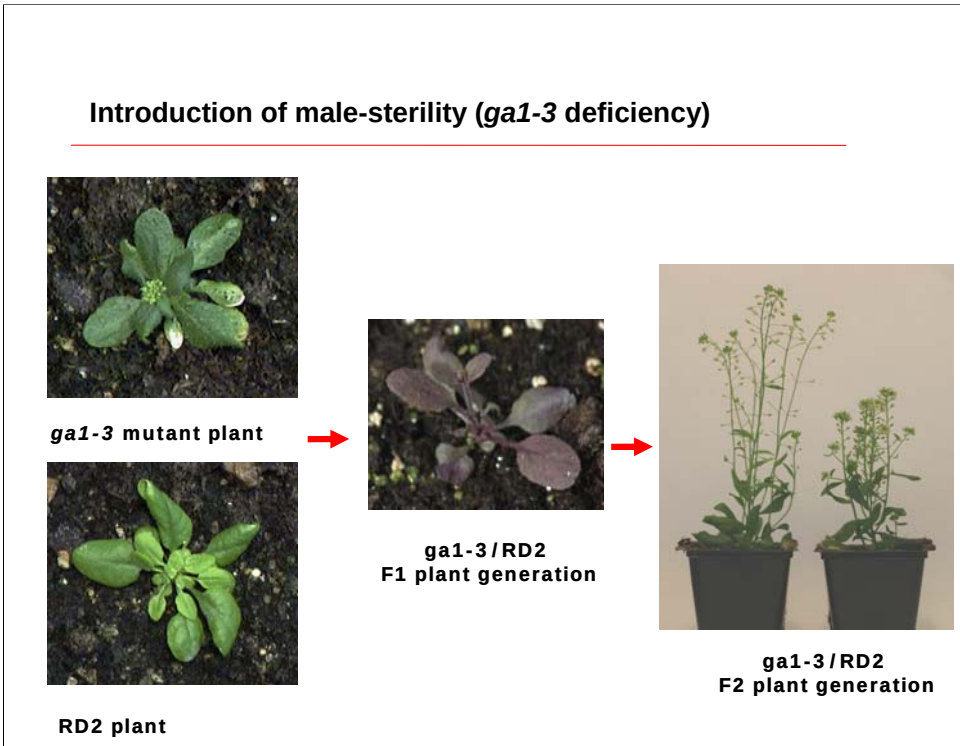
+GA

Fewer flowers and larger fruit

Delayed fruit harvest

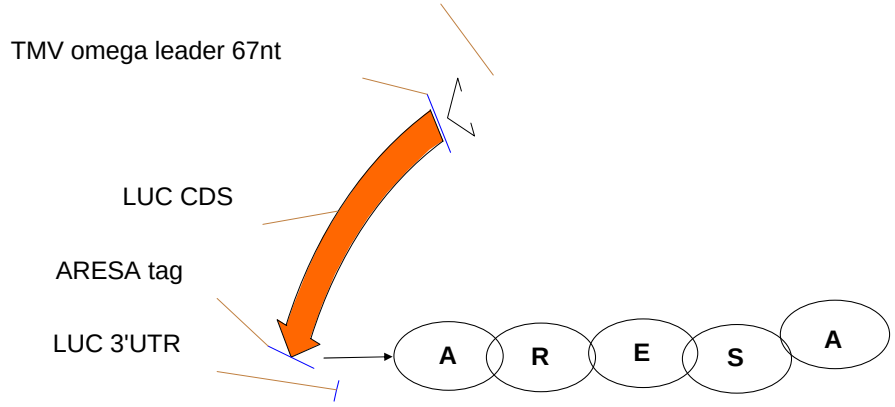
Increased fruit size

GAs are used commercially to increase fruit size in table grapes and to regulate citrus flowering and rind maturation



Crosses it becomes red.

DNA tag at the end of the maker gene



Operational procedures for use of the system

Establishing a grid system boxes (manual de-mining and/or flails)

Removal of vegetation (vegetation cutters, controlled burning or herbicides)
Establishing water supply systems
Dispersion of seeds
4 weeks growth period
Clearance of boxes

The Danish EOD forces are working out practical methodologies.

All realistic, creative and constructive critique for practical application is very welcome.

Practical partnerships are also welcome.

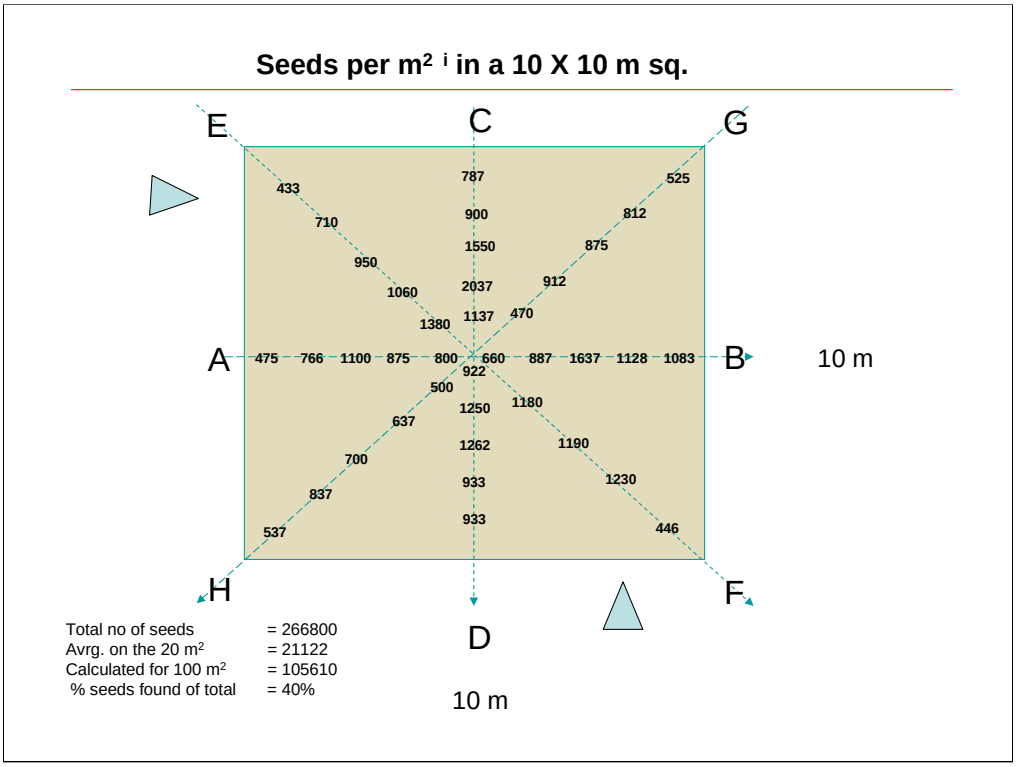
Works on agricultural land.

Pelleted Arabidopsis seeds for distribution tests

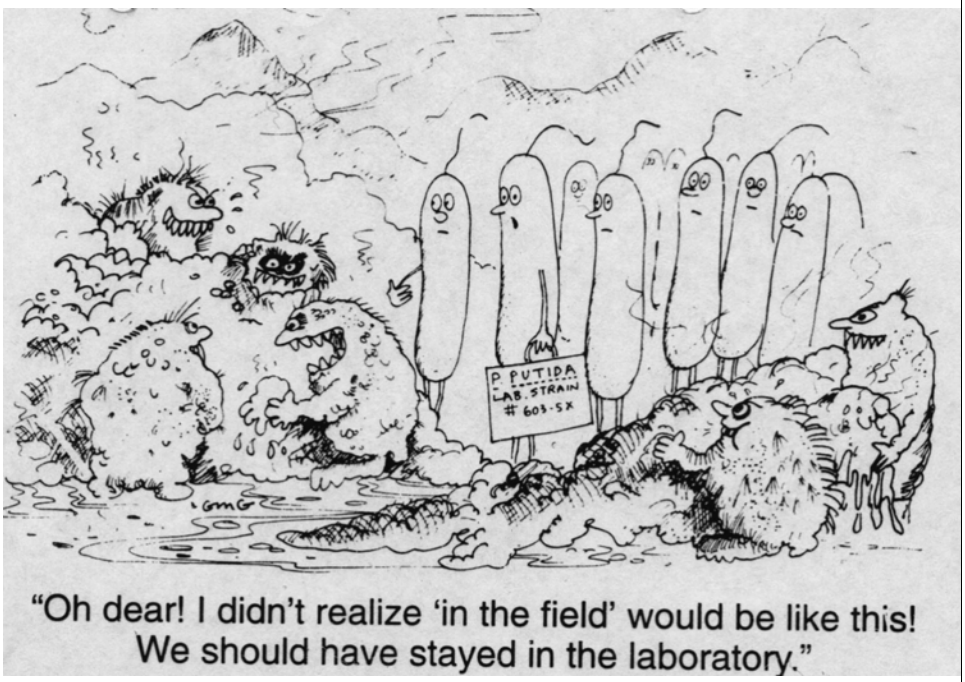
The clay coat Contains:
Seed,
GA3 hormone,
Fertilizer for plant growth
Dye depending on the color of the soil in the working area

Angola 2003

Initial setup of dispersion, add whatever to seeds



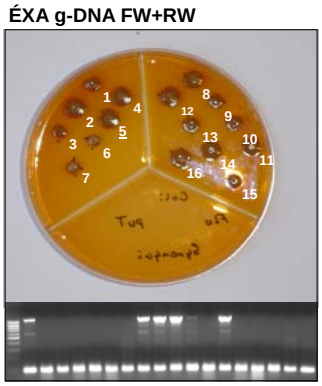
Dispersion test



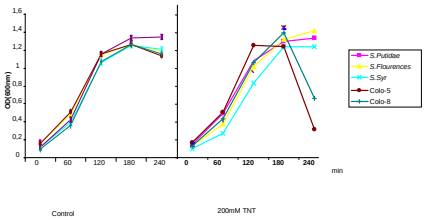
"Oh dear! I didn't realize 'in the field' would be like this!
We should have stayed in the laboratory."

Colony PCR on isolated bacteria

ÉXA g-DNA FW+RW

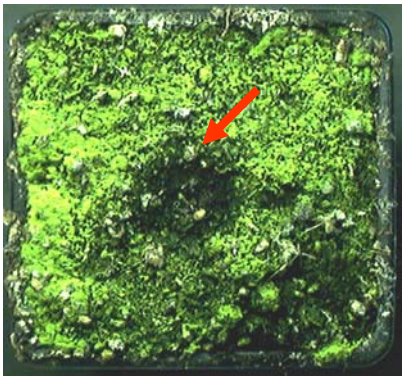


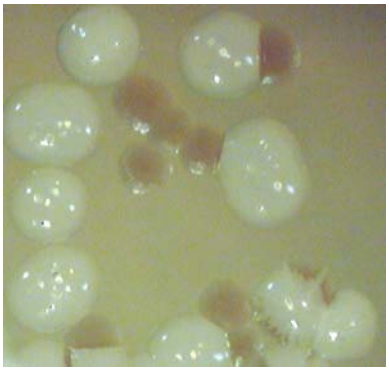
1 g of std. soil



You need bacteria in the soil, are the there? Yes always.

0,25g TNT (12 weeks incubation)





Sampels	Con	TNT
Avrg 6 samples	48	2881

Ditto in normal soil

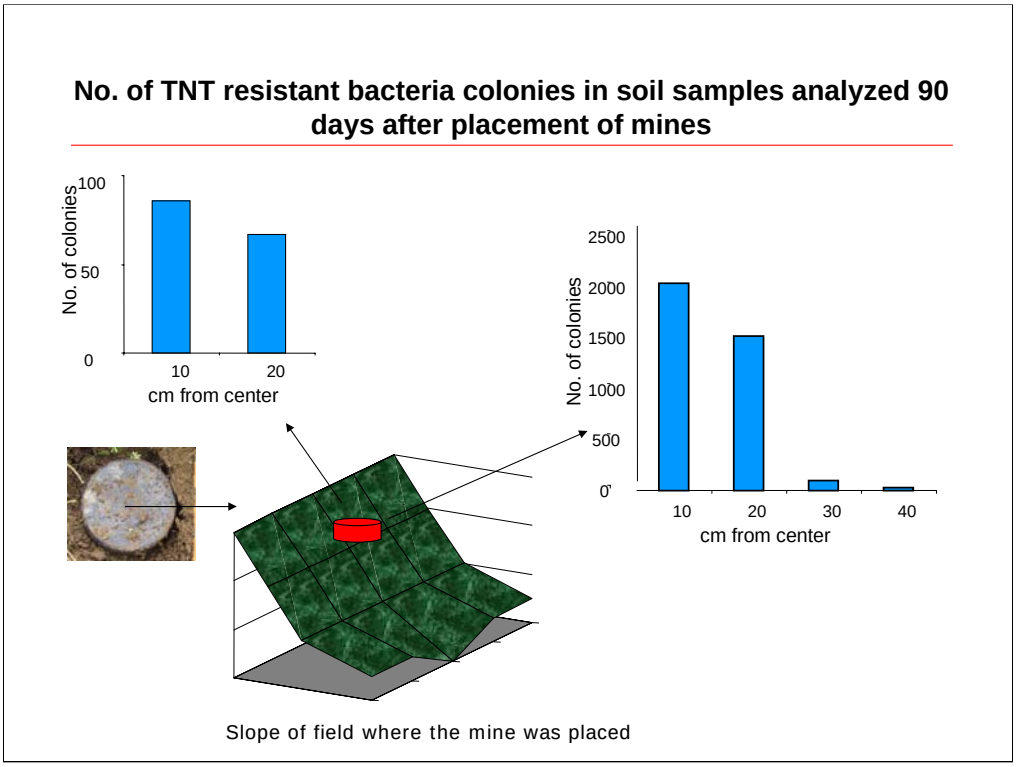
Soil samples from a mine field in Bosnia controlled by the Norwegian People Aid

Bosnia 2004

Soil samples from center of the mine

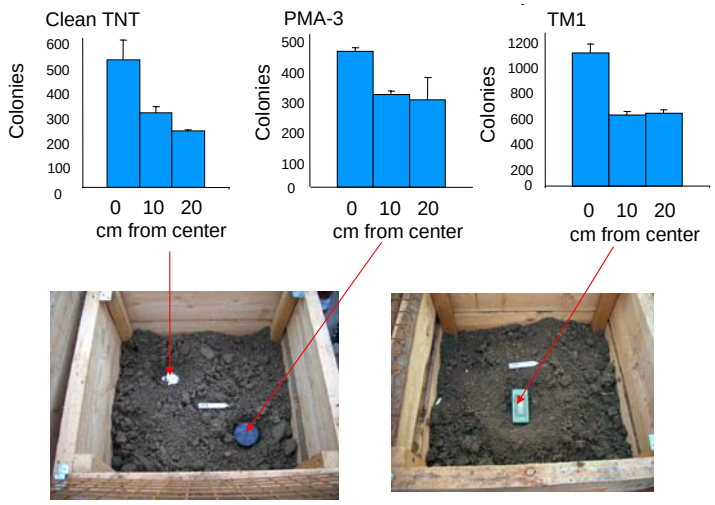
Mine (PMA-3) exposed after removal of the soil

Are there bacterias in wild life


No. of TNT resistant bacteria colonies in soil samples analyzed 90 days after placement of mines
No. of colonies
100
50
0
10
20
cm from center
No. of colonies
2500
2000
1500
1000
500
0
10
20
30
40
cm from center
Slope of field where the mine was placed

yes

Colony no. 60 days after placement of TNT targets



Mine test boxes at Risø, Denmark

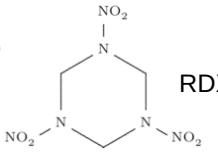
Bacteria gradient.

Test area in Denmark

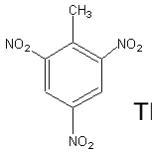
Target placment in the test area

Explosives being used in Danish EOD forces test

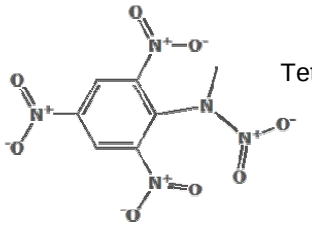
Hexanitro stilben (HNS-NSE-853)
Nitropenta (PETN)
Com B (RDX & TNT)
Tetryl piller (used for detonators)
C4 (RDX derivative)
TNT (trotyl)
RDX (oldest plastic explosive)
AT mine M56
AP mine PMA-3



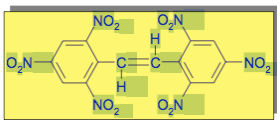
RDX



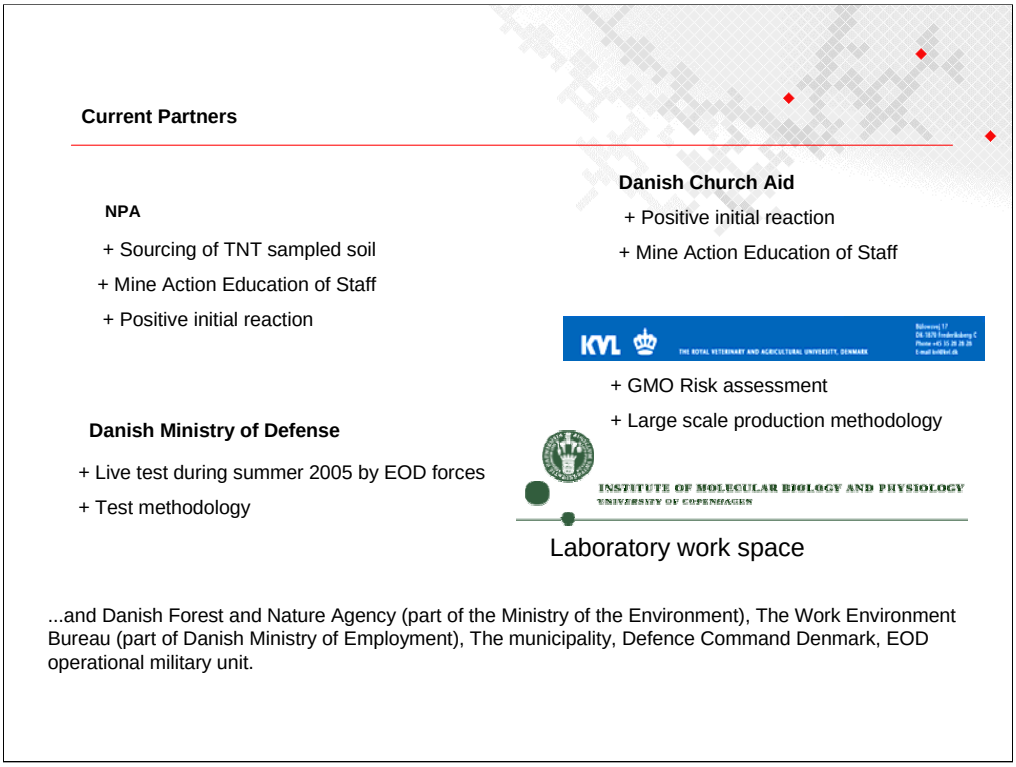
TNT



Tetryl



Hexanitrostilben



The plant project needs all the constructive critique possible by de-mining experts, including - how do we setup a methodology for the practical usage economically viable for affected areas? We are not the experts here, and would like to use the opportunity to ask for all relevant input.

Aresa is currently in active partnerships with Norwegian Peoples Aid, Danish Church Aid and the Danish Army/Ministry of Defense, with respect to logistics, tests and methodologies, staff education and more.

We would like to use this opportunity to send our warm regards and special thanks for their aid.

Aresa is searching for further partnerships with states parties and NGOs.

Working Crew

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Anders Søndergaard

Researcher
Brian Olszak

Liaison
Bernino Lind

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The PFA Staff of Copenhagen University