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Forschungsanstalt Agroscope Changins-Wädenswil ACW

Society for Medicinal Plant and Natural Product Research
Permanent Committee on Breeding and Cultivation of Medicinal Plants

Genetic Resources, Conservation and Breeding

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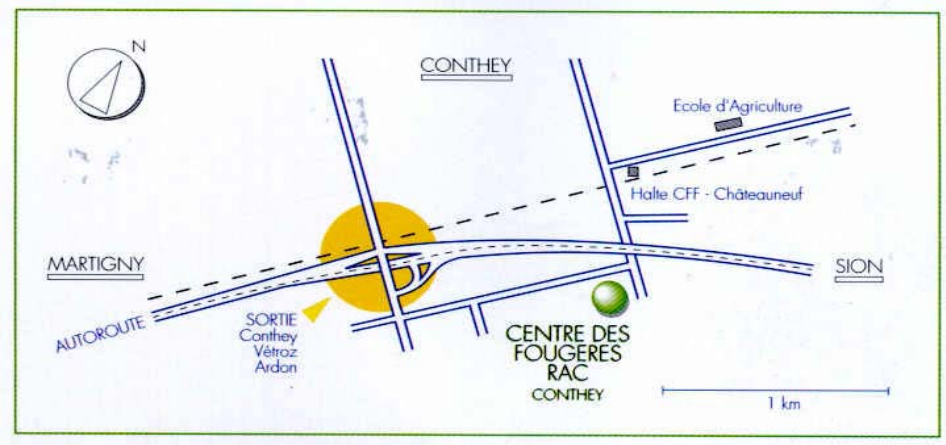
Agroscope Changins-Wädenswil ACW Research Station

Research Centre Conthey

-35 persons
-25 ha in Conthey
- 1 ha in Aproz
- 1 ha in Arbaz
- 2 ha in Bruson
- different labs



Agroscope Changins-Wädenswil ACW
Research Station Recherche Centre Conthey

Medicinal and aromatic plants

Small fruits

Fruit-growing

Glasshouse Crops

M E D I P L A N T

Research Centre for Medicinal and Aromatique Plants

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www.mediplant.ch
mediplant@acw.admin.ch

mainly projects from private organisation

supported by Agroscope ACW and the 'Canton du Valais'

6 persons

Research in medicinal and aromatic plants (Agroscope ACW / Mediplant)

Breeding new cultivars or testing cultivars	**Quality**
Optimising cultivation techniques and plant protection	**Expertises Consulting Teaching**

Research in medicinal and aromatic plants (Agroscope ACW / Mediplant)

xavier.simonnet@acw.admin.ch

Structure of the presentation: Breeding of MAP

- Introduction
  - Market and cultivation of medicinal plants
  - Importance of agronomic research for the supply and quality of medicinal plants
  - Importance of conservation, characterisation, evaluation of genetic resources (biodiversity) for breeding
- Breeding examples: projects of Agroscope ACW and Mediplant:
  - Breeding for increased levels of desired compounds
  - Breeding for higher resistance
  - Breeding for better homogeneity
  - Breeding for decreasing undesired compounds
- Conclusions and Perspectives

Global Market of medicinal plants

- 422‘000 flowering plants on the planet
- 21‘000 (WHO, 1980) to 52‘000 (FAO, 2002) species ➔ medicinal plants; most of them used as traditional medicine for primary health care needs
- 2‘500 species in international trade (FAO, 2002)
- ~ 200 species are cultivated (FAO, 2002)
- For companies trading medicinal plants, 60 – 90 % of the volume of medicinal plants are from cultivated production (Laird and Pierce, 2002)

Why cultivation of medicinal plants

+ continuing supply of raw material is guaranteed
+ production volume and price can be agreed for longer periods
+ genotypes can be standardised and improved (by breeding)
+ quality standards are easy to maintain with optimal cultivation procedures and post-harvest handling
+ certification is possible (i.e. oragnic farming)
+ no risk of a decrease of biodiversity due to over-harvesting of wild plants (especially for plants with a high demand)
- it needs substantial investments before and during production

➔ R & D is essential for a successful cultivation:

botany, conservation of genetic resources
breeding, optimising cultivation procedures

Why biodiversity is important for breeding ?

- The wild relatives of cultivated plants provide a reservoir of potentially important genes for crop improvement programmes.
- To efficiently use these genetic resources, it is important that biodiversity is further studied, characterised, evaluated, conserved and available for breeders.
- This will allow to find new genotypes of wild species and local/neglected varieties with key genes for important traits suitable for developing improved and new cultivars for agricultural production.
- This will also allow to promote domestication of wild species for product innovation and new bioactive compounds .

Breeding of medicinal plants

Exemplarily will be presented projects of Agroscope ACW and Mediplant:

Breeding for increased levels of desired compounds (*Artemisia annua, Thymus vulgaris*)

Breeding for higher resistance against biotic factors (*Hypericum perforatum*)

Breeding for better homogeneity (*Salvia officinalis*)

Breeding for decreasing undesired compounds (*Artemisia unbelliformis*)

Artemisia annua: Breeding for increased levels of desired compounds

Plant: ***Artemisia annua*** **L. (annual wormwood;** ***Asteraceae*** **)**

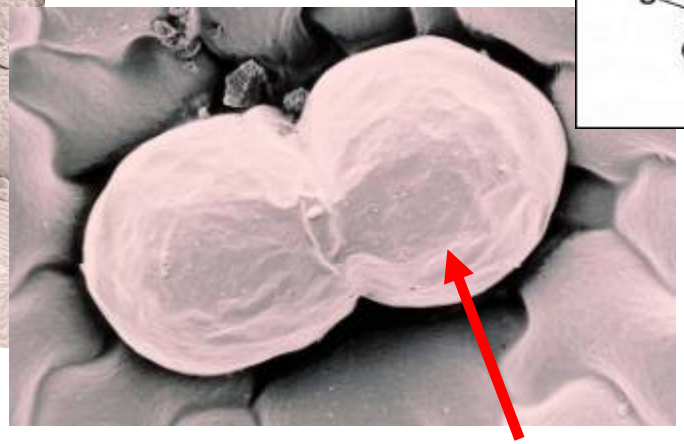
Properties: highly efficient against ***Plasmodium falciparum*** **(pathogen of Malaria)**

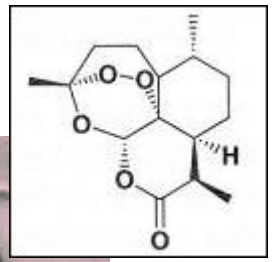
Drug: Artemisinin, extracted from leaves of ***Artemisia annua.*** **It is the only source of artemisinin at the moment.**

Artemisia annua: Breeding for increased levels of desired compounds

Active compounds: Artemisinin (sesquiterpene lactone endoperoxid), is a highly effective anti-malarial compound.

It is also efficient against multidrug-resistant strains of *Plasmodium falciparum*. WHO officially adopted artemisinin-based combination therapies (ACTs) as the most efficient treatment against malaria





Artemisia annua: Breeding for increased levels of desired compounds

Breeding aim: **Cultivars with increased content of Artemisin in the leaves.**

Breeding tools:

testing available genetic resources,

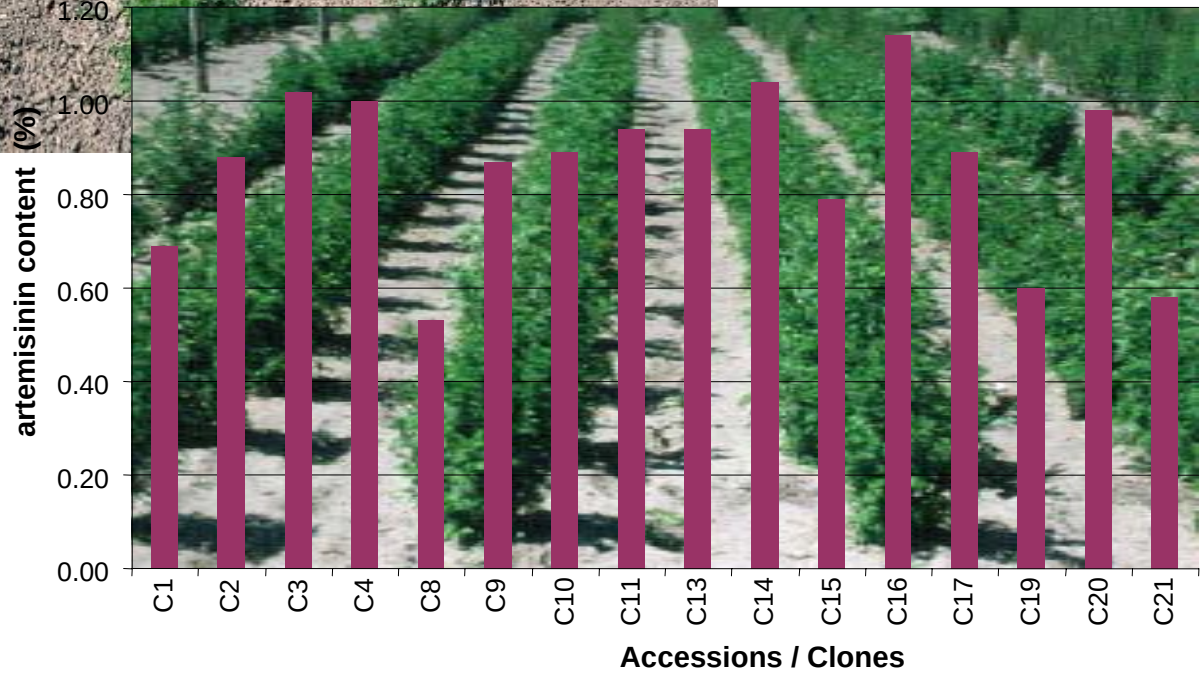
floral biology,

classical breeding techniques,

in vitro techniques

Artemisia annua: Breeding for increased levels of desired compounds

Testing of available Genetic resources (accessions /clones



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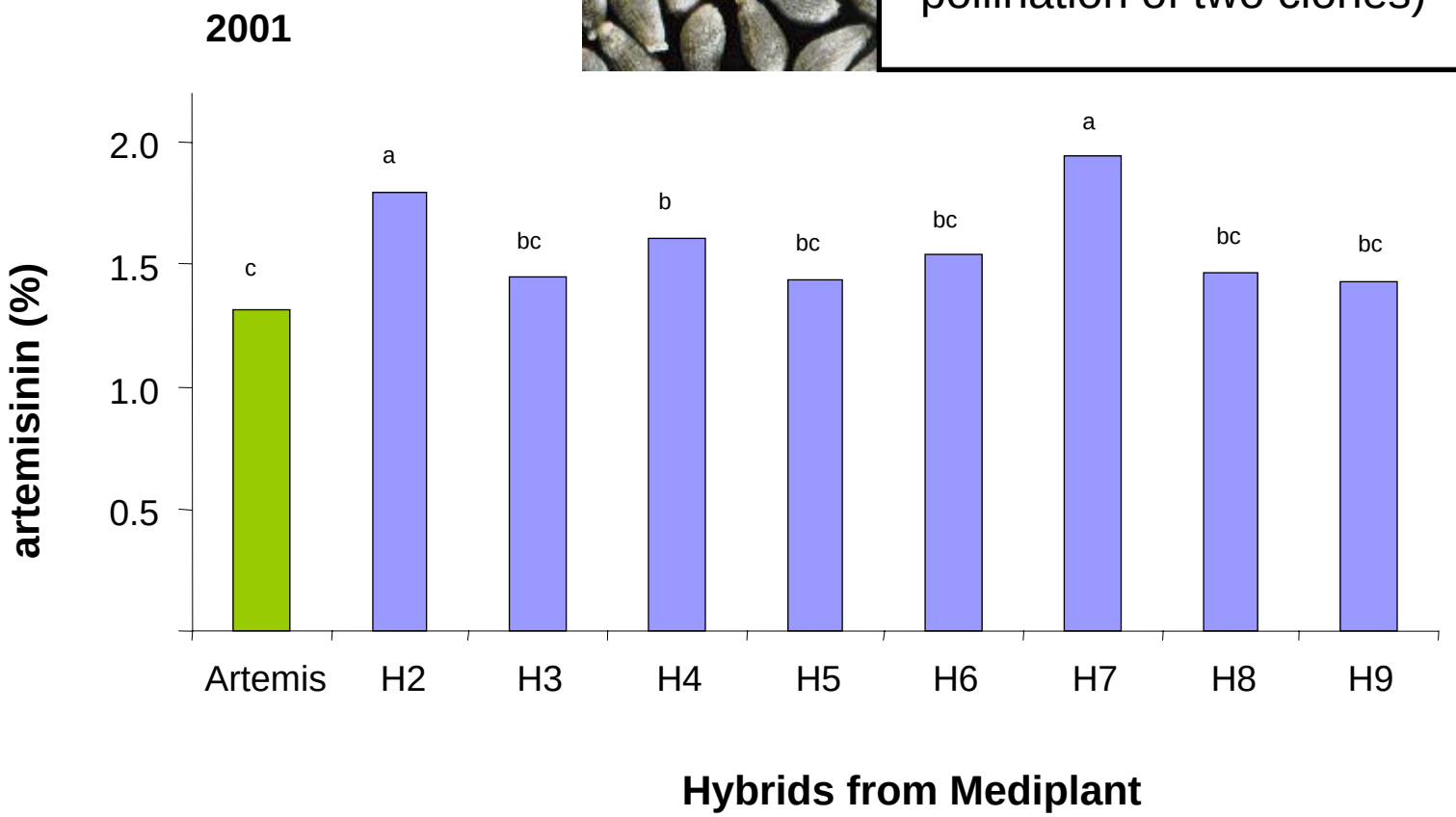
Artemisia annua: Breeding for increased levels of desired compounds

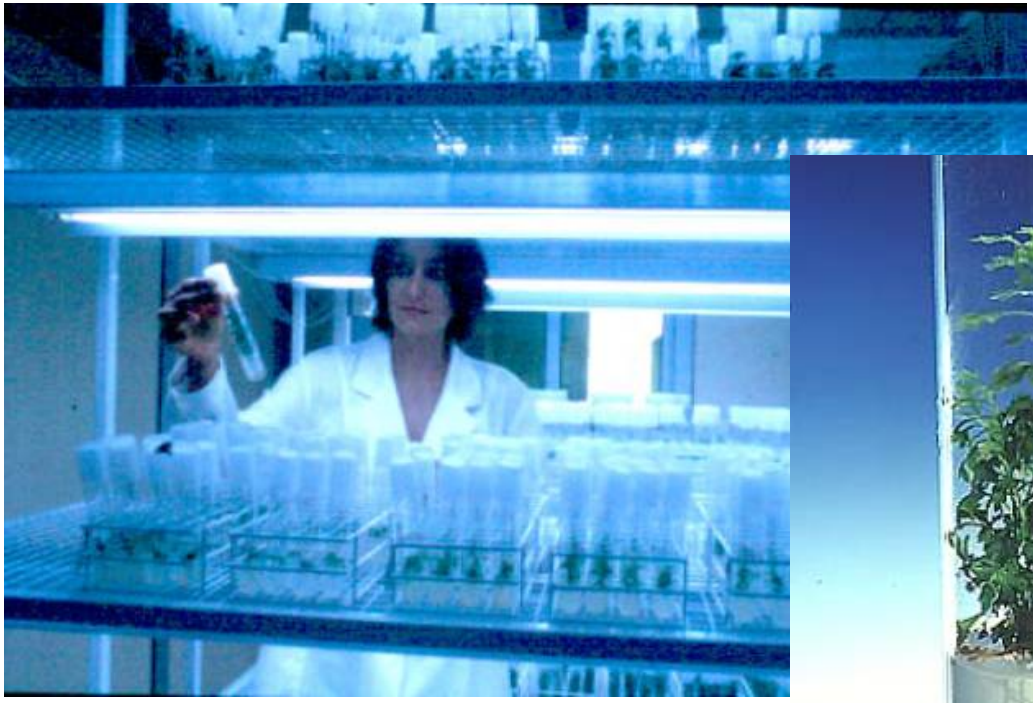
Origine	Type	Artemisinin content (%DW)	Reference
Germany	wild	**0.02**	Singh *et al*., 1988
USA (Connecticut)	wild	**0.06**	Charles *et al*., 1990
Argentina	wild	**0.10**	Acton *et al*., 1985
India	cultivar	**0.11**	Sharma *et al*., 1991
China	wild	**0.14**	Charles *et al*., 1990
USA (Dakota)	wild	**0.21**	Charles *et al*., 1990
Spain	wild	**0.24**	Delabays *et al*., 1993
Vietnam	wild	**0.46**	Wallaart *et al*., 1999
Netherlands	tetraploid	**0.52**	Wallaart *et al*., 1999
China	wild	**0.60**	Liu *et al*., 1979
China	wild	**0.79**	Anonymous, 1980
Vietnam	wild	**0.86**	Woerdenbag *et al*., 1994
China	wild	**1.07**	Delabays *et al*., 1993
Switzerland	hybrid	**1.38**	Delabays, 1997; Cultivar Artemis

Artemisia annua: Breeding for increased levels of desired compounds

Testing of combination ability of the most interesting clones still continues (by cross-pollination of two clones)

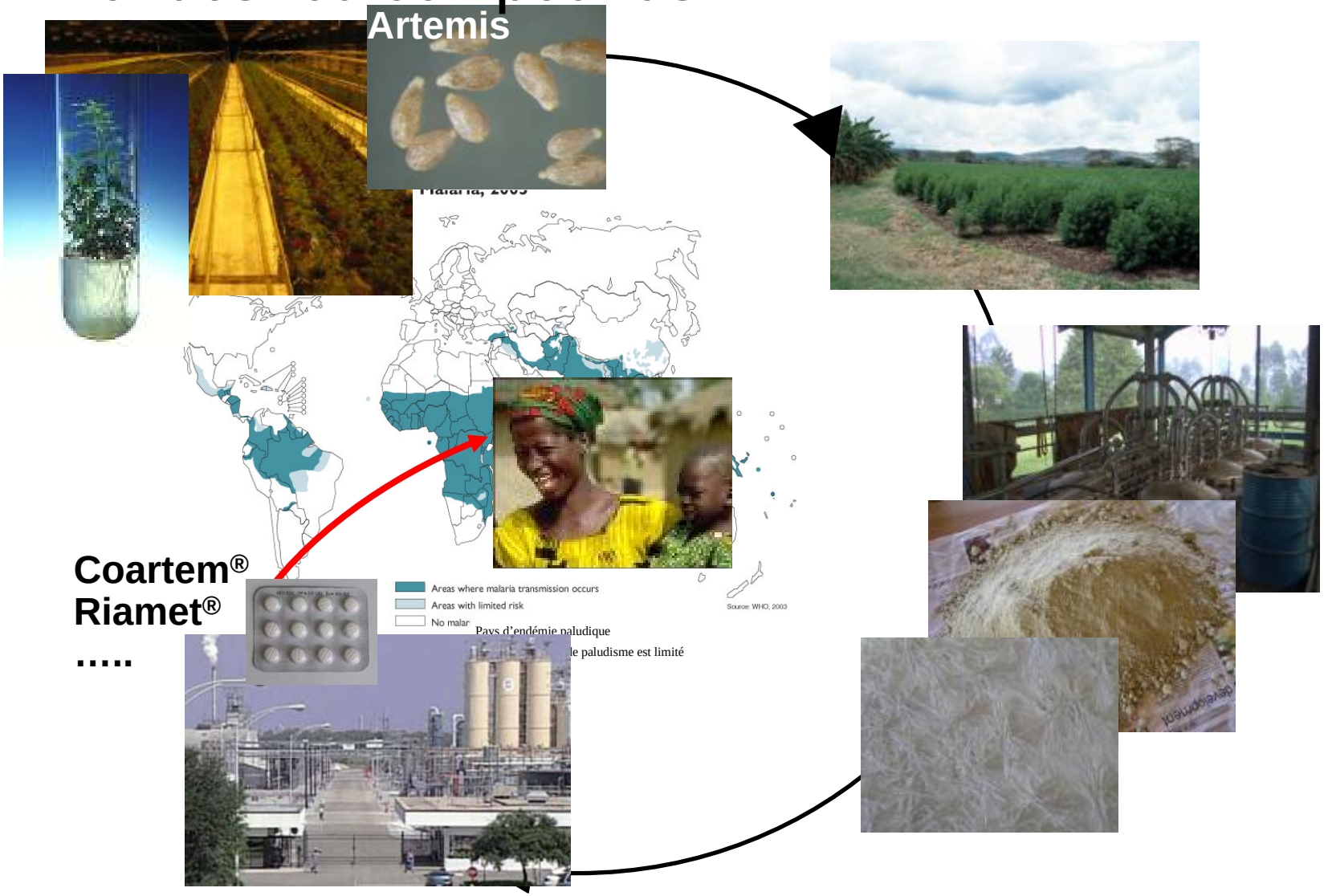


Artemisia annua: Breeding for increased levels of desired compounds



In vitro techniques for conservation and multiplication of interesting parental lines

Artemisia annua: Breeding for increased levels of desired compounds



Thyme: Breeding for increased levels of desired compounds

Plant: ***Thymus vulgaris*** **L. (thyme)**

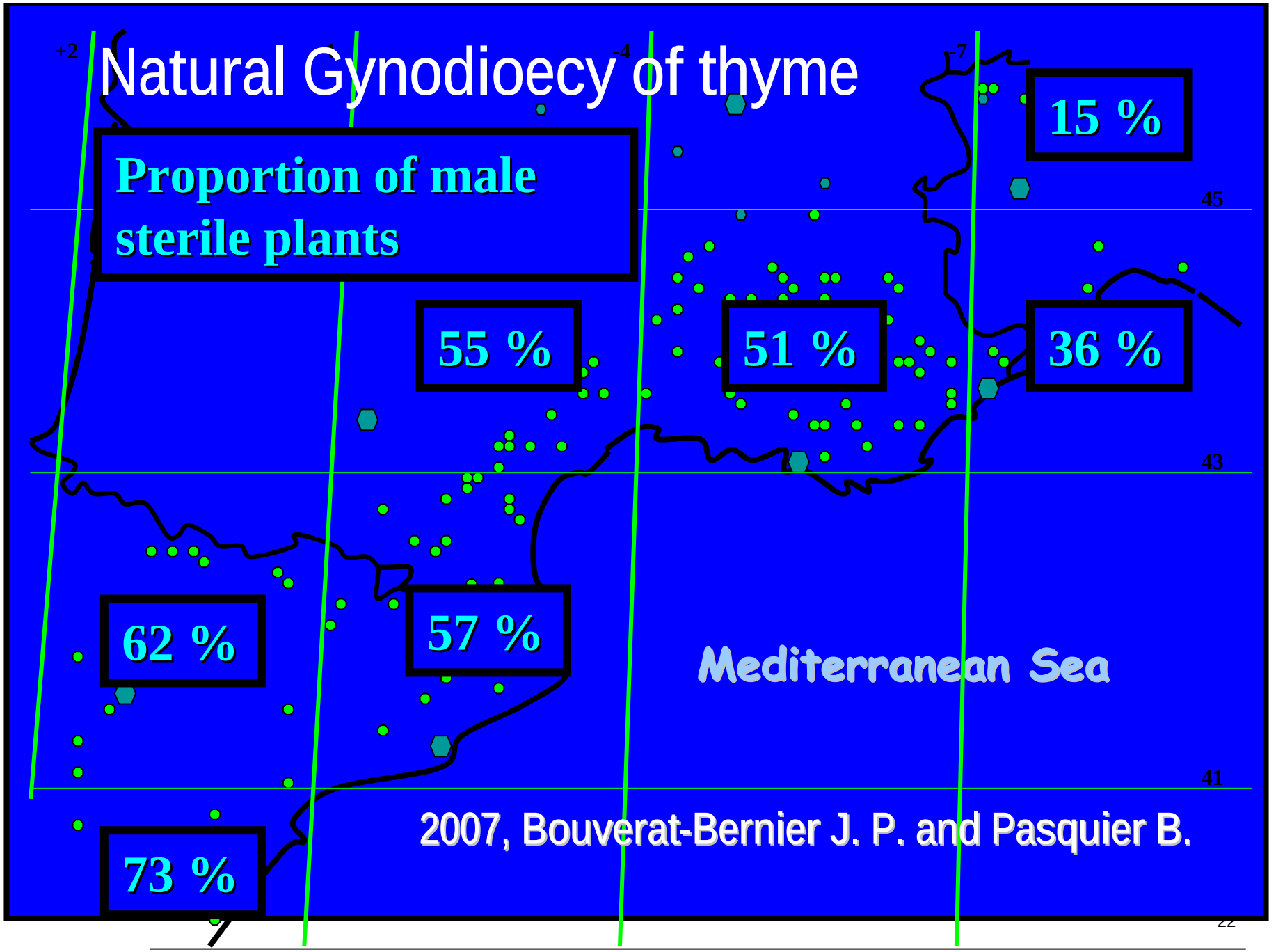
Properties: The essential oil is used as a flavour additive, as well as in antimicrobial and antioxidative products

Thyme: Breeding for increased levels of desired compounds

Gynodioecy of thyme flowers

Male sterile plant

Male fertile plants
(hermaphroditic plants)


Natural Gynodioecy of thyme
Proportion of male sterile plants
15 %
55 %
51 %
36 %
62 %
57 %
73 %
Mediterranean Sea
2007, Bouverat-Bernier J. P. and Pasquier B.
+2
-1
-4
-7
45
43
41

Thyme: Breeding for increased levels of desired compounds

Breeding goals:

high yield in essential oil,

homogeneity,

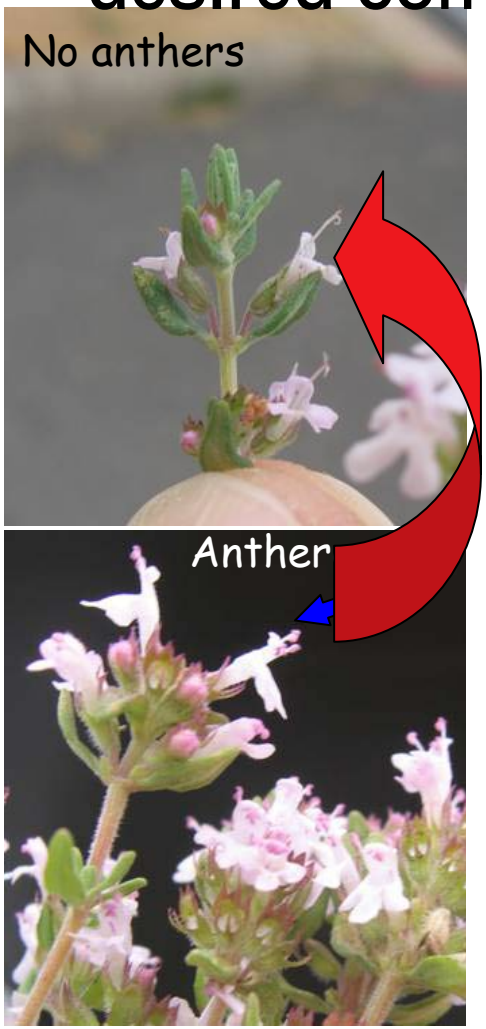
winter hardiness

Breeding method:

Crossing male sterile and male fertile plants

(MS-plants x MF-plants)

Thyme: Breeding for increased levels of desired compounds



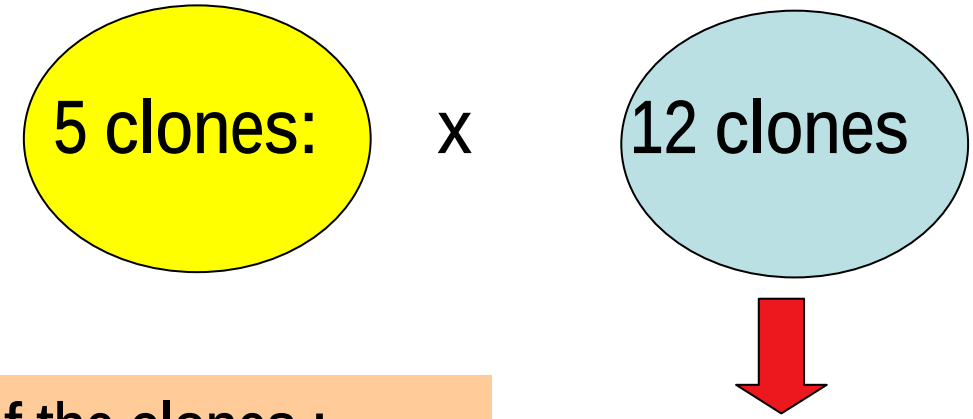
Male sterile plants (MS)

Male fertile plants (MF)

MF-clone x MS-clone

Seeds on MS-clones → cultivar

Thyme: Breeding for increased levels of desired compounds



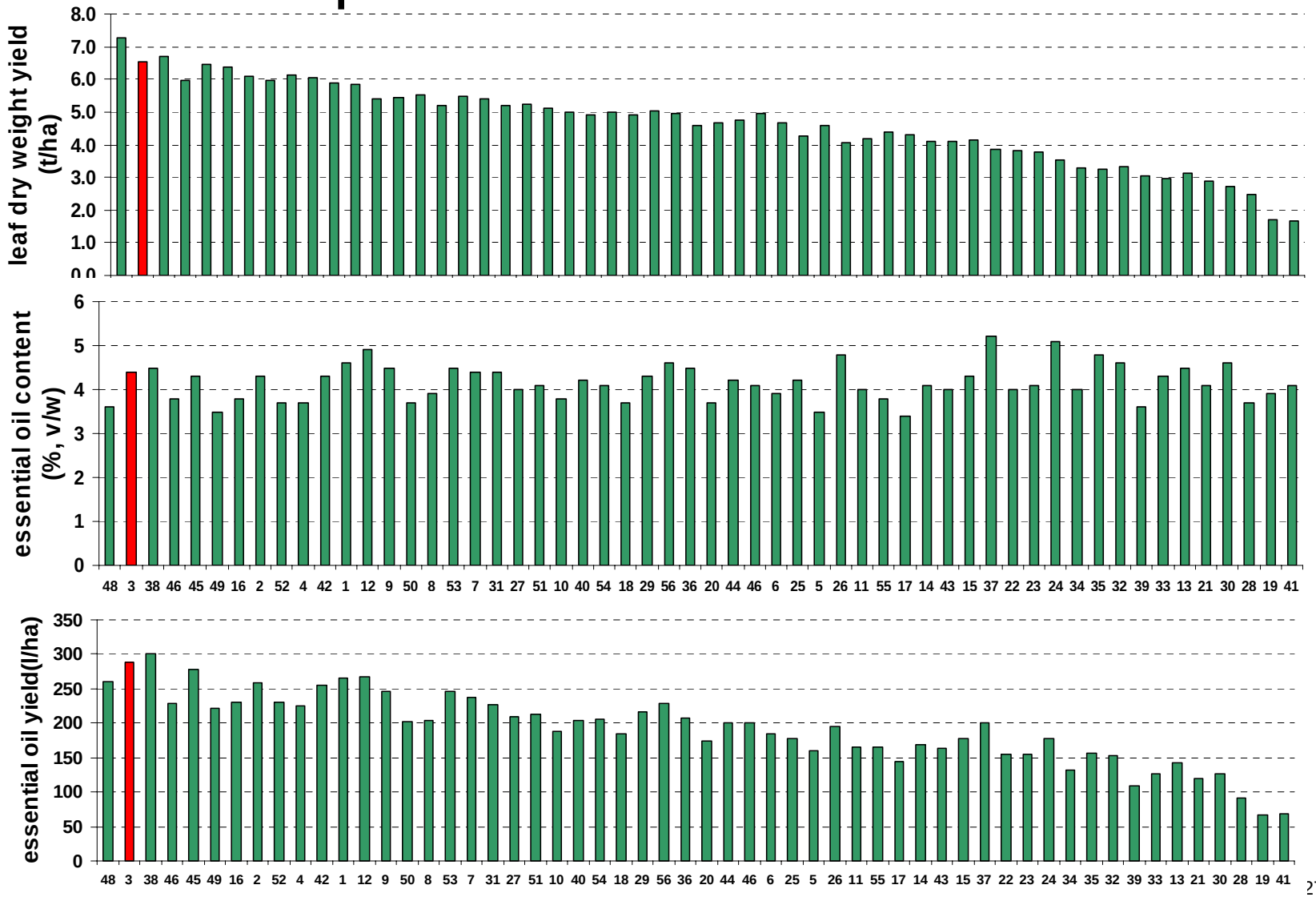
Origin of the clones :

- Wild populations in the Aosta Valley
- Breeding material from Agroscope ACW
- Old cultivar ‘Deutscher Winter’

Thyme: Breeding for increased levels of desired compounds

- Period: 2000 - 2002
- Planting date: beginning of May 2000 (plantlets)
- Planting density: 5.7 plants/m^2 (4 X 25 plants per hybrid)
- Fertilisation kg/ha: 60 N, 30 P_2O_5, 100 K_2O et 15 Mg
- Irrigation, if necessary
- 5 harvests: 4.9.00; 5.6.01 und 11.09.01; 18.6.02 und 24.09.02
- Organic farming
- 4 replications

Thyme: Breeding for increased levels of desired compounds



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Thyme: Breeding for increased levels of desired compounds

‘Varico 3’ compared to other cultivars

Leaf yield, content and yield of essent. oil

(3 harvests and 4 replications)

Cultivar	Origin	dw-leaf yield (t/ha) ∑ 07-08	Content of essent. Oil (%) ∅ 07-08	Essent. oil yield (l / ha) ∑ 07-08
Varico 3	*Hybrid*	**3.9** ab	**4.9** b	**191** a
Varico 2	*Hybrid*	**4.2** a	**3.5** c	**146** b
Dt. Winter	*Population*	**3.3** c	**2.9** d	**97** c

St. John‘s wort: Breeding for higher resistance

Plant: ***Hypericum perforatum*** **(St. John‘s wort, Hypericacaea)**

Properties: antidepressive, antiviral, vulnerary,
mainly used for mild depressions, cancer treatments, ..

Drug: flower horizon

Active compounds: Hypericin, Pseudo-hypericin, hyperforin,
flavonoides

St. John‘s wort: Breeding for higher resistance

Disease on stem base:
Dieback (*Colletotrichum gloeosporioides)*

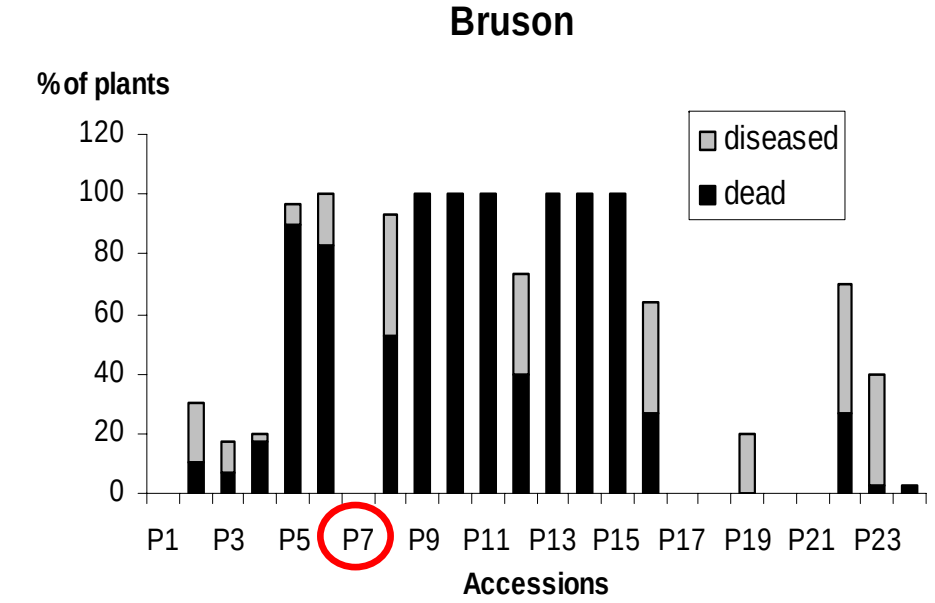
Breeding aim: **Increasing resistance to *Colletotrichum gloeosporioides* fungus, yield, phytochemical profile similar as the standard cultivar Topas,**

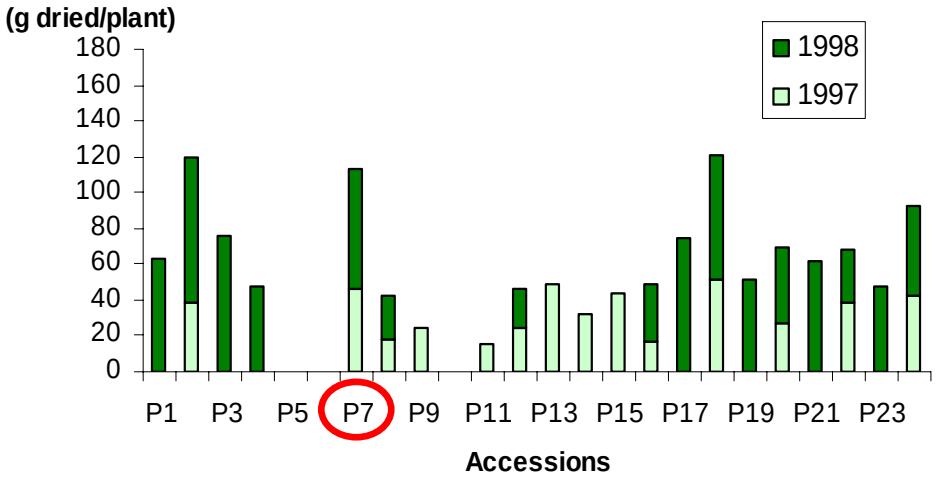
Breeding tools: **Comparison of different ecotypes (40, mainly from nature and some from botanical gardens)**

St. John‘s wort: Breeding for higher resistance

Comparison of different accessions on resistance to *Colletotrichum gloesporiodes*

St. John‘s wort: Breeding for higher resistance





P7: Cultivar Hypervivo 7 selectes by Mediplant

St. John‘s wort: Breeding for higher resistance

Heilkräuter / Plantes médicinales / Piante medicinali

82 Johanniskraut / Millepertuis (Hypericum perforatum L.)

1999-03-29	1999-05-31	9)	Bioforce AG	HYPERIVO 7
99-82-1487	2002-06-30		Postfach 76, 9325 Roggwil	
		10)	Nicole Debrunner, c/o Médiplant, 1964 Conthey	
		11)	idem	

Sage: Breeding for better homogeneity

Plant: **Salvia officinalis**

Properties: **antibiotic, antifungal, astringent, antispasmodic, estrogenic, ..., management of mild to moderate Alzheimer's disease (cholinergic binding properties)**

Drug: **Essential oil, leaf extracts**

Active compounds: **Essential oil (cineole, borneole, thujone,...);**
Leaf extracts (tannic acid, oleic acid, chlorgenic acid, caffeic acid,…..)

Sage: Breeding for better homogeneity

Breeding aim: **Homogeneity**, **winterfrost resistance, quality, yield**

Breeding tools: **testing of natural populations and accessions, biology of flowers, combining male sterile clones with male fertile clones**

Sage: Breeding for better homogeneity

- **Flower biology**

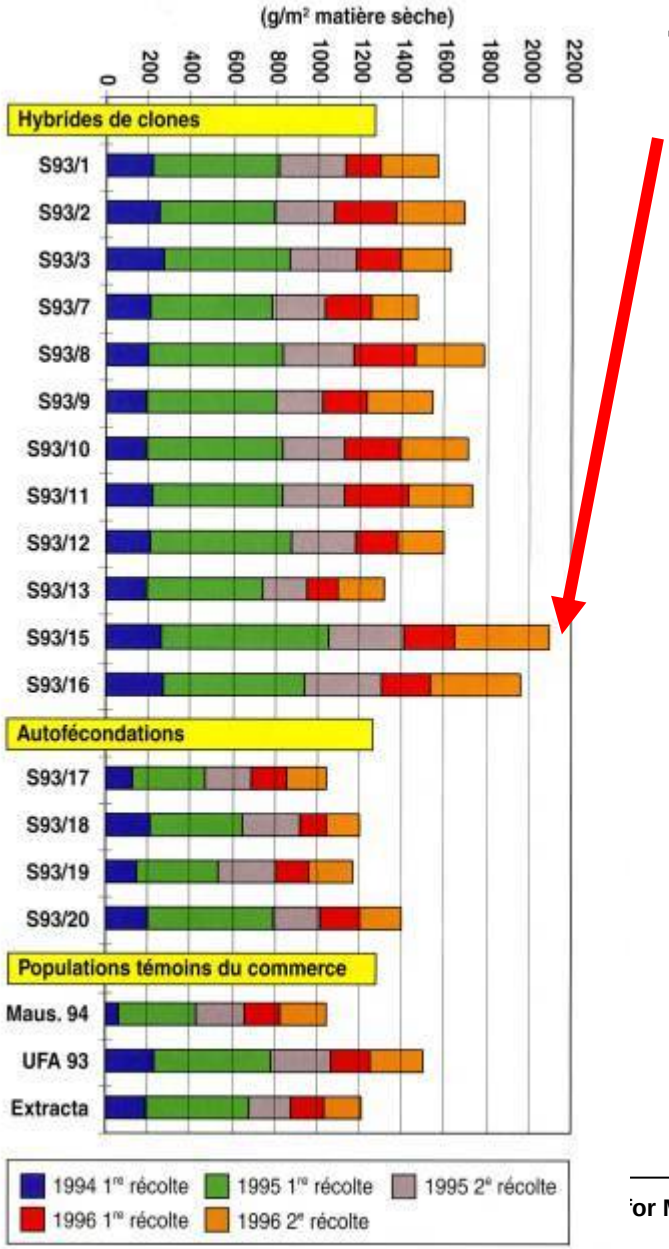
Male fertile plants
(MF, Hermaphrodite)

Male sterile plants (MS)

- **Crosses: MS X MF**

- **Seeds from the male sterile plant ⟶ F1 hybrid (cultivar)**

Sage: Breeding for better homogeneity



The most interesting hybrid was called Regula and is multiplied by DSP

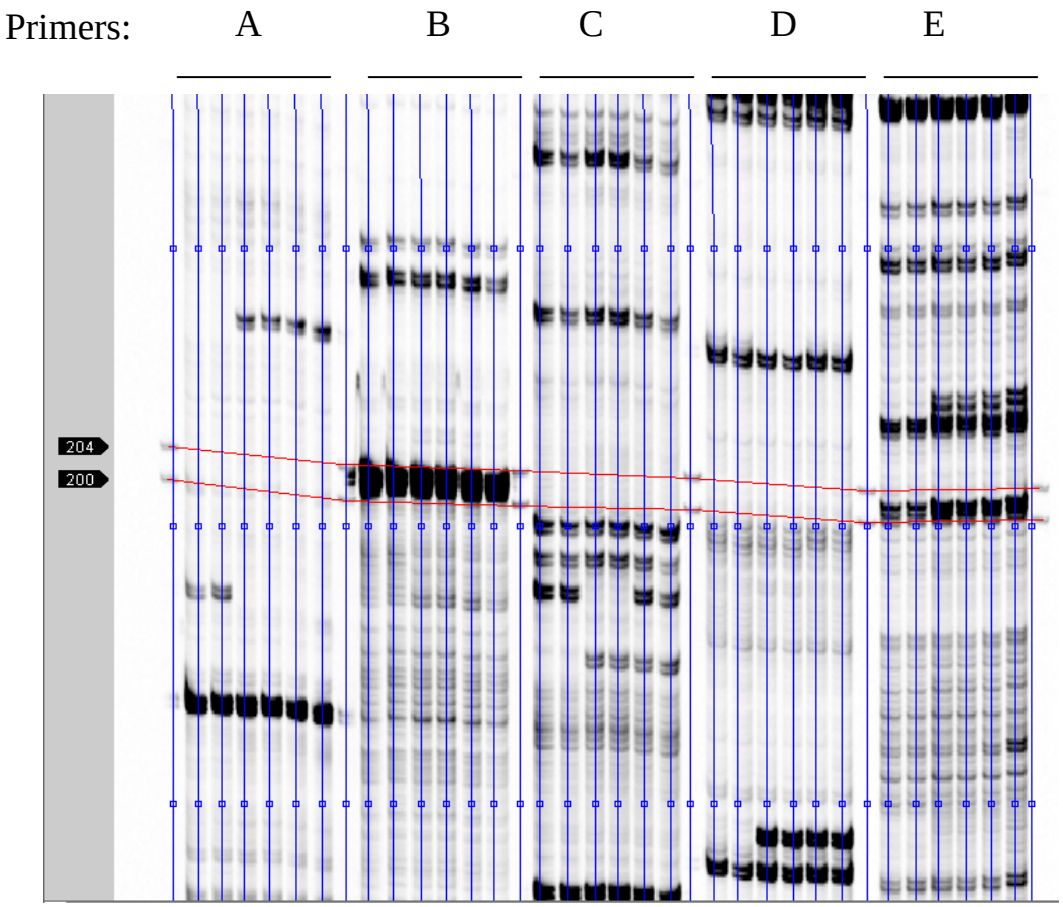
Sage: Breeding for better homogeneity

Comparison of the variety Regula with the variety Extracta, widely used in Europe

Locations	Cultivars	Yield (t/ha) ∅02-04	Part of leaves (%) ∅ 02-04	Content of essential oil of leaves (%) ∅ 02-04	Yield of essential oil (l / ha) ∅ 02-04	flower trusses per linear metre ∅ 03-04
Arbaz	Regula	10,79	77 a	2,04 a	156,0 a	35,8 b
	Extrakta	9,56	71 b	1,74 b	102,3 b	77,2 a
Bützberg	Regula	7,87	78 a	2,05 a	122,4 a	12,8 b
	Extrakta	7,66	73 b	1,78 b	91,3 b	51,1 a

Sage: Breeding for better homogeneity

Genetic analysis by AFLP (Amplified Fragments Length Polymorphism) of the MS mother clone, the MF father clone and the F1-hybrid Regula



1,2 MS (Mutter-Klon)
3,4 MF (Vater-Klon)
5,6 Hybride F1

Genepi: Breeding for decreasing undesired compounds

Plant: Artemisia umbelliformis (alpine wormwood, white genepi; Asteraceae)

Drug: Flower trusses (used in liquor, tonic properties, digestive)

Active compounds: *essential oil* (pinene, cineole, thujone, borneole,….), bitter compounds, flavonoides

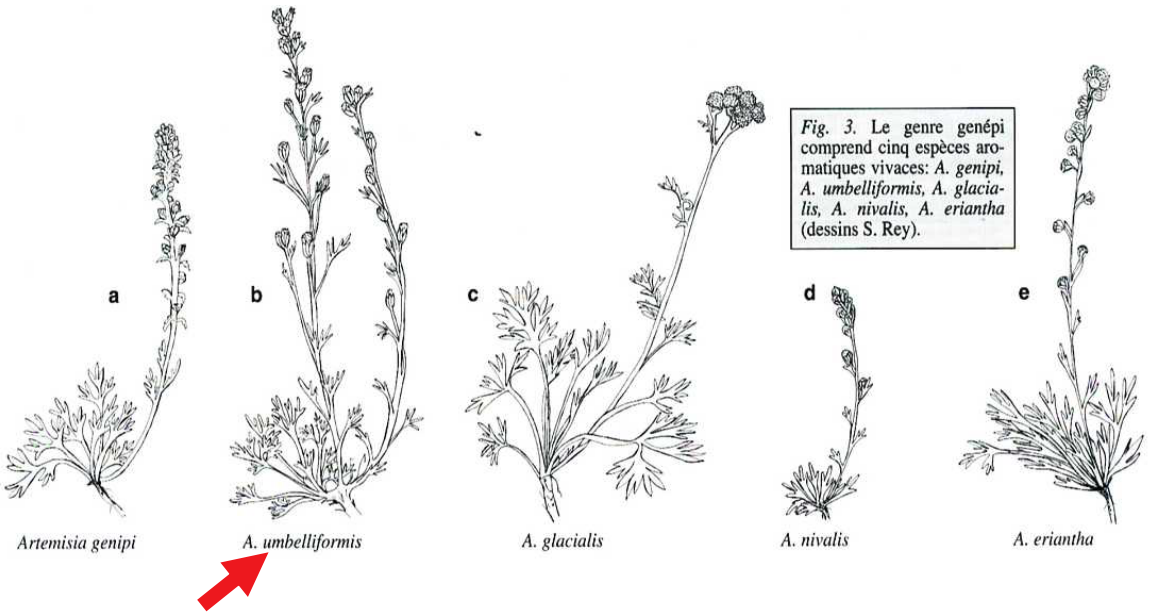
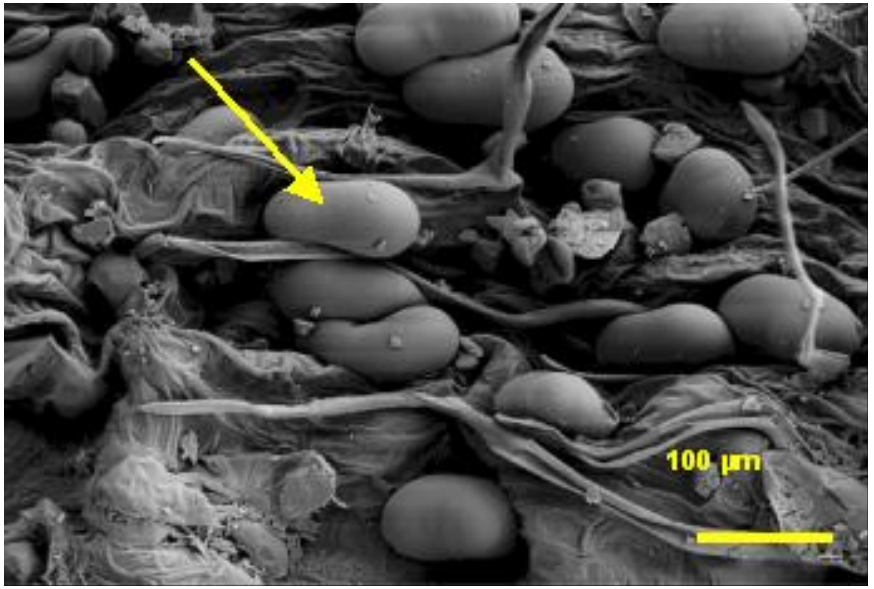


Fig. 3. Le genre genépi comprend cinq espèces aromatiques vivaces: *A. genipi, A. umbelliformis, A. glacialis, A. nivalis, A. eriantha* (dessins S. Rey).

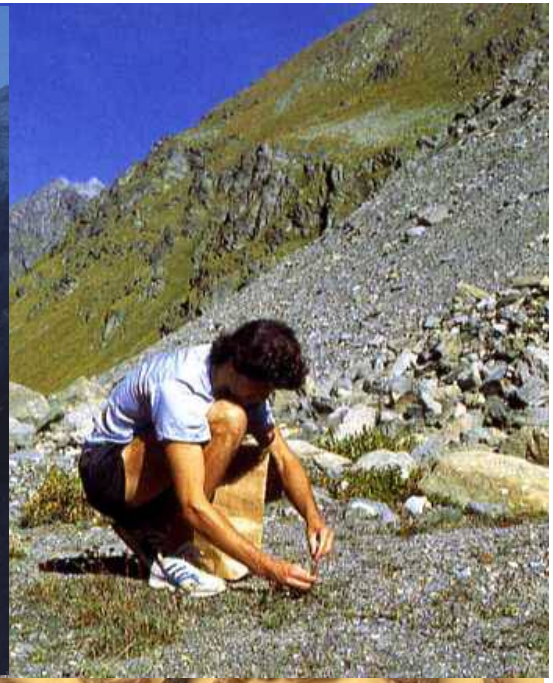
Genepi: Breeding for decreasing undesired compounds

Breeding aim: no thujone (neurotoxic effects), erect growth habitus, yield,

Breeding tools: test and analysis of different ecotypes, cross pollination of selected clones.



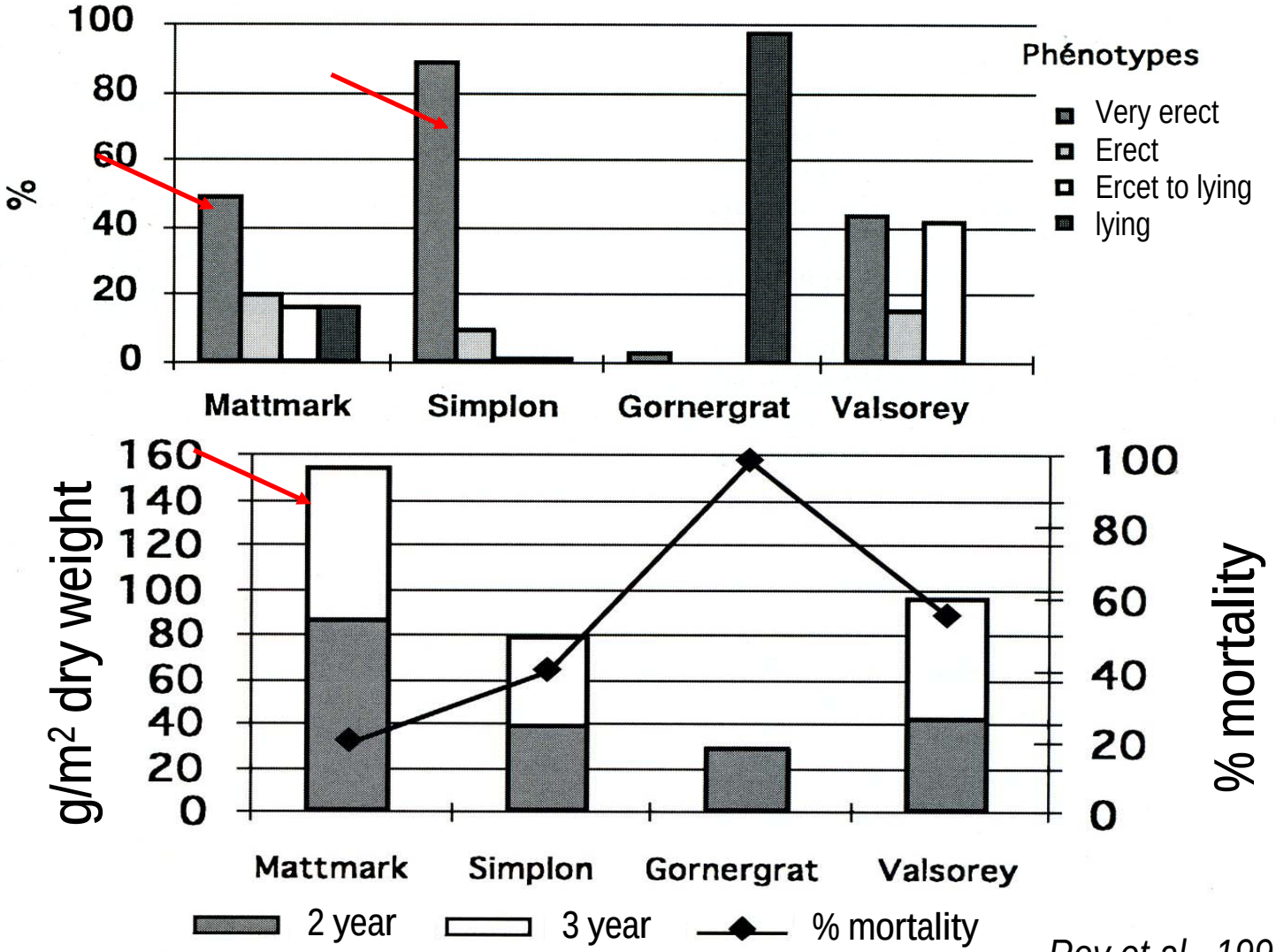
Genepi: Breeding for decreasing undesired compounds



Collection of seeds of different alpine sites
Comparison of 100 plants per site and describing the variability

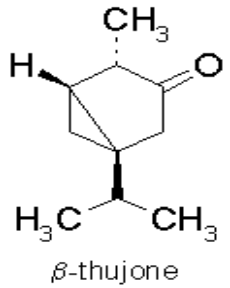
Genepi: Breeding for decreasing undesired compounds

Genepi: Breeding for decreasing undesired compounds



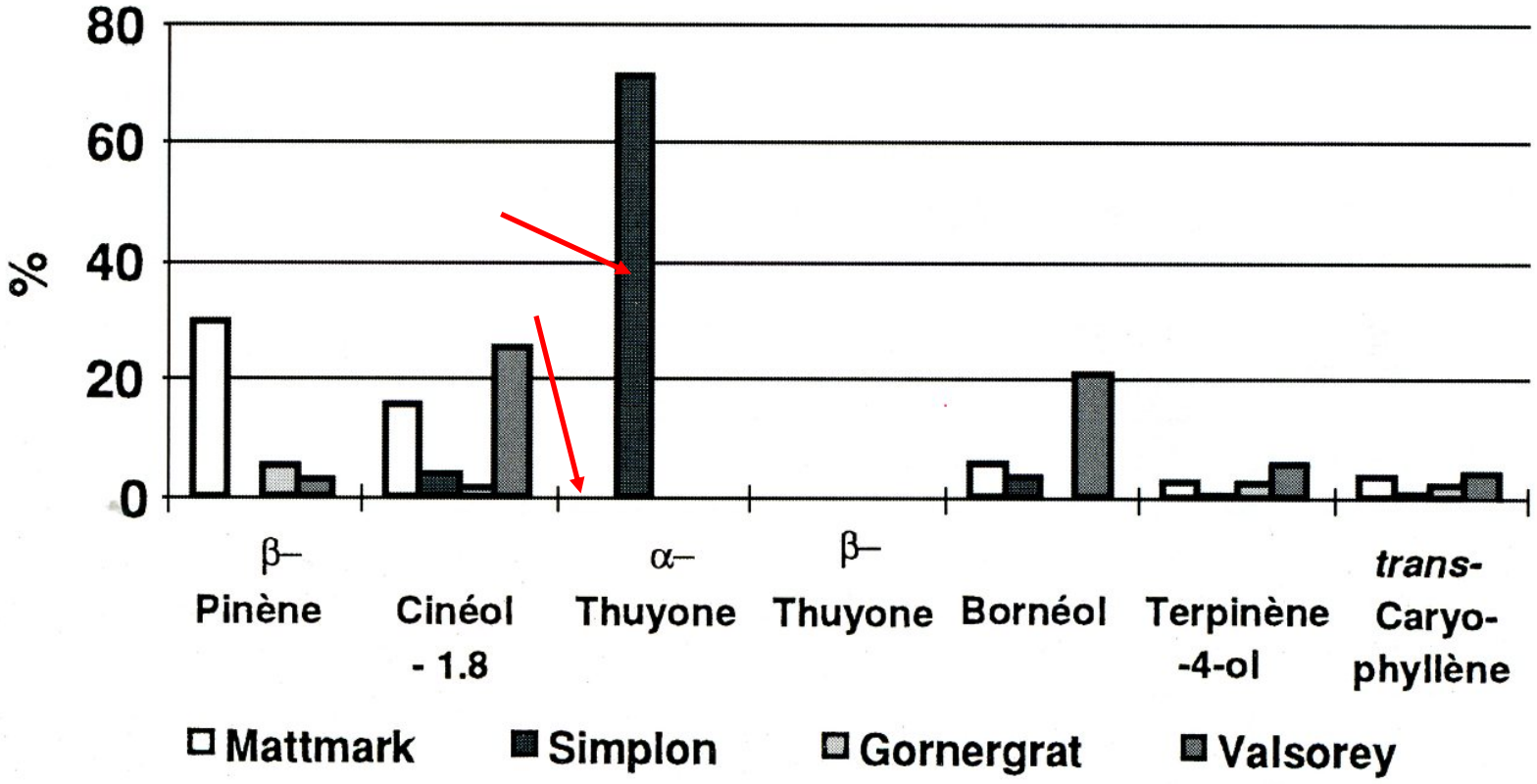
Rey et al., 1997, RSVAH, 29 I.

Genepi: Breeding for decreasing undesired compounds



Interreg IIIA, Italie-Suisse, Projet no. 6 A)

Genepi: Breeding for decreasing undesired compounds



Rey et al., 1997, RSVAH, 29 I.

Genepi: Breeding for decreasing undesired compounds

- Selection of adapted plants, then *in vitro* multiplication
- Transplanting of these plants in the field to get the seeds for new cultivars (population cultivar)
- Cultivars: - RAC 12 (without thujone, origin Mattmark)
  - RAC 10 (with thujone >60 %, origin Simplon)

Optimising cultivation procedures to get a better quality

What is the best

- cultivar?
- planting procedures?
- harvesting stage?
- harvesting procedures?
- methods to protect the plants?
- harvesting technique?
- fertilisation treatments?
- drying techniques?
- storage conditions?
- extraction procedures?
- .....

Research and development is important to answer these questions and to formulate good agricultural practices (GAP) to enhance and standardise the quality of medicinal plants

Conclusions

- Cultivation of medicinal plants is essential to supply the increasing global demand.
- Agronomic research plays an essential role to optimise the cultivation of medicinal plants and the quality of the raw material by efficient breeding programs
- Therefore it is very important that biodiversity and genetic resources (e.g. new genotypes of wild species and local/neglected varieties) is further characterised, evaluated, conserved and available to take the advantages of genetic and chemical diversity within species over wide geographical areas.
- For each created cultivar, the development of optimised cultivation procedures is necessary to formulate good agricultural practices (GAP) and to improve the benefit of medical plants.

Perspectives for botanical and agronomic R & D in medicinal plants

- Preservation of species and genetic diversity *in-situ* by a sustainable use of medicinal plants from natural habitats

- Conservation and multiplication of genetic resources *ex-situ*: botanical gardens, gene banks, as well as micropropagation (*in vitro*) are stille important to multiply and conserve endangered genotypes and interesting genotypes for breeding

- Conventional breeding prevails in MAP because it is a cheap methods and the high natural variability of most MAP species provides a good basic to achieve high breeding progress in short time.

- However gene/molecular techniques are more and more important for breeding better cultivars (e.g. molecular markers). Interest on detailed under-standing of pathways of desired compounds is increasing for the identification of the concerned genes (that can be used eventually for genetic engineering).

Perspectives for botanical and agronomic R & D in medicinal plants

- Besides the classical use of MAP, new interesting markets can be developed in the future such as cosmetics, functional foods, veterinary medicines, as well as plant protection products.
- Therefore conservation of genetic resources programmes, as well as domestication and breeding programmes need to be expanded.

Thank you for your attention!

Special thanks to

different private COMPANIES,
different NGOs

Agroscope ACW/OFAG,
Canton du Valais/HES-SO

for their financial and technical support