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Cultivars' propagation

virus testing, grafting & certification scheme

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- Conclusion

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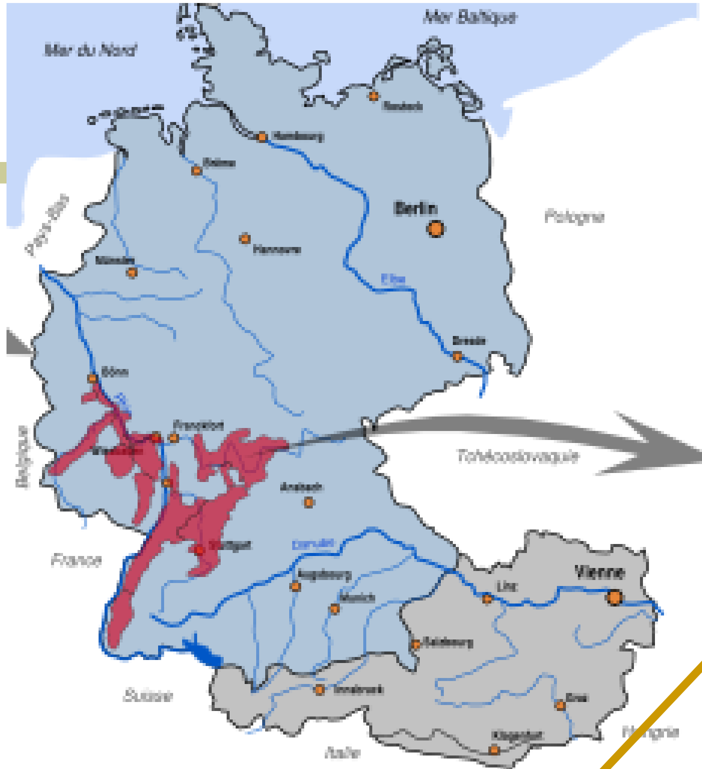
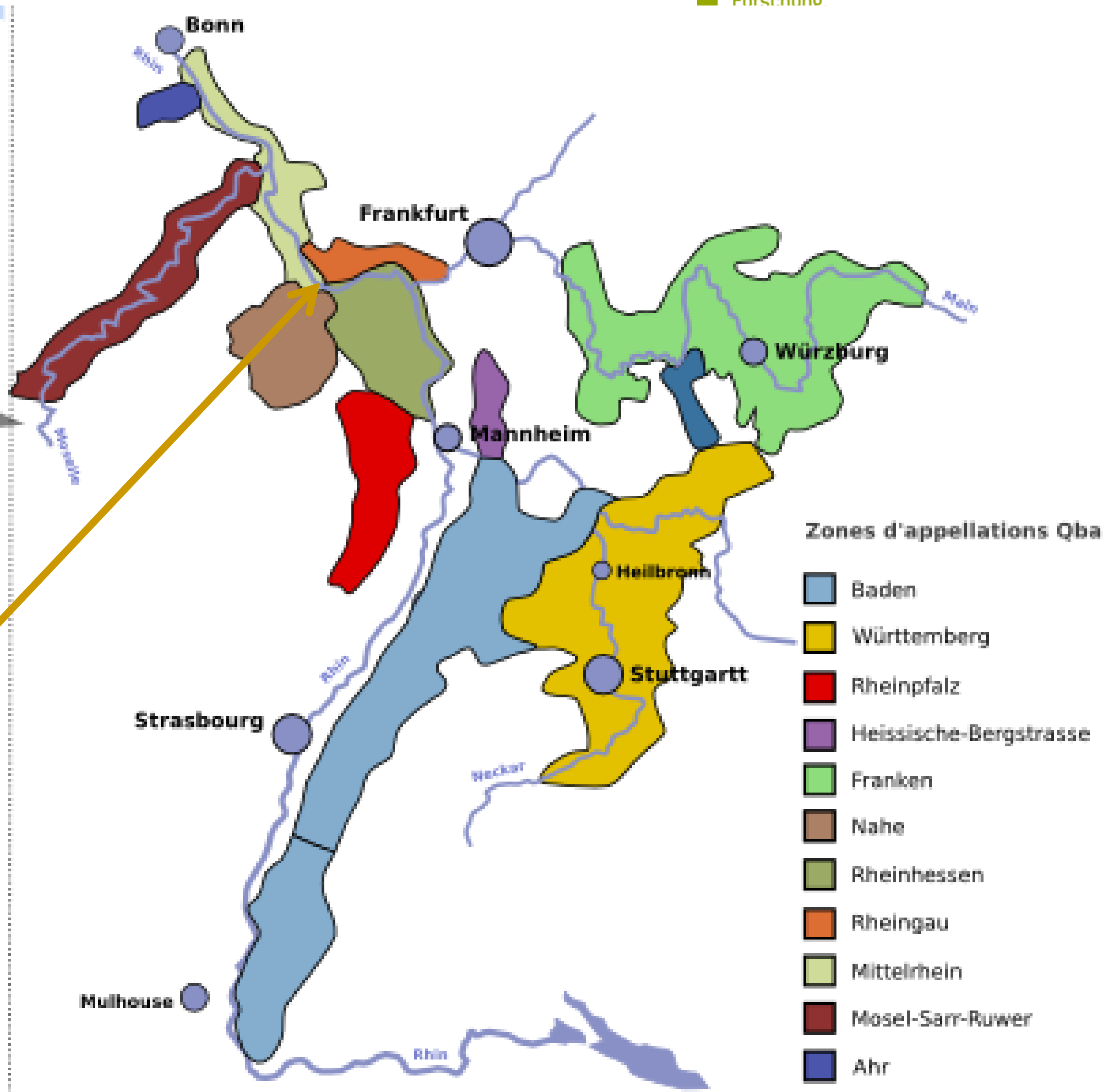
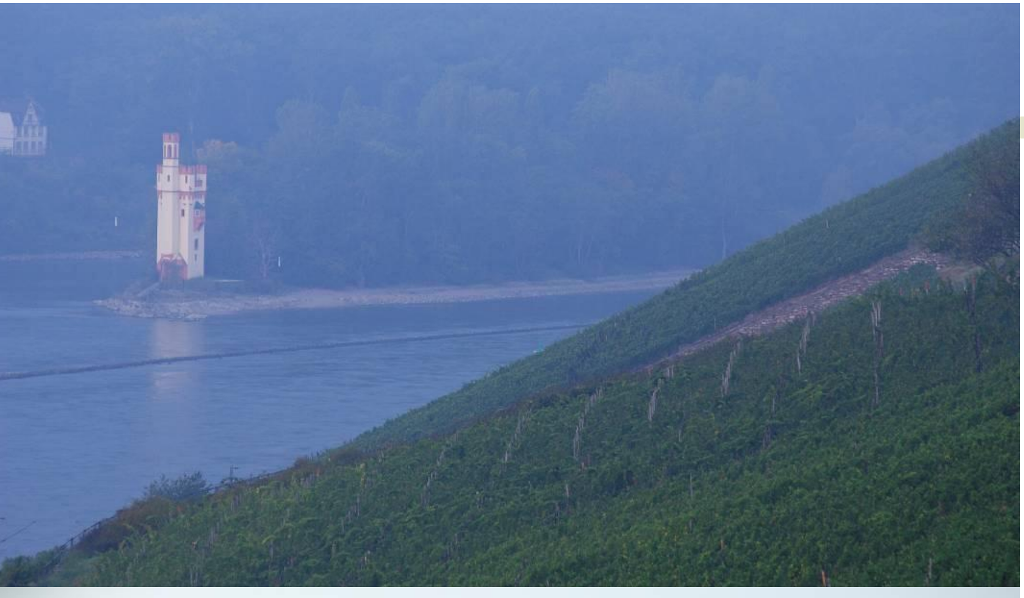

Mer Baltique
Mar du Nord
Berlin
Pologne
Pays-Bas
Tchécoslovaquie
Vienne
France
Suisse
Italie


Bonn
Rhin
Frankfurt
Main
Würzburg
Moselle
Mannheim
Heilbronn
Stuttgartt
Strasbourg
Neckar
Mulhouse
Zones d'appellations Qba
Baden
Württemberg
Rheinpfalz
Heissische-Bergstrasse
Franken
Nahe
Rheinhessen
Rheingau
Mittelrhein
Mosel-Sarr-Ruwer
Ahr

Geisenheim



Where are we?

Entrance to UNESCO world heritage site upper rhine valley

Photos DWI

Rheingau (3000 ha), a region of castles and monastries

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Château Johannisberg

Since the 9th century

Since 1720 only Riesling

Late harvest discovery 1776

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Châteaux Vollrads, 13th century

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Monastry Eberbach 1116-1135 founded by the monks of Clairvaux, Burgundy

Photos DWI

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Images

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Images

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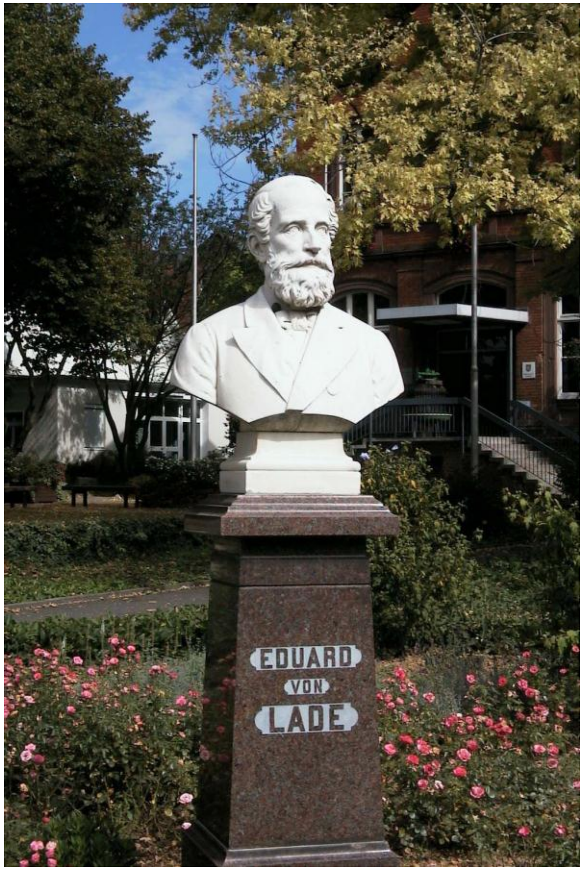
Eduard von Lade



- Applied Reasearch in Horticulture and Viticulture
- Foundation: 1872
- Today: 13 departments with ~350 staff

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Study programmes Geisenheim Campus

Each B.sc. can be followed by a M. Sc. programme

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Sum of students	**2010**	**637**
Viticulture/Oenology (B.sc.)		362
Beverage Technology (B.sc.)		98
International Wine Business (B.sc.)		177
Sum of students		**347**
Horticulture (B.sc.)		145
Landscape architecture (B.eng.)		202
Total number without Master students		**984**


Central Campus Building


Center for Studies in Beverage Technology


Lab Course in Microbiology


Field Studies in Organic Viticulture

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Special Study Programmes

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Since July 2009 Erasmus Mundus Status

2. Year currently 60% of the students are in Geisenheim, 32 places, currently 305 applicants

Introduction clonal selection

- The beginning
- Roman times: Virgil, Columella –
  - loss of performance, degeneration
- Middle ages: Monasteries?
- Beginning of *modern* clonal selection
  - Gustav Froelich, 1876
- Virus as pathogen: W. M. Stanley: Tobacco mosaic virus – 1935
- Aim: Fighting virus problems in vines

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Reason for variation Focus of selection

- Genetic variation
  - Mutations
  - Chimeras
  - Preserved by vegetativ propagation
- Sanitary selection
  - Virus
  - Bacteria

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The enemy

- Nepo-Viruses:
  - Grapevine fanleaf virus - GFLV
  - Arabismosaic virus - ArMV
  - Raspberry ringspot virus - RRV
  - Tomato black ring virus - TBRV
  - Strawberry latent ringspot virus - SLRV
  - Tomato ringspot virus - TRSV
- Chlostero-Viruses:
  - Grapevine leaf roll virus:
    - Typ-I: Central Europe
    - Typ-II: Damage?
    - Typ-III: Mediterranean, more damaging
- Corky bark, Rupestris Stem Pitting, Fleck . . .
- Viroids, Agrobacterium, Phytoplasm . . .

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The enemy

- Most damaging and common
  - Grapevine fanleaf virus: GFLV
  - Arabismosaic virus: ArMV
  - Grapevine leaf roll virus Type-I: GLRaV-1
  - Grapevine leaf roll virus Type-III: GLRaV-3
- Focus of EU-legislation

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Why pathogen-free propagation material?

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- Reduced yield and grape quality in virus infected vines
  - Walter & Martelli 1996, 1997
  - Ipach, 2004
  - etc.
- Mostly observations
- Not identical genotypes used

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Effect of virus infection on vine performance

R. Credi, A.R. Babini: Am. J. Enol. Vitic. 48, 7-12

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- Fiedl trial 4 replicates in Emilia Romagna
  - Albana clone AL 14T
  - Trebbiano Romagnolo clone TR 7T
  - Rootstocks: Kober 5BB, SO4; Spacing: 3.5 x 1.7 m

- Healthy vine infected via chip budding
  - GFLV — grapevine fanleaf virus
  - GLRaV-1(3) — grapevine leafroll virus 1 (3)
  - KSG — Kober stem grooving
  - RPS — Rupestris stem pitting
  - VN / VM — vein necrosis / vein mosaic
  - FK — Fleck

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Effect of virus infection on vine performance of Trebbiano Romagnola

R. Credi, A.R. Babini: Am. J. Enol. Vitic. 48, 7-12

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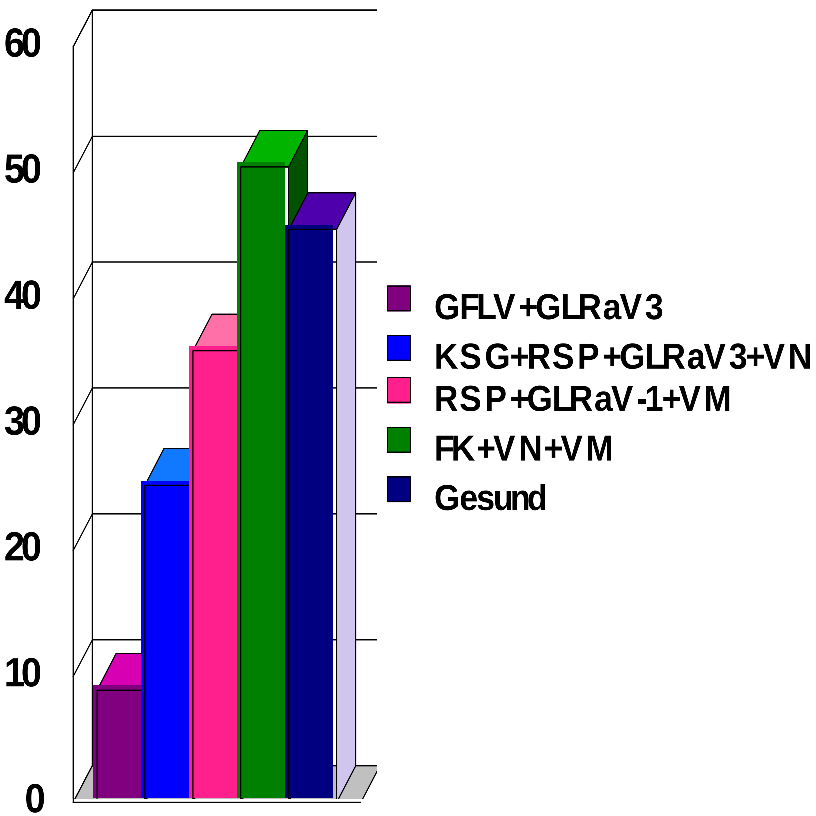
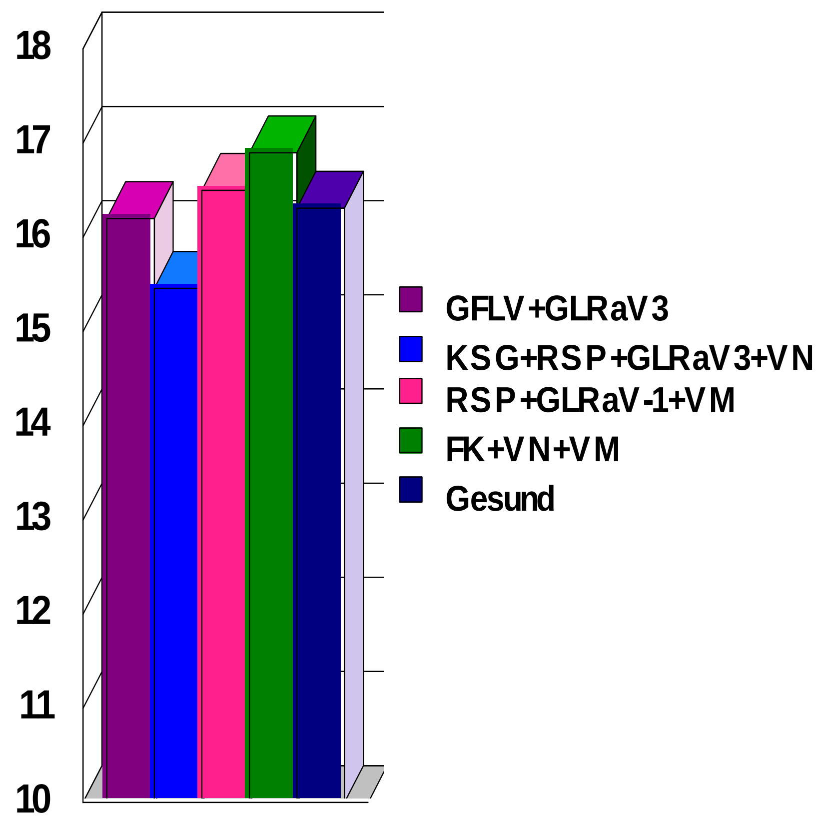
Ertrag [kg 87 bis 93]



Zuckergehalt [° Brix]



Virus effects on vine performance

- Virus infection can dramatically reduce
  - Yield
  - Quality

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Ways to virus 'free' vines

Option 1:

- Clean material from the beginning

Option 2:

- 'Removing' virus from vines
  - Thermotherapy
  - Shoot apex culture
  - Somatic embryogenesis

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Thermotherapy

- Goheen et al. 1969, 1972, 1973
- Idea:
  - Viruses do not multiply in hot conditions
  - Plants are grown at ~38°C [100°F]
  - New growth is virus-free
  - Cuttings from shoot top are virus-free

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Tissue culture with or without heat therapy

- Galzy 1969
- Barlass 1977, 1978, 1980 a, b: 'Fragmented shoot apex culture'
  - Idea:
    - No virus in meristematic tissue
    - Shoot tip (~1mm) removed
    - Sterilized and cut in ~40 little pieces
    - Pieces are cultured *in vitro*
    - May be combined with thermotherapy

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Somatic embryogenesis

- Morgana et al. 2004,
- Gambino et al. 2006, 2009,
- Borroto-Fernandez et al. :
- Idea:
  - No virus in meristematic tissue
  - E.g. Anthere culture
  - Embryogenic callus
  - Somatic embryos

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Possible problems of virus removal

- Permanent changes in behaviour of material after virus removal
- Caused by absence of virus
- Caused by tissue culture
  - Mix-ups of material
  - Juvenility
  - Plant hormons in culture media
  - Mutation/chimeras
    - Many generations in tissue culture
- Multiplication of viroids during heat therapy

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Performance change after virus elimination

- Mannini et al. 1995
- Clonal comparison with Nebbiolo clones
- Leaf shape and size

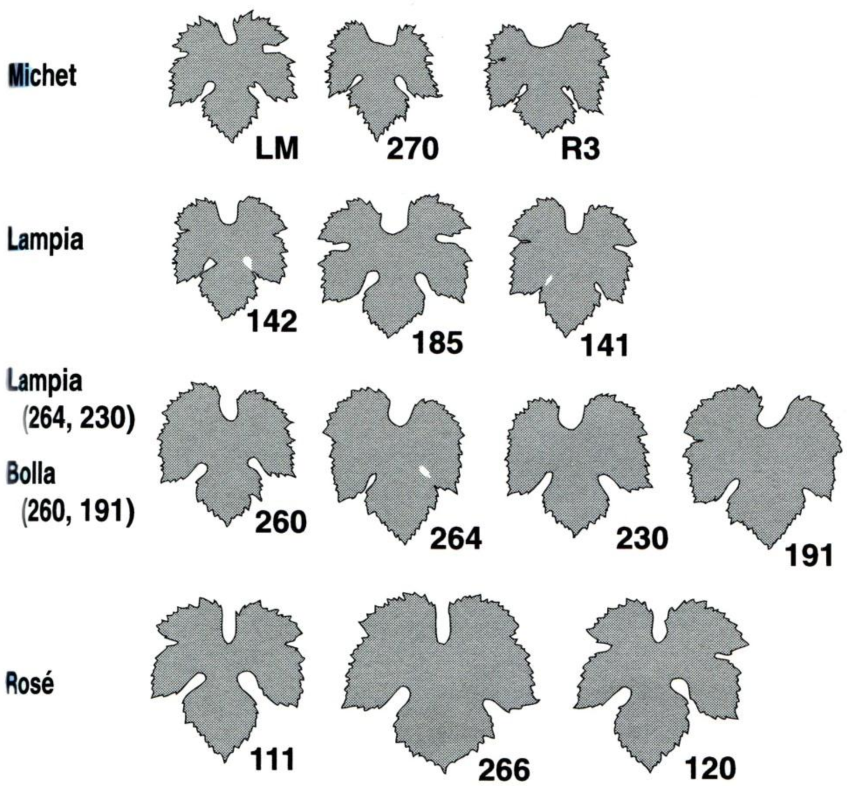

Fig. 2. Typical leaf profiles of different clones of Nebbiolo and supposed sub-variety membership.

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Performance change after virus elimination

- Mannini et al. 1995
- Clonal comparison with Nebbiolo clones
- Discriminant analysis with phyllometric parameters (leaf measurements)

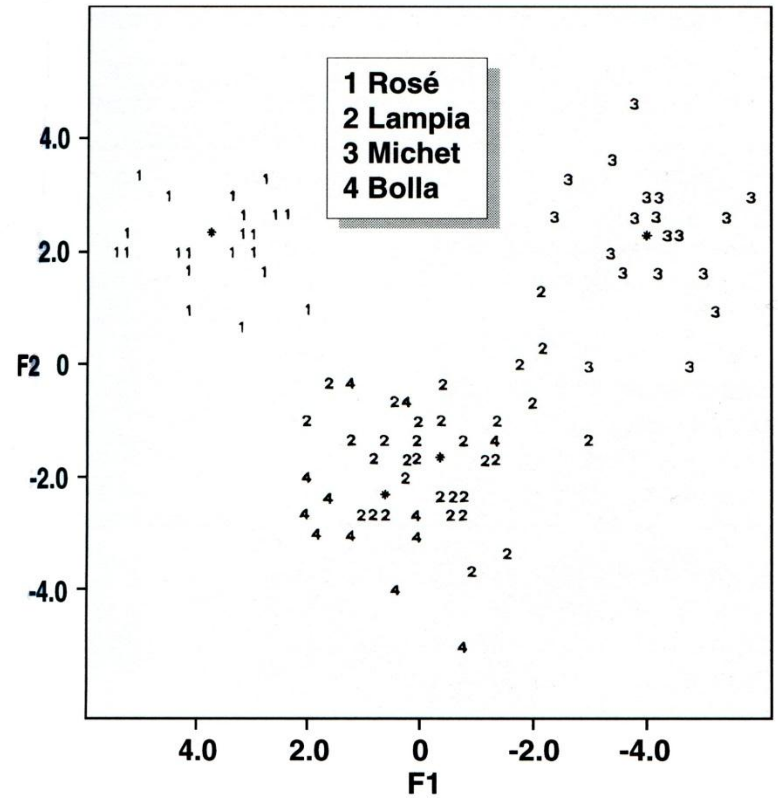

Fig. 1. Distribution of individuals (single vines) grouped according to Nebbiolo traditional sub-varieties resulting from discriminant analysis of phyllometric parameters (* = centroid of the groups).

More virus-infected sub-varieties?

- Yellow Silvaner
  - (common: Green Silvaner)
- Yellow Riesling
  - (common: White Riesling)
- Question: Earlier ripening?

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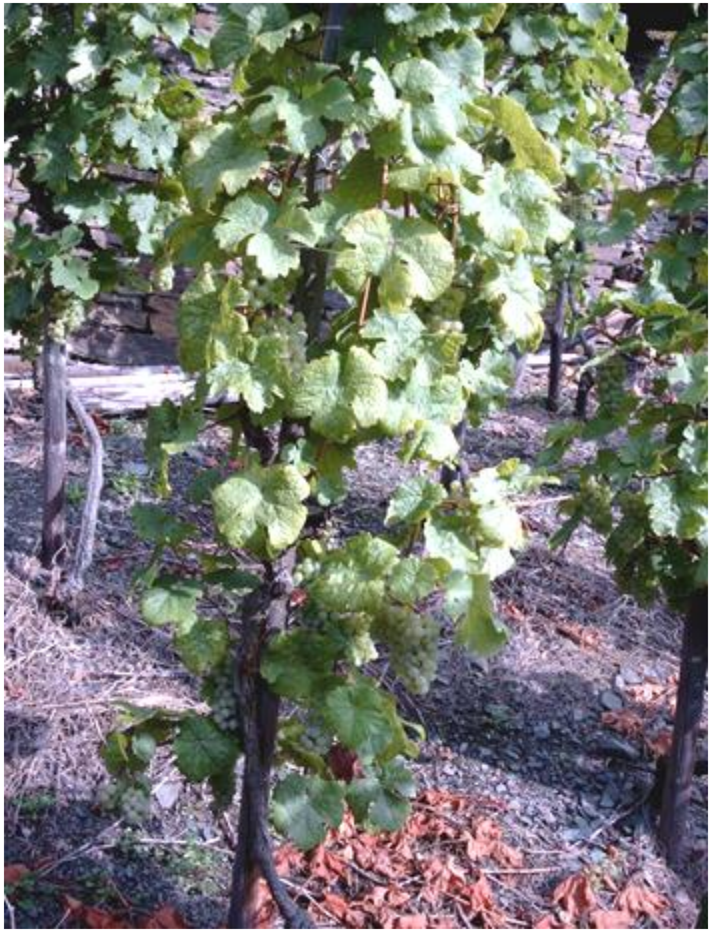
Yellow Riesling

- Yellow, downward rolling leaves
- Slightly stunned growth
- Small, yellow grapes
- Taste
  - ☹ sour
  - ☹ unripe – less sugar
  - ☹ bland, tasteless
  - ☹ Riesling atypical
- Virus test:
  - ☹ GLRaV-1 positiv



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White Riesling

- Green leaves
- Vital appearance
- Small, greenish grapes
- Taste
  - ☺ sweet
  - ☺ ripe
  - ☺ fruity, aromatic
  - ☺ typical Riesling
- Virus test:
  - ☺ healthy

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Breeding for virus-freedom: Geisenheim as example

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- Preservation of genetic diversity within cultivars
- Search for already virus free material
- If not available: Use of somatic embryo genesis for virus elimination

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Preserving genetic diversity within cultivars

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- In Germany clonal selection since 1876
- Since 1950 mostly clonal material planted
- Less than 500 ha none-clonal vineyard
- Number decreasing

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Geisenheim as example

- Collection of material in old vineyards
- Virus tests for GFLV, ArMV, GLRaV1, 3
- Germplasm collection at 2 locations (3 to 5 vines ea.)
- Phenotypic data sampled
- Interesting? → Further evaluation (25 to 50 vines)
- Still interesting? → Single vine as source for new clone
- Virus test for +/- all known viruses

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- Leafroll (Grapevine leafroll associated viruses: GLRaV1, GLRaV2, GLRaV3, GLRaV4, GLRaV5, GLRaV9)
- GVA (Grapevine Vitiviruses)
- GVB (Grapevine Vitiviruses)
- GfkV-A (Grapevine fleck virus - variants A)
- GfkV-B (Grapevine fleck virus - variants B)
- GFLV (Grapevine fanleaf virus)
- RG (Grapevine rootstock stem lesion associated virus - Red Globe Leafroll Virus)
- RRSV (Raspberry ringspot virus)
- Phytoplasms (e.g. grapevine yellows)
- Agrobacterium vitis

PCR testing method on phloem extractions of dormant wood samples

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Further measures

- Phytosanitary lines
  - Every ~20 years:
    - Subclones: ~5 separate lines
    - Kept separate, visual and ELISA-testing
- Material identification and traceability
  - In combination with phytosanitary lines
  - Back-tracking possibly infected material

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Geisenheim as example

Virus testing German legislation

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- Pre-base blocks:
  - Sample of 5 vines every 5 years
- Base blocks:
  - Sample of 10 vines every 6 years
- Certified blocks:
  - Sample of 10 vines, every 20th vine tested, every 10 years

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Geisenheim as example
Maintaining a clone

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- Further consequences:
- Small pre-base plantings: 10-50 vines at the Geisenheim Research Center
- Small base plantings: 50-1000 vines (1000 for rootstocks)
- Certified blocks: Thorough visual checks every year (breeder and officials)
  - only best plantings kept and tested in year 10

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SEE-ERA project

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2010/2011	cuttings tested	virus found	virus free	grafted
Croatia	159	81	78	56
Montenegro	56	38	18	26

2011/2021	cuttings tested	virus found	virus free	grafted
Croatia	70	55	10	22
Montenegro	54	14	40	31

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Grafting Croatia 2011

Grafted vines Croatia - 2010/2011						Grafted vines Croatia - 2010/2011					
Scion	**clone**	**rootstock**	**grafted**	**in nursery**	**grown**	**Scion**	**clone**	**rootstock**	**grafted**	**in nursery**	**grown**
Sansigot	HR 3-1	5 BB Kl. 13-3	20	17	7	Zlatarica Blat	ZLA 7	5 BB Kl. 13-2	20	1	1
Diseca ranina	HR 4-7	5 BB Kl. 13-3	20	17	3	Zlatarica Blat	ZLA 9	5 BB Kl. 13-2	11	3	2
Diseca ranina	HR 4-10	5 BB Kl. 13-3	36	25	9	Zlatarica Blat	ZLA 12	5 BB Kl. 13-2	15	3	3
Diseca ranina	HR 4-11	5 BB Kl. 13-3	41	27	11	Zlatarica Blat	ZLA 15	5 BB Kl. 13-2	25	10	7
Diseca ranina	HR 4-12	5 BB Kl. 13-3	25	14	4	Zlatarica Blat	ZLA 34	5 BB Kl. 13-2	6	4	3
Diseca ranina	HR 4-13	5 BB Kl. 13-3	30	16	5	Zlatarica Blat	ZLA 37	5 BB Kl. 13-2	18	10	7
Diseca ranina	HR 4-18	5 BB Kl. 13-3	22	16	3	Zlatarica Blat	ZLA 50	5 BB Kl. 13-2	17	16	8
Diseca ranina	HR 4-19	5 BB Kl. 13-3	31	6	3	Zlatarica Blat	ZLA 51	5 BB Kl. 13-2	15	4	2
N.N.	HR 7	5 BB Kl. 13-3	23	20	5	Zlatarica Blat	ZLA 56	5 BB Kl. 13-2	18	8	5
Jarbola	HR 12-2	5 BB Kl. 13-3	25	1	1	Dobricic	DOB 2	5 BB Kl. 13-2	17	8	2
Jarbola	HR 12-5	5 BB Kl. 13-3	25	4	2	Dobricic	DOB 3	5 BB Kl. 13-2	20	13	1
Jarbola	HR 12-6	5 BB Kl. 13-3	26	1	1	Dobricic	DOB 7	5 BB Kl. 13-2	15	3	1
Jarbola	HR 12-8	5 BB Kl. 13-3	30	2	2	Dobricic	DOB 10	5 BB Kl. 13-2	16	10	3
Jarbola	HR 12-10	5 BB Kl. 13-3	10	1	1	Dobricic	DOB 11	5 BB Kl. 13-2	25	2	1
Jarbola	HR 12-11	5 BB Kl. 13-3	26	1	1	Diseca ranina	HR 4-17	5 BB Kl. 13-2	20	18	5
Jarbola	HR 12-12	5 BB Kl. 13-3	32	5	3	N.N.	HR 6	5 BB Kl. 13-2	23	18	6
Jarbola	HR 12-13	5 BB Kl. 13-3	25	2	1	N.N.	HR 9	5 BB Kl. 13-2	23	12	2
Jarbola	HR 12-14	5 BB Kl. 13-3	30	13	3	Stara bellina	HR 10	5 BB Kl. 13-2	37	29	6
Jarbola	HR 12-15	5 BB Kl. 13-3	30	16	8	N.N.	HR 11	5 BB Kl. 13-2	28	22	4
N.N.	IP 161	5 BB Kl. 13-3	30	5	4	Stara ranina	HR 14	5 BB Kl. 13-2	25	0	1
Sokol	SOK 2	5 BB Kl. 13-3	30	24	2						
Sokol	SOK 6	5 BB Kl. 13-2	19	11	1	**Total:**		**43**	**1001**	**458**	**151**
Sokol	SOK 11	5 BB Kl. 13-2	21	20	1						

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Grafting Montenegro2011

Grafted vines Montenegro - 2010/2011					
Scion	**clone**	**rootstock**	**grafted**	**in nursery**	**grown**
Toatal		**25**	**346**	**261**	**155**
MNE 2	2-4	1103 Paulsen	16	5	4
MNE 2	2-5	1103 Paulsen	11	10	9
MNE 2	2-6	1103 Paulsen	11	11	8
MNE 2	2-10	1103 Paulsen	17	11	9
MNE 7	7-4	1103 Paulsen	14	13	8
MNE 12	12-3	1103 Paulsen	10	10	6
MNE 12	12-4	1103 Paulsen	11	3	2
MNE 13	13-1	1103 Paulsen	14	8	8
MNE 15	15-2	1103 Paulsen	18	13	10
MNE 15	15-3	1103 Paulsen	13	9	6
MNE 15	15-5	1103 Paulsen	12	12	6
MNE 16	16-1	1103 Paulsen	17	10	8
MNE 21	21-1	1103 Paulsen	11	11	6
MNE 21	21-2	1103 Paulsen	13	12	9
MNE 21	21-3	1103 Paulsen	12	10	3
MNE 21	21-4	1103 Paulsen	11	10	3
MNE 21	21-5	1103 Paulsen	12	10	7
MNE 1	1-4	1103 Paulsen	13	9	4
MNE 1	1-7	1103 Paulsen	18	18	8
MNE 7	7-2	1103 Paulsen	23	13	3
MNE 7	7-3	1103 Paulsen	22	18	6
MNE 15	15-4	1103 Paulsen	11	10	4
MNE 17	17-3	1103 Paulsen	13	10	7
MNE 17	17-4	1103 Paulsen	10	6	4
MNE 20	20-1	1103 Paulsen	13	9	7

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Virus types in samples

Croatia

- GLRaV-1: 24
- GLRaV-3: 48
- GFV: 10
- ArMV: 3
- GLRaV-1+3: 21
- GFV+GLRaV-3: 10
- ArMV+GLRaV-3: 2
- ArMV+GLRaV-1: 1
- GFV+GLRaV-1+3: 6

Montenegro

- GLRaV-1: 12
- GLRaV-3: 2

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What's needed in future?

- Virus tested clones of all autochthonous varieties
- Large clonal range with different characters
- Reliable rootstock supply
  (base, pre-base material)
- Competitive nursery industry
- Awareness in industry
  (importance of clonal selection)
- Financial support: Government, industry, royalties…

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Conclusion

- Pathogen-free grapevine propagation material is essential for reliable vine performance (yield, quality, longevity)
- Development of new clones based on already virus-free propagation material
- Virus elimination only, if virus-free plants are not available
- Strategies are required for long-term preservation of genetic diversity within traditional varieties (virus-free)
- Reducing pre-base and base plantings to their minimum helps to keep costs down and still insure genetic diversity.

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Thank you very much for your attention