Genetic structure and epidemiology of *Plasmopara viticola* populations from Australian grape growing regions

Diploma Thesis
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Abstract

Plasmopara viticola is the causal agent of grapevine downy mildew. In wet years, direct crop losses in Australia may be as high as 64 million AUD. *P. viticola* was introduced at different times into the states on the east and west coast (Victora: 1917; New South Wales: 1918-1920; Western Australia: 1998).

The principal objective of this diploma thesis was to assess the contribution of primary vs. secondary inoculum to epidemic development of grapevine downy mildew in Australia, and to better-define the period during which the overwintering spores (oospores) release inoculum. Therefore 789 lesions were collected from an unsprayed vineyard of the table grape cultivar “Ribier” in Caversham (Swan Valley, Western Australia) from 12 November 2004 until 19 January 2005 and genetically analyzed by four different microsatellite markers. 32 distinct genotypes could be identified from 709 successfully analyzed samples. The most salient findings of this study comprehend both the early dominance of secondary inoculum (95%) and the continuous occurrence of putatively caused primary lesions (4-6), suggesting that the primary inoculum is released much longer than generally assumed. 17 different genotypes produced one lesion each, 94 genotypes produced 2-22 lesions each, and four genotypes produced a high number of lesions (69-315).

A second objective of this thesis was to assess the genetic diversity of the following Australian populations: Caversham (cav(all), WA), Henley Brook (heb, WA), Pemberton (pem, WA), Lovedale/Pokolbin (hun, NSW), Yarra Glen (yar, Vic). With normalized Shannon indices E_H of 0.28 (cav), 0.33 (heb), 0.34 (pem), 0.62 (hun) and 0.84 (yar), genotypic diversity of populations was considerably low in Western Australia, elevated in New South Wales and high in Victoria, later being close to the average diversity found of American and European populations. Genetic distances within Australian populations are large (F_{st} 0.20-0.22), especially between east and west coast. There are three possible explanations. Firstly, the populations seem to have had low genetic exchange between each other, thanks to natural barriers and interstate quarantine measures. Secondly, *P. viticola* was introduced at different times into the states on the east and west. Thirdly, the Western Australian climate is adverse to the development of the pathogen. The fungus is forced to go through regular cycles of extinction and recolonization.

Introduction

Plasmopara viticola, (Berk. & Curt.) Berl. et de Toni, causal agent of grapevine downy mildew, is a heterothallic, diploid, obligate-biotrophic oomycete and is considered as one of the most important grape pathogens worldwide (Wong, *et al*. 2001). The estimated annual crop loss in an average year in Australia is 22.5 million AUD, with an additional 10 million AUD spent on control measures. In wet years, direct crop losses may be as high as 64 million AUD (Magarey and Butler, 1998).

Downy mildew had a much shorter evolution time in Western Australia, where it firstly was confirmed in 1998 (McKirdy *et al*., 1999). *P. viticola* first appeared in Rutherglen, Victoria, in 1917, coming in with vine planting material being brought in from Europe (de Castella F & Brittlebank CC, 1917). It was found 'soon after' in the New South Wales (Hunter Valley), South Australia and Queensland (Laffer HE, 1918). In Tasmania, downy mildew was found only in 1959 (Emmett RW *et al*., 1998).

The life cycle of the pathogen consists of one sexual cycle in autumn and several asexual cycles throughout the grapevine growing period. In the sexual stage, two mating gametes form a genetically unique oospore, which overwinters on dead leaf material and berries. In spring, with temperatures above 10°C and enough soil moisture, the oospores germinate and produce macrosporangia which release zoospores as primary inoculum. Zoospores are rain-splashed onto young grapevine leaf tissue, where they swim through the water film to stomata and penetrate them. Early symptoms, so-called oilspots, appear as yellowish lesions on the upper leaf surface. Generally 5-10 days after the intial infection, depending on factors such as temperature, humidity and light, microsporangia are produced on the lower leaf surface containing several genetically identical zoospores. Secondary disease cycles can happen under suitable infection conditions after sporulation during humid nights (Blaeser & Weltzien 1979; Lafon & Clerjeau 1988; Schruft & Kassemeyer 1999).

In order to predict the magnitude of primary and secondary infections, disease forecasting models were developed. Many forecasting systems base on the assumption that overwintering oospores mature synchronously in spring and are exhausted after a brief period of germination just before bloom of grapevine. Following an initial oosporic infection event, an epidemic is driven by massive clonal

multiplication (Lalancette et al., 1988a,b; Blaise et al., 1999). However, present models often fail to quantify the intensity and the spread of the inoculum and thus to predict the quantitative development of epidemics within a single vineyard. Forecasting models need to be more regionally adapted, e.g. to Western Australia, where downy mildew firstly is still a new disease (introduction in 1998, McKirdy *et al.*, 1999), and secondly seems to have a different biology than elsewhere (Smith, 2004).

Recent DNA microsatellite analysis have suggested that oospores mature and cause infection over a much longer period than previously assumed (at least two months or even more), and that secondary (clonal) spread may sometimes be a minor component of the overall epidemic (Gobbin, *et al.,* 2003b; Eugster, 2003). In this study, aforementioned DNA microsatellite technique was used to focus the life cycle and genetic structure of *P. viticola* populations in Australia. The principal objective in this present study was to better-define the period during which the overwintering spores (oospores) release inoculum, and to assess the relative contribution of primary vs. secondary inoculum to epidemic development, in order to further improve forecasting models and consequently disease management strategies of grapevine downy mildew. This study may help to adapt a model to Western Australia, where no state downy mildew weather forecasting system yet exists.

A second objective of this diploma thesis was to study the genetic structure of the pathogen in a secondary center with a relatively short evolution time and probably low gene flow from the centre of origin. Studies of the genetic variability of *P. viticola* have been reported from North America (Eugster, 2003), which is believed to be the center of origin of the pathogen (Lafon & Clerjeau 1988), and several European sites (Gobbin, *et al.*, 2003b; Rumbou & Gessler 2004), but never from Australia.

For the present study, five downy mildew populations from three different Australian states (Western Australia, New South Wales, Victoria) could be selected and their genetic diversity as determined by a microsatellite analysis compared to the ones of North American and European populations of *P. viticola*. With different introduction times to eastern and western states, immense natural barriers (desert, sea) as well as cyclic adverse environmental conditions (hot and dry weather in WA), it is possible

that P. viticola populations were subjected to bottleneck situations, followed by founder effects and population differentiation, resulting in a lower genetic diversity than in the pathogens centre of origin. Therefore, phylogenetic relationships, on the one hand between Australian and European populations, on the other hand and between Australian and American populations, were explored.

Materials and Methods

Vineyards

Epidemiology

The trial for the epidemiological studies was conducted in an unsprayed vineyard of the Ribier table grape cultivar in Caversham (Swan Valley, Western Australia, table 1) from 12th November 2004 trough 19th January 2005. The vines were planted in 1980 (3.25 m between rows, 2.75 m between vines), trained in a cordon system and manually pruned. For the experimental plot three rows of 13 vines per row were selected, with three missing plants reaching a total of 36 vines. All adjacent vineyards were regularly treated against downy mildew. Hourly temperature, rainfall and relative humidity were recorded at the weather station at Caversham AQMS (by courtesy of the Air Quality Management Branch, Department of Environment, WA), at about than one kilometer away from the experimental vineyard.

The meteorological variables recorded by the meteo station at Caversham AQMS are following:

- Air temperature at 2m and 10m - Met One (Met One Instruments Inc., Grants Pass, OR, USA), model 062, aspirated by electric fan
- Relative humidity (at 10m height) - Rotronic MP100 (Bassersdorf, Switzerland)
- Rainfall – Rimco (Mulgrave, Vic, AUS) tipping bucket at ground level with 0.2mm bucket.

In order to map the location of lesions within the vineyard as they were collected, spatial coordinates for each individual lesion were recorded as X (horizontal distance from trellis wire in the row) and Y (horizontal distance down the row), with the coordinates “0,0” being a pole next to the first vine in row one, and Z (vertical distance from the ground) with a precision of measurement on the “cm” level (Fig. 1).

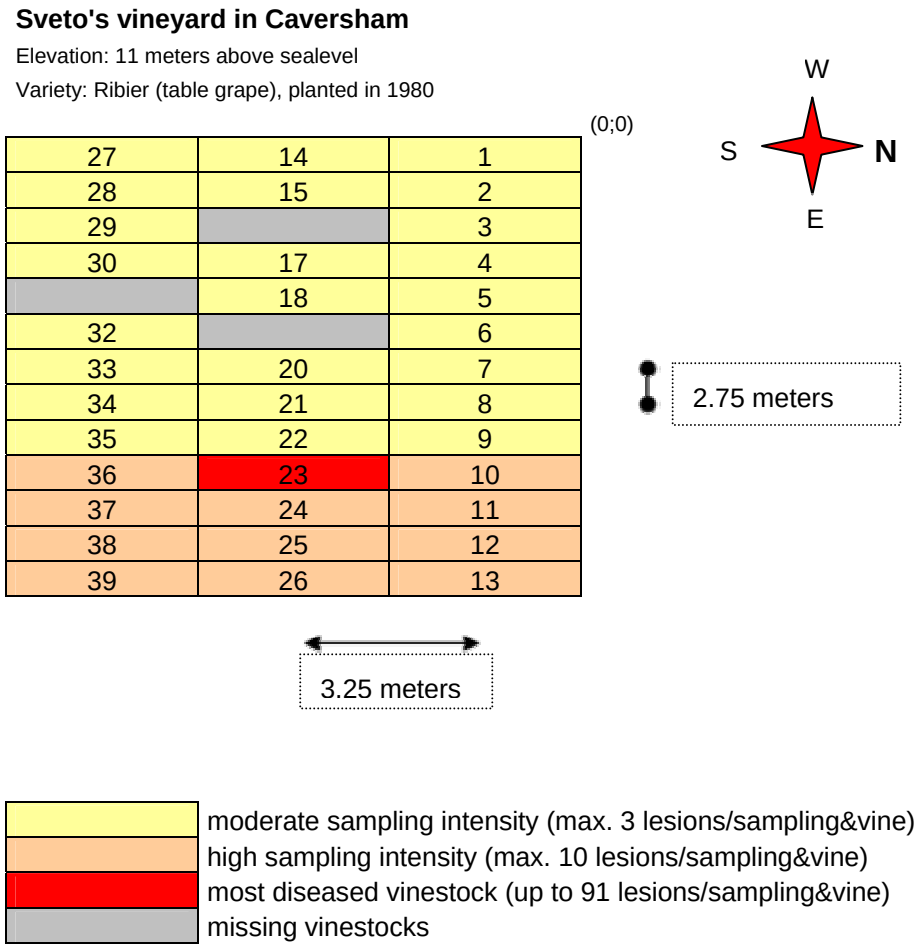


Fig. 1: Vineyard for epidemiological studies in Caversham, Swan Valley, WA

Population Genetics

The vineyards of the following locations could be selected to study the genetic structure of the downy mildew pathogen in a secondary center:

Nr on map	Vineyard site	State	Longitude	Latitude	Altitude	Annual Rainfall
1	Caversham	WA	115.98	-31.88	11	648.8 mm [a]
2	Henley Brook	WA	115.60	-31.48	20	765.0 mm [b]
3	Pemberton	WA	116.04	-34.45	174	1199.5 mm [b]
4	Lovedale/Pokolbin	NSW	151.23	-32.43	62	750.8 mm [b,c]
5	Yarra Glen*	Vic	145.53	-37.68	131	1020.1 mm [b]
6	Lenswood	SA	138.81	-34.95	450	1032.1 mm [b]

Table 1: Vineyard location, State, GPS, altitude and annual rainfall of selected vineyards for population genetic studies. [a]: annual rainfall in 2004, [b]: average annual rainfall, [c]: info from Cessnock (Nulkaba) meteorological station; * all info from Healesville meteorological station. (Sources: Department of Environment, WA; Australian Bureau of Meteorology)

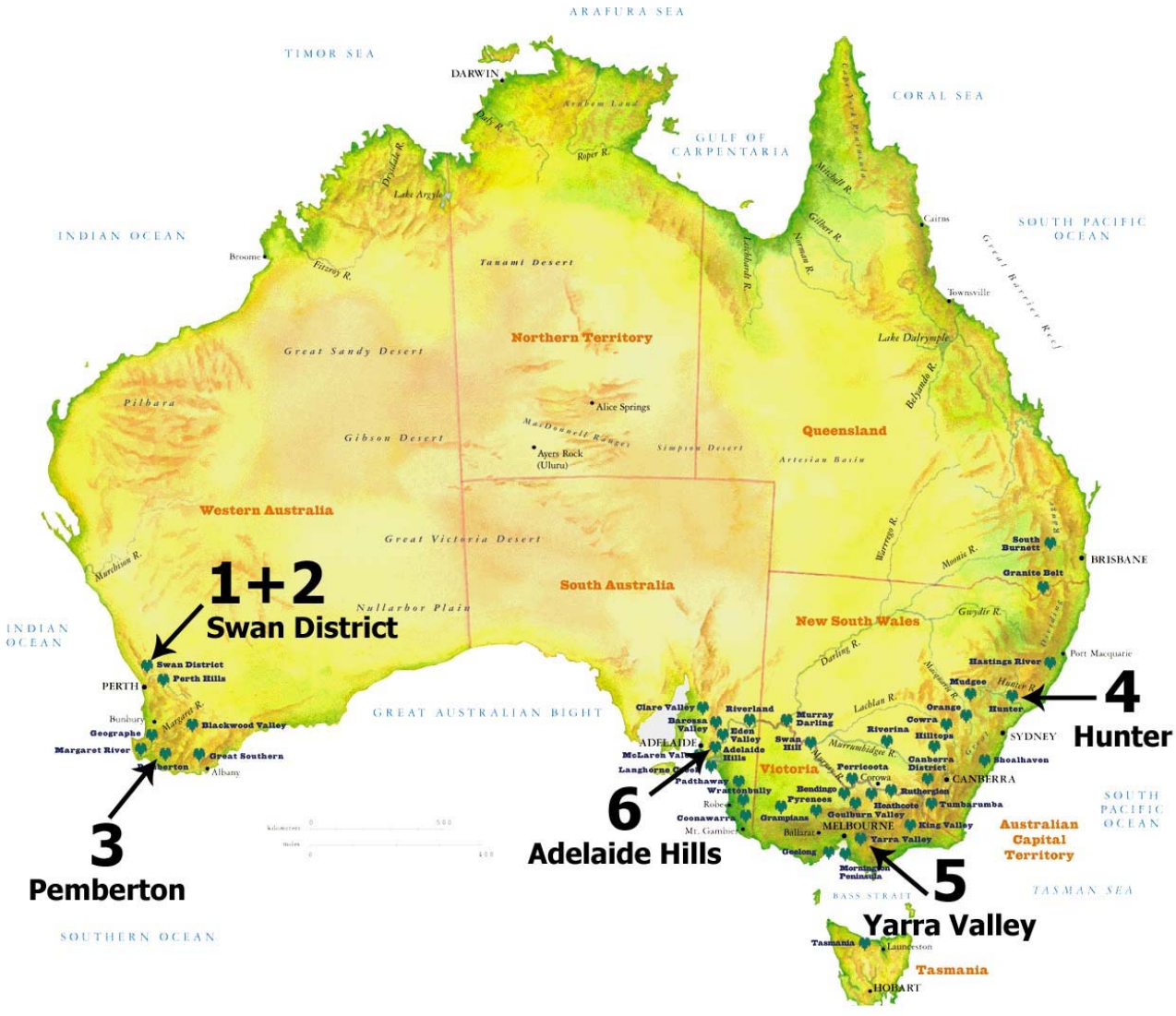


Fig.2: Map of wine producing regions in Australia (by courtesy of the Australian Wine Bureau). Caversham (cav, WA) and Henley Brook (heb, WA) belong to the wine producing region “Swan District”, Pemberton (pem, WA) belongs to “Pemberton”, Lovedale/Pokolbin (hun, NSW) to “Hunter”, Yarra Glen (yar, Vic) to “Yarra Valley” and Lenswood (len, SA) to “Adelaide Hills”.

Sampling

Epidemiology

Sampling dates were determined by three weekly control walks in the Caversham site and collecting when a new series of lesions appeared (Table 1). Collection of infected plant material for pathogen DNA analysis was performed essentially as described by Gobbin, et al. (2003a). A one-cm square of infected leaf tissue was collected from each identifiable lesion. The remainder of the lesion (about half) was left in the vineyard to allow the genotype to reproduce in the vineyard. The sampling method reported by Gobbin, *et al* (2003a) was modified as follows: (i) sampled lesions were assigned a unique identity number following a prior tagging of every single vine stock branch with flagging tape, and (ii) to reduce the possibility of cross-contamination, surgical blades and cutting surfaces (Petri plates) were sterilized with ethanol (70%) after every use and discarded regularly.

The vineyard was divided into three areas with different sampling intensities:

1. Plants 1-9 of each row (three plants missing; 24 vines): moderate sampling intensity (max. three lesions per sampling and vine),
2. Plants 10-13 of each row (12 vines): high sampling intensity (max. 10 lesions per sampling and vine; Fig. 1).
3. Intensive sampling on the most heavily infected vine 23 on 19/01/05.

All 1.5 ml Eppendorf tubes with collected lesions from the Caversham site were frozen immediately upon arrival at the UWA in Perth (University of Western Australia) in a -80°C freezer. Before their transport to Adelaide, the samples were freeze-dried for 20 hours (FD4, Heto Lab Equipment, Denmark). After their transfer from Eppendorf tubes into 96 deep-well racks, the lesions were again freeze-dried (see chapter Sample processing and genotype identification).

Population Genetics

Lesions at all vineyards were collected within one day. Samplings at Henley Brook and Pemberton were identical to the one described above for the Caversham site.

Sampling procedure of the remaining populations was as follows:

- Yarra Glen (Vic): Collection in Eppendorf tubes, freezing to -80°C, transport on dry ice by express mail to Adelaide, storage in -80°C freezer (by courtesy of CSIRO SA).
- Hunter Valley (NSW): Collection of entire leaves in zip-lock bags (one leaf per bag), transport by express mail to Adelaide, storage in -80°C freezer (by courtesy of the Department of Primary Industries NSW and of the CSIRO SA).
- Lenswood (SA): Collection of entire leaves in zip-lock bags (one leaf per bag), transport to Adelaide, storage overnight in fridge, sample processing the following day.

Sample processing and genotype identification

All laboratory work (DNA extraction, PCR, panelling and micro satellite analysis) was performed at the agrf (Australian Genome Research Facility Ltd.), located in the Plant Genomics Centre at the Waite Campus in Adelaide, SA. High throughput DNA extraction and PCR amplification by four different micro satellites ISA, CES, GOB and BER was performed on the collected samples as described by Gobbin *et al.* (2003a). Deep-well racks (NucleoSpin Multi-96 Plant kit, Macherey Nagel, Düren, Germany) with 8-strip micro dilution tubes were prepared, each tube containing a grinding ball (Tungsten Carbide Beads, 3mm, (Qiagen, Hilden, Germany)). Infected plant tissue was transferred into the 8-strip micro dilution tubes (1.2ml, Scientific Specialities Inc., CA) and freeze-dried overnight (Alpha 2-4 LSC, Christ, Osterode, Germany). The samples were capped with 8-strip dilution tube caps (Scientific Specialities Inc., CA) and homogenized on a Retsch MM 300 Mill (Haan, Germany). Total DNA extraction was performed according to manufacturer's protocol (CTAB method) with the following modifications: Using Multidrop DW (ThermoLabsystems, Finland, Type 833), plant material was lysed with 0.4ml C1, containing 10 µl pre-added RNase A (at

room-temperature). Retsch MM 300 Mill (Qiagen) was used a second time to mix tube content at 30/s frequency for 30 seconds. The racks were centrifuged at 1500 rpm for two minutes on a Sigma 4-15C centrifuge, equipped with a Qiagen 2x96 plate rotor (09100F, buckets 09366e) in order to precipitate cellular debris. The tube caps were secured with masking tape. Incubation of the masked racks was at 56°C and 1200 rpm for one hour in Titramax 1000 Incubator (Heidolph, Germany). Multidrop DW was used to mix 0.3 ml of the clear supernatant with 0.3 ml of the binding buffer C4 and 0.2 ml 100% ethanol in order to let the DNA bind to the silica membrane. Capped tubes content was mixed by vigorous shaking for 15-30 seconds and centrifuged for 30 seconds at 1500 rpm to collect any sample from cap strips. DNA was applied on the silica membrane. Firstly, washing was performed adding 0.5 ml of CW buffer to each well of the NucleoSpin Plant Binding Plate containing sample DNA with Multidrop DW. The plate was sealed with a new plastic cover and centrifuged for three minutes at 6000 rpm. Secondly, 0.9ml of C5 buffer were added to each well with Multidrop DW and centrifuged for three minutes at 6000 rpm. In order to remove ethanol traces the plates were placed into the Titramax 1000 Incubator for 40 min at 37°C. Finally DNA was eluted two times in 80 µl CE buffer (10mM). Incubation at room temperature was for three minutes, centrifugation at 6000 rpm for three minutes. The second elution was used for PCR.

Three separate PCR amplifications (ISA, GOB, CES&BER (multiplexed)) per sample were performed in a 10 µl volume containing 1 µl of DNA solution (average 2.8 µg/µl, SpectraMax Gemini XS, Molecular Devices, CA) and 9 µl of Mastermix, containing 1 µl of 1x reaction buffer (1.5mM $MgCl_2$, Qiagen, Hilden, Germany), 1.0mM $MgCl_2$ (total 2.5 mM, Qiagen, Hilden, Germany), 0.20 mM dNTP (Finnzymes), 0.25 µM of dye-labeled forward primer, 0.25 µM of reverse primer and 0.05 U of Taq Polymerase (Hot Star Taq™ Buffer Set, Qiagen, Hilden, Germany). Forward primers were labeled with the following dyes: ISA_f and BER_f with HEX, GOB_f with 6-FAM (GeneWorks Pty Ltd, Adelaide) and CES_f with NED (Applied Biosystems, CA). 1 µl of DNA solution and 9 µl of Mastermix were distributed amongst PCR plate wells with a single-channel Corbett Robot (CAS-1200, Corbett Robotics, Mortlake NSW, AUS) and a multi-channel Corbett Robot (CAS-3800, Corbett Robotics, Mortlake NSW, AUS), respectively. PCR plates were sealed with pierceable sealing foil sheets (AB-Gene®

Thermo-Sealer, Surrey, UK) and centrifuged at 1500 rpm for 1 min prior to start PCR. PCR was performed in a PTC-225 Peltier Thermal Cycler under the following conditions: 15 min at 95°C, 40 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 min at 72°C with a final elongation of 10 min at 72°C.

To determine the exact allele sizes, fragment analysis was performed on a 96-capillary ABI Prism 3700 Genetic Analyzer. Therefore, at first, 3 µl of PCR product of each primer ISA, GOB and CES/BER (multiplexed) were mixed with 147 µl H_2O. Secondly, all markers were panelled together, using the following volume of the 1/50 dilution plates: 18 µl of GOB and 1 µl of ISA and CES/BER, respectively. Finally, 3 µl of the mixture were added to 5 µl deionized formamide (containing 0.05 µl of size standard 500 ROX^{TM} labeled 35-500 bp ladder (Applied Biosystems, Warrington, UK)) in a 96-well sequencing plate (Applied Biosystems, CA). The data were evaluated visually with Genotyper 3.7 (Applied Biosystems). For each marker, different fragment lengths were considered as different alleles. Combining the information provided by the scoring of the four loci, two samples showing identical allele pattern were interpreted as clonal progeny, deriving from the same primary lesion. Different genetic profiles of two oil-spots were interpreted as two individuals derived from independent oosporic infections.

Data analysis

Employing the pivot table report option in a Microsoft Excel spreadsheet (version 2002), the number of genotypes (N_{gen}) and the number of times that a particular genotype was collected (tN_{obs}) were calculated for each sampling period (Appendix 1). The genotypes were categorized according to the number of times they occurred (occurrence class, tN_{obs}) as single genotypes (SG), other genotypes (OG), fourth most frequent genotype (FMFG), third most frequent genotype (TMFG), second most frequent genotype (SMFG) and most frequent genotype (MFG).

Furthermore, Microsoft Excel was used to calculate and map the data of the following sections: meteorological data, spatio-temporal dispersion patterns of the epidemic in the experimental plot, average height and standard deviation of Caversham vineyard lesions, relative contribution of oosporic and sporangial inoculum.

A parameter (t_inf) was created in order to calculate the time when the weather was suitable for a possible downy mildew infection. Time was counted when the following conditions were fulfilled: Temperature between 14 and 22°C (14<T<22), relative humidity over 96% (rH>96) in 10m height.

Allele frequencies were obtained by counting the number of individuals with a particular allele and dividing it by twice the total number of individuals (for a diploid organism). Gene diversity H_{exp} and H_{obs} (Nei, 1973) were calculated for each locus with clone corrected data using Identity 1.0 software (Wagner H. W. and Sefc K. M., 1999). Genotypic diversity was estimated without clone correction by calculating the Shannon index (*H*), which accounts for both abundance and evenness of the genotypes distribution. It was calculated according to the formula (Krebs, 1989):

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the frequency of isolates with the i^{th} genotype and *S* the number of individuals in the sample.

To compare populations with different sample size (*S*) the Shannon Index (*H*) was normalized by calculating Shannon's equitability (E_H) dividing *H* by H_{max} (here $H_{max}=\ln S$).

$$E_H = \frac{H}{H_{\max}} = \frac{H}{\ln S}$$

Independent from the numbers of lesions collected, Shannon's equitability (E_H) gives a good idea of the genetic diversity of all the populations. Equitability assumes a value between 0 and 1 with 0 being complete evenness. A E_H value close to 1 is equal to a high degree of genotype diversity.

The following data sets for the Caversham site were used in epidemiological and populations genetic studies:

- 1 – cavall (including all collected lesions during samplings 1-7)
- 2 – cavcc (clone corrected)
- 3 – cavmax3 (including max 3 samples per vine during samplings 1-6; three lesions per sampling and vine stock were randomly selected from area 2 (max. 10 lesions/sampling and vine), in order to get an even sampling intensity for the whole vineyard in Caversham),
- 4 – cavmax10 (including all collected lesions during samplings 1-6: area 1 (max three lesions/sampling and vine) + area 2 (max 10 lesions/sampling and vine).
- 5 – cavmdv (most diseased vine 23), equal to sampling 7.

For population genetic studies, both clone corrected (e.g. “hebcc)) and non-clone corrected data (e.g. “heb”) was used. Population differentiation indices F_{st} and R_{st} were calculated with the program Genepop on the Web (Curtin University of Technology, WA, Australia). The pairwise test of differentiation (F_{st} values) was calculated with the programme Fstat (Goudet J, version 2.9.3.2 (2.2002)).

An unrooted Neighbour-joining tree based on D_{sw} (Shriver *et al.*, 1995) was drawn, depicting phylogenetic relations amongst the five Australian populations, two Swiss (CH), two French (F), two Italian (I), two Greek (H), two German populations (D) and one American population (Geneva NY, USA).

Results

Epidemiology

Meteorological conditions, epidemic development, sample collection

Foliar symptoms (yellow, non-sporulating lesions) of downy mildew were already present in the vineyard during the first visit at the Caversham site (08/11/04), when flowering began (grapevine growth stage 19). From this moment on the disease was sampled (Table 2). The rainfall at Caversham during the 12 month period 01/01/04 to 31/12/2004 was 648.8mm, whereof 33.4mm fell during the surveying period (08/11/04-19/01/05). Between November 04 and January 05, there were six daily rain events over 1mm, whereof three before the first sampling (09-11/11/04). Rainfall on 25/11/04 was the only event over 10mm (11.6mm, Fig. 3). Before sampling 4 (08/12/04), minor rain events (0.8mm on 04/12/04 and 0.2mm on 05/12/04), high maximum relative humidity (88% on 04/12/04; Fig. 4) and average temperatures around 18°C occurred. Shortly after that, the disease "exploded" (06/12/04) – literally overnight, downy mildew oil-spots appeared on young leaf tissue everywhere in the Swan Valley, no matter if the vineyards were well treated or not.

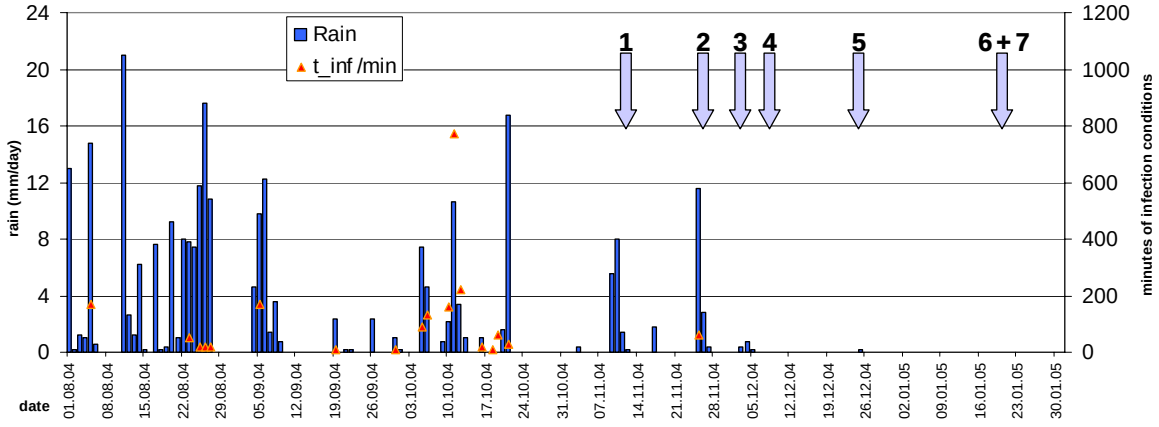


Fig. 3: Daily rain events (in mm) and minutes of infection condition at the Caversham site (meteorological data by courtesy of the Air Quality Management Branch, Department of Environment, WA). Time with fulfilled conditions for a possible downy infection [(t_inf): 14<T<22, rH>10m height] are represented by triangles. Arrows represent the sampling dates: 1=12/11/04, 2=26/11/04, 3=03/12/04, 4=08/12/04, 5=25/12/04, 6+7=19/01/05.

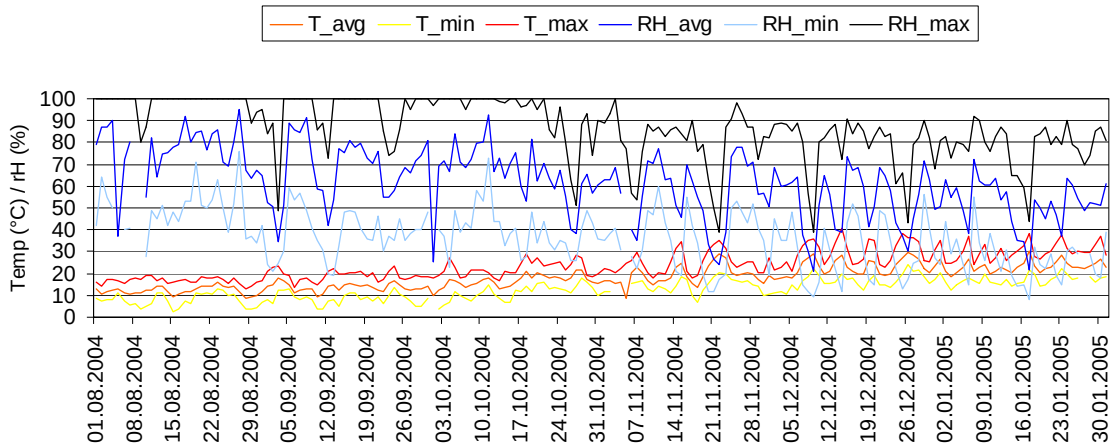


Fig. 4: Daily measures of average temperature (T_avg), minimum temperature (T_min), maximum temperature (T_max), average relative humidity (RH_avg), minimum relative humidity (RH_min) and maximum relative humidity (RH_max) at the Caversham site (meteorological data by courtesy of the Air Quality Management Branch, Department of Environment, WA).

Average daily temperatures were around 17°C during the first sampling, increasing gradually to a level of up to 30°C during Christmas, and then varied between 19°C and 30°C over the course of the epidemic survey in January 05. Maximum relative humidity was mostly around 80 percent, with an absolute minimum of 39 % and a maximum of 98 % during the survey period. Disease severity was on a steady increase until the fourth sampling (08/12/04; grapevine growth stage 31 (berry pea size)), then decreased quite abruptly because of a period of dryness.

Sampling

In the period 12/11/04-19/01/05, a total of 789 samples were collected and genetically analyzed. 709 samples produced results for all four sets of primers and could therefore be used for genotype analysis (data set cavall; Table 2). The remaining 10% of samples could not be included in statistical programs for diploid organisms, either because they did not yield sufficient PCR product, or because the genotypes showed zero, three of four alleles. Of the 80 excluded isolates, 16 samples showed an apparently triploid and four an apparently tetraploid allelic pattern for the marker GOB. The data set cavmax10 included 636 samples, the data set cavmax3 included 450 samples, the clone corrected data set cavcc included 32 samples and the data set for the most diseased vine cavmdv included 73 samples.

Table 2: Overview of collected samples at the Caversham site, including sampling, date of sampling, days from sampling 1 ("Days"), number of collected lesions ("NL"), cumulative number of lesions of samplings 1-7 ("cNL"), collection site ("Vines"), and grapevine growth stage. Sampling 7 was performed on the most diseased vine (MDV).

Sampling	Date	Days	NL	cNL	Vines	Grapevine growth stage
1	12.11.2004	0	127	127	all	21/30% caps off (bloom)
2	26.11.2004	14	68	195	all	25/80% caps off (bloom)
3	03.12.2004	21	56	251	all	31/berries pea-size
4	08.12.2004	26	174	425	all	31/berries pea-size
5	25.12.2004	43	78	503	all	33/berries still hard and green
6	19.01.2005	68	133	636	all	35-37/veraison
7	19.01.2005	68	73	709	MDV	35-37/veraison

Allelic diversity in the Caversham population

In the Caversham population (data set cavall), SSR analysis revealed different degrees of polymorphism amongst micro satellite markers in the Caversham population. Two alleles were detected at locus ISA, 15 at GOB, one at CES and two at BER. The most frequent alleles at each locus (fragment lengths detected in base pairs, bp) were ISA-110.6, ISA-129.7, GOB-293.3, CES-137.4, BER-175.3 and BER-177.3. The locus ISA was always heterozygote, BER mostly heterozygote (705 out of 709 isolates; Table 3).

Table 3: Locus name, number of alleles (*), number of SSR core units (Repeat nr.), allele frequency (AF) and length in bp of the alleles found in the Caversham population. Data set used: cavall.

ISA	2		GOB	15		CES	1		BER	2	
Repeat nr.	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**
7	0.5	110.6	75	0.00	202.5	33	1.00	137.4	11	0.5	175.3
16	0.5	129.7	82	0.00	217.6				12	0.5	177.3
			115	0.00	283.1						
			116	0.01	285.3						
			118	0.14	289.3						
			119	0.00	291.3						
			120	0.35	293.3						
			121	0.00	295.2						
			122	0.16	297.5						
			123	0.00	299.3						
			124	0.29	301.4						
			125	0.00	302.5						
			126	0.03	305.5						
			127	0.00	307.5						
			128	0.01	309.6						

Nei's (1973) gene diversity H_{exp} (expected heterozygosity) and H_{obs} (observed heterozygosity) showed differences for the different loci in the Caversham data sets. H_{exp} and H_{obs} for ISA and BER were 0.5 and 1.0 in all the data sets, respectively (Table 4). For GOB, the most diverse locus, H_{exp} and H_{obs} in the clone corrected data set cavcc were different to H_{exp} and H_{obs} from the other Caversham data sets. H_{exp} was highest in cavcc (0.89), whereas H_{obs} was highest in cavall/cavmax3 (0.97). H_{exp} and H_{obs} could not be calculated for the locus CES, due to the presence of one allele.

The genotypic diversity (normalized Shannon index E_H) varied between 0.28 (cavall) and 0.31 (cavmax3) of the data sets without clone correction (Table 4.).

Table 4: $H_{exp,}$ $H_{obs,}$ E_H of the four different Caversham data sets (cavall, cavcc, cavmax3, cavmax10).

	Heterozygosity (expected/observed)				
Data set	**ISA**	**GOB**	**CES**	**BER**	**E_H**
cavall	0.50/1.0	0.75/0.97	-	0.5/0.99	0.28
cavcc	0.50/1.0	0.89/0.78	-	0.50/0.88	1
cavmax3	0.50/1.0	0.76/0.97	-	0.5/0.99	0.31
cavmax10	0.50/1.0	0.77/0.97	-	0.50/0.99	0.29

Genotype contribution to the epidemic – entire Caversham vineyard

Combining the information from the four loci, 31 distinct genotypes (cavmax10 data set) were identified in all samplings. The genotypes that were detected once throughout the surveying period (tN_{obs}=1, SG, single genotypes) represented 52% of the total genotypes collected (16/31) and 2.5% of the total number of lesions collected (16/636, Table 5a). From the total 31 genotypes, four of them were identified 60 times or more. The most frequent genotype (MFG, “dgulosse”) was identified 268 times (tNobs=268) and accounted for 42.1% of the total number of samples collected. The last group (OG=other genotypes) with genotypes found between two and 25 times amounted to 13.5% (tNobs=86) of the overall sample size.

Using the cavmax3 data set, 26 genotypes were found. The five missing genotypes in cavmax3 with respect to the cavmax10 data set belonged to the SG-group. However, the relative contribution of the SG-group to the total sample size did not change much (2.4%). Compared to the descending percentage order in the whole vineyard, SMFG and TMFG changed their position: “dgulesse”, second most frequent genotype (SMFG) in the data set cavmax10, was the third most frequent genotype (TMFG) in the data set cavmax3, because “dgilesse” was more dominant in cavmax3 (SMFG; Table 5a,b).

Table 5a,b: Genotype Group, number of times a genotype was collected (occurrence class, tN_{obs}), number of genotypes (N_{gen}), number of lesions (NL) and contribution to total sample size (%tNL) per genotype occurrence class for the whole vineyard with different data sets used: cavmax10, cavmax3.

* Groups: SG = Single Genotype, found only once; OG = Other Genotypes, found between two and 25 times; FMFG = Fourth Most Frequent Genotype; TMFG = Third Most Frequent Genotype; SMFG = Second Most Frequent Genotype; MFG = Most Frequent Genotype.

Data set: cavmax10

Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	16	16	**2.5**
OG	2	2	4	
OG	3	1	3	
OG	4	1	4	
OG	5	1	5	
OG	6	1	6	
OG	8	2	16	
OG	10	1	10	
OG	17	1	17	
OG	21	1	21	**13.5**
FMFG - dgilosse	63	1	63	**9.9**
TMFG - dgilesse	90	1	90	**14.0**
SMFG - dgulesse	113	1	113	**17.8**
MFG - dgulosse	268	1	268	**42.1**
		31	636	100

Data set: cavmax3

Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	11	11	**2.4**
OG	2	2	4	
OG	3	3	9	
OG	5	2	10	
OG	7	2	14	
OG	12	1	12	
OG	17	1	17	
				14.7
FMFG - dgilosse	48	1	48	**10.7**
TMFG - dgulesse	61	1	61	**13.6**
SMFG - dgilesse	68	1	68	**15.1**
MFG - dgulosse	196	1	196	**43.6**
		26	450	100

A first visit to the Caversham site revealed an already advanced status of a downy mildew infection with the presence of yet seven clonal lineages (see Appendix 1). The initial infection, took place before the first sampling (12/11/04).

In data set cavmax3, SGs remained at a constant low level throughout the entire sampling period, reaching an average of three in a hundred lesions. OGs were mainly present at the beginning of the infection ("@", 2 out of 6 initial oospores), slumping down to 10% with the first sampling and only recovering to 15% towards the final sampling (Fig. 5b; for figures of subdivided sampling areas see Appendix 2).

The four most frequent genotypes (MFG, SMFG, TMFG, FMFG) were present since the beginning of the epidemic and held a constant proportion of the total number of individuals sampled at each stage. These clonal lines already reached a dominant level during the first sampling and remained at a constant level of 84.0% (534 lesions) of the total number of lesions collected (Fig. 5a,b).

The most frequent genotype (MFG, “dgulosse”) started out with a 33% share in the first sampling and gradually increased its contribution towards the overall epidemic, reaching a level of 43% in the final sampling (Fig. 5b).

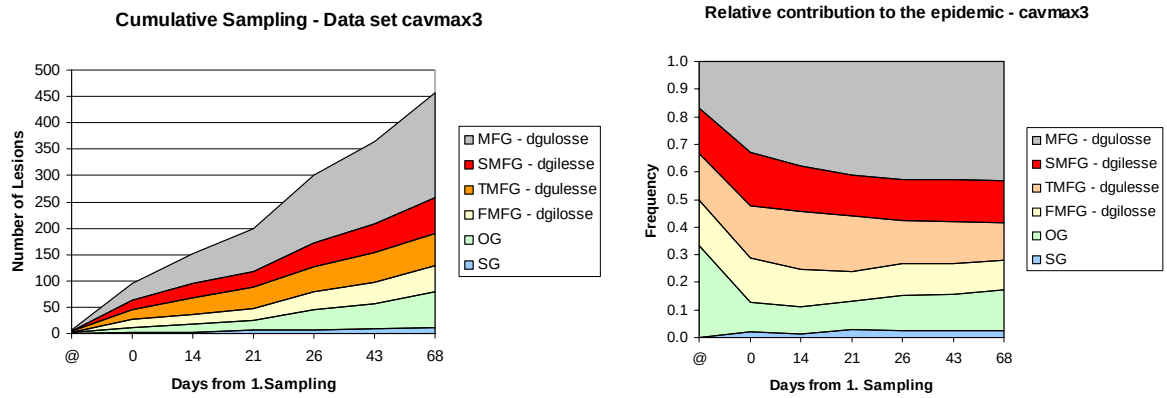


Fig. 5a,b: Cumulative number of lesions and percentage of lesions of the downy mildew infection in Caversham, caused by the most frequent genotype (MFG), the second (SMFG), third (TMFG) and fourth (FMFG) most frequent genotype, other genotypes (OG*) and single genotypes (SG). The day of the first infection is indicated with the symbol “@”. Data set used: cavmax3.

*OG: Genotypes which occurred between two and 25 times.

Genotype contribution to the epidemic – most diseased vine (MDV)

On the most diseased single vine (MDV), nine different genotypes were identified amongst 73 lesions collected in a last intensive sampling on 19/01/05 (sampling 7, table 6, data set: cavmdv). The dominant genotype on that vine, which was at the same time the most frequent genotype (MFG, “dgulosse”) in the whole vineyard, was detected in 64.4% of all lesions collected. The SG group in this case showed 4.1% presence (three lesions).

Table 6: Genotype Group, number of times a genotype was collected (occurrence class, tN_{obs}), number of genotypes (N_{gen}), number of lesions (NL) and contribution to total sample size (%tNL) per genotype occurrence class for the most diseased vine. Data set used: cavmdv.

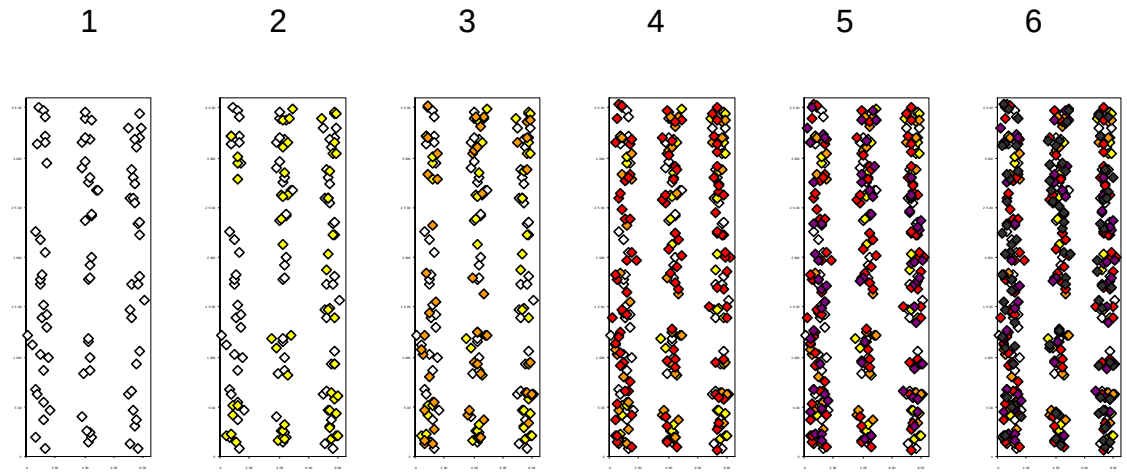
* Groups: SG = Single Genotype, found only once; OG = Other Genotypes, found between two and four times; FMFG = Fourth Most Frequent Genotype; TMFG = Third Most Frequent Genotype; SMFG = Second Most Frequent Genotype; MFG = Most Frequent Genotype.

Data set: cavmdv

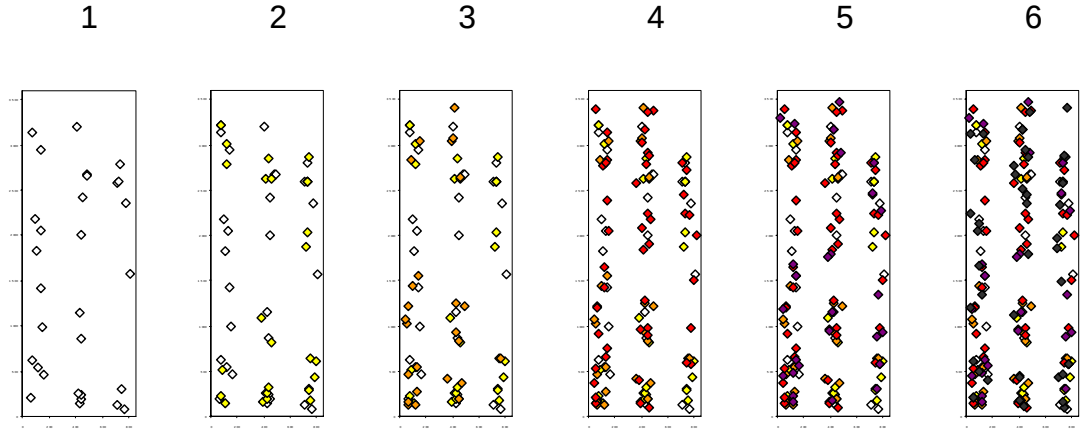
Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	3	3	**4.1**
OG	3	2	6	**8.2**
FMFG - dgilesse	5	1	5	**3.8**
SMFG - dgilosse	6	1	6	**8.2**
SMFG - dgulesse	6	1	6	**8.2**
MFG - dgulosse	47	1	47	**64.4**
		9	73	100

Spatial distribution of genotypes in the vineyard

The cavmax3 data set shows a random distribution of the total disease in the vineyard from the first sampling on (Fig. 6a). The most frequent genotype (MFG) shows a random distribution constant over time (Fig. 6b), whilst the third most frequent gene (TMFG) is the only one that shows an aggregated distribution on the east side of the vineyard (Fig. 6c). Fig. 6d shows all single genotypes (SGs) in a sole diagram. For spatial distribution of the second (SMFG) and the fourth most frequent genotype (FMFG), refer to Appendix 3.

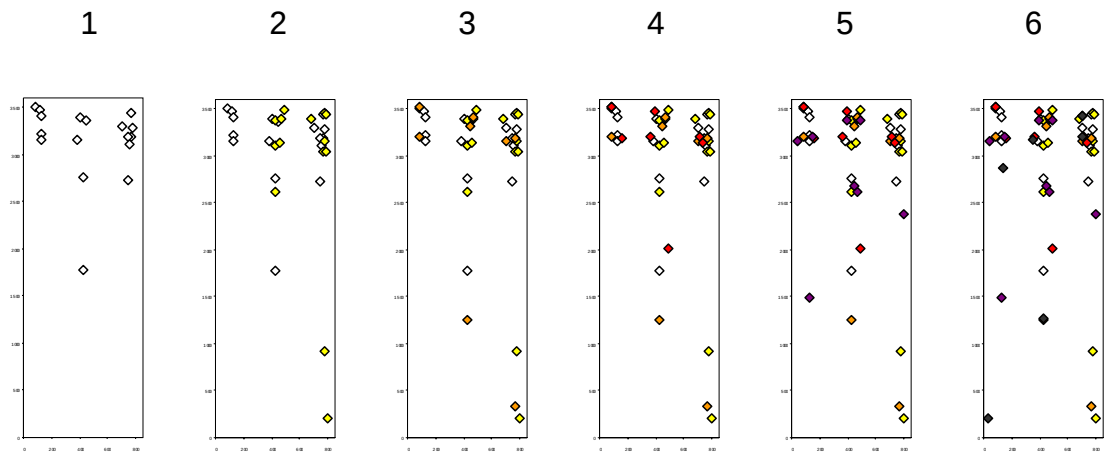


all genotypes



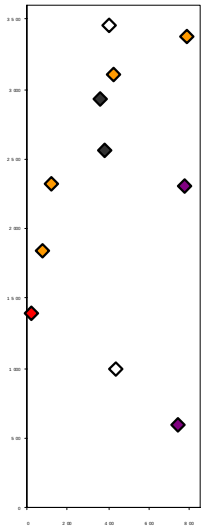
most frequent genotype (MFG)

Fig. 6a,b: Spatio-temporal dispersal of all genotypes (total disease, Fig. 6a) and of the most frequent genotype (MFG, Fig. 6b) in the Caversham vineyard. The vineyard is represented by a scaled rectangle. Top numbers represent the sampling dates. Lesions found at different sampling dates are shown in different colours, changing in the following order: white, yellow, orange, red, purple and black. Sampling dates: 1=12/11/04, 2=26/11/04, 3=03/12/04, 4=08/12/04, 5=25/12/04, 6=19/01/05. Moderate sampling intensity for the whole vineyard (max. three lesions/sampling and vine). Data set used: cavmax3.



third most frequent genotype (TMFG)

1-6

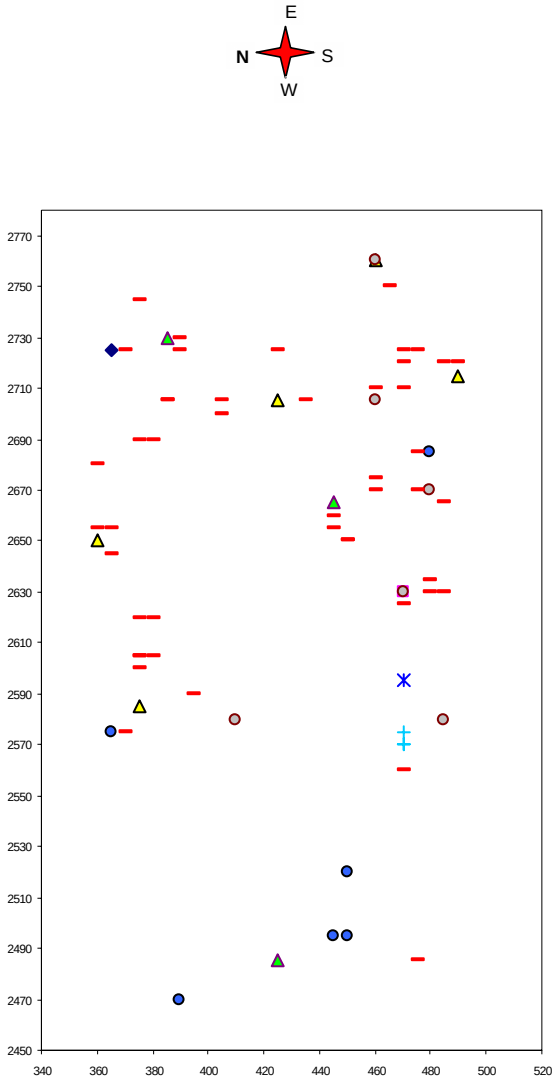


single genotypes (SGs)

Fig. 6c,d: Spatio-temporal dispersal of the third most frequent genotype (TMFG, Fig. 6c) and all single genotypes (SGs, Fig. 6d) in the Caversham vineyard. The vineyard is represented by a scaled rectangle. Top numbers represent the sampling dates. Lesions found at different sampling dates are shown in different colours, changing in the following order: white, yellow, orange, red, purple and black. Sampling dates: 1=12/11/04, 2=26/11/04, 3=03/12/04, 4=08/12/04, 5=25/12/04, 6=19/01/05. Moderate sampling intensity for the whole vineyard (max. three lesions/sampling and vine). Data set used: cavmax3.

Spatial distribution of genotypes in a single vine (MDV)

A slight trend of more lesions on one half (east side) can be observed in Fig. 7, mainly due to the clustered distribution of the most frequent genotype (MFG). The last intense sampling on the same plant shows the spatial aggregation of the most frequent genotype (MFG), whereas other genotypes could be found evenly distributed over the entire vine stock.



all genotypes found in last intense sampling on MDV (73 lesions)

Fig. 7: Spatial dispersal of all genotypes found in the last intense sampling (19/01/05) on the most diseased vine (MDV) in the Caversham vineyard. The vine stock (MDV) is represented by a scaled rectangle. The nine found genotypes are depicted with different symbols.

No clearly defined trend in the height, measured from the soil surface, of lesions was identified at the Caversham site (Fig. 8). New lesions were always found on young leaf material. No canopy labor was performed throughout the sampling period; as a consequence young branches grew in all directions.

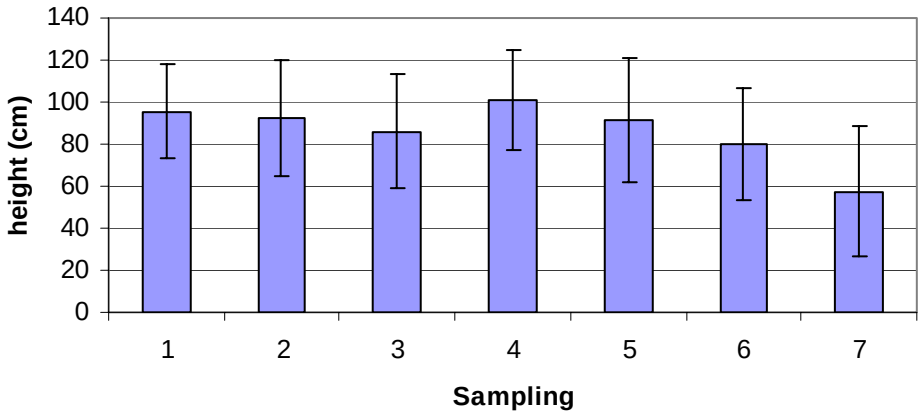


Fig. 8: Average height and standard deviation of found Caversham vineyard lesions. Sampling 7 is equal to the last intense sampling on the most diseased vine stock (MDV). Data set: cavmdv.

Relative contribution of oosporic and sporangial inoculum

Calculations were carried out with the data set cavmax3 by considering the first appearance of a genotype as an oosporic infection. Any subsequent observations of a given genotype were considered secondary (sporangial).

Multiple detections of seven genotypes already began with the first sampling (12/11/04). Oosporic infection of these particular genotypes may have taken place at an earlier stage (delineated with the symbol "@" on the time scale).

The relative contribution of primary inoculum quickly plunged to a level of 10 % with the first sampling (Fig. 9b). At day 14, no primary inoculum was found, whereas during the consecutive sampling dates a steady number of four up to six lesions were caused by oospores. The relative contribution of cumulative primary inoculum appeared to stabilize between five and seven percent after day 14.

In the course of the infection, multiple detection of genotypes increased. The production of secondary inoculum kept increasing until the end of the survey, ending in a 95% contribution at day 68 (613 lesions, Fig. 9a).

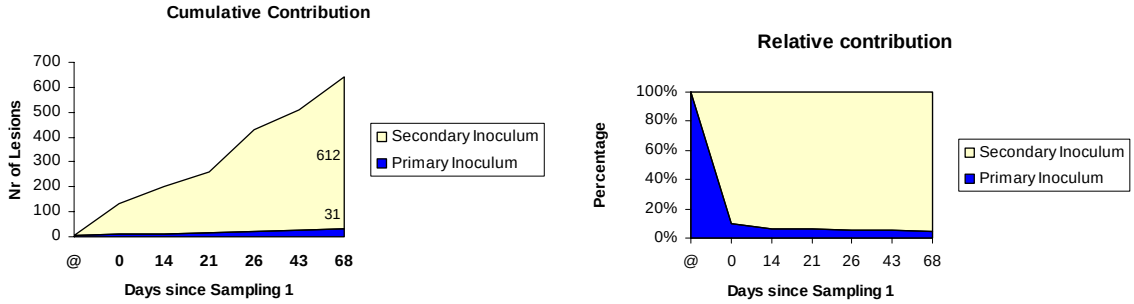


Fig. 9a,b: Number of lesions (Fig. 9a) and relative contribution of primary and secondary inoculum to the epidemic (Fig. 9b). Moderate sampling intensity for the whole vineyard (max. three lesions/sampling and vine). Data set used: cavmax3.

Population Genetics

Allelic diversity of Australian populations

Allelic diversity in Western Australia is low: For the locus CES, only one allele was found throughout the whole state, which is up to six times less than in the east coast states (Table 7). Two (Henley Brook, Caversham) up to three (Pemberton) alleles were found for the locus ISA, whilst BER remained at a level of two alleles throughout all Australian states (Table 7). The most diverse locus was GOB. The number of alleles for the locus GOB varied between seven (Pemberton) and 15 (Caversham). In Yarra Glen, Yarra Valley, the number of alleles was the highest for all four primers: five for ISA, 30 for GOB, six for CES and two for BER, which remained the same.

In Pemberton (WA) and Yarra Glen (Vic), a similar amount of lesions had been collected in the vineyards (120 vs. 125; Table 8). The number of GOB alleles found in Pemberton was less than a fourth of the correspondent number detected in Yarra Glen (7 vs. 30). H_{exp} and H_{obs} values for the locus BER were lower in the east states. H_{obs} values (BER) of clone corrected data sets varied between 0.67 (Pemberton) and 1.0 (Henley Brook) in Western Australia, whereas H_{obs} reached levels of 0.15 in Yarra Glen and 0.16 in the Hunter Valley. The same phenomenon could be observed for the locus ISA: despite of having a higher number of alleles, the observed heterozygosity H_{obs} was smaller on the east coast than in Western Australia (Table 7).

In the Swan Valley, a sole combination of two different ISA alleles (repeat numbers 7 and 16, Table 3) was found, therefore a large difference between H_{exp} and H_{obs} was shown. The more diverse populations from the east coast (Yarra Valley, Hunter Valley) show narrower ranges between H_{exp} and H_{obs} for all SSR markers.

The Lenswood population was excluded for further population genetics studies, due to its low number of genotypes.

The null allele frequency estimation was the highest in Caversham (cavcc, 6% for the locus GOB) and in Yarra Glen (yarcc, 6% for the locus BER). For the locus CES, the null allele frequency was always zero (Table 7).

Table 7: Summary of genetic variation and null allele frequency estimation (Nafe,%) within each of the six clone corrected *P. viticola* populations (Pvp) of Australia. NG refers to the number of genotypes found and k to the number of SSR marker of the individuals studied. SSR markers are listed in the headings. Stars represent significant ("*": P<0.01) deviations from the Hardy-Weinberg equilibrium. Null allele frequency estimation per SSR marker is given as percentage. [a]: not enough genotypes to calculate the Hardy-Weinberg equilibrium. *P. viticola* populations: cav=Caversham, heb=Henley Brook, hun=Hunter Valley, pem=Pemberton, yar=Yarra Glen. "cc" after a location refers to a clone corrected population.

Clone corrected

		Number of alleles (k)				Heterozygosity (expected/observed)				Nafe (%)			
Pvp	**NG**	**k_{ISA}**	**k_{GOB}**	**k_{CES}**	**k_{BER}**	**ISA**	**GOB**	**CES**	**BER**	**ISA**	**GOB**	**CES**	**BER**
cavcc	32	2	15	1	2	0.50/1.0*	0.89/0.78	-	0.50/0.88*	0	6	0	0
hebcc	12	2	8	1	2	0.50/1.0*	0.83/0.83	-	0.50/1.0*	0	0	0	0
pemcc	15	3	7	1	2	0.53/0.60	0.64/0.60	-	0.44/0.67	0	3	0	0
huncc	43	4	20	5	2	0.74/0.72	0.82/0.77	0.64/0.81	0.15/0.16	1	3	0	0
yarcc	79	5	30	6	2	0.69/0.73	0.94/0.96	0.72/0.84	0.22/0.15	0	0	0	6
lencc[a]	3	4	1	1	2	0.67/1.0	-	-	0.50/1.0	0	0	0	0

Genetic diversity of Australian populations

The six populations distinguished themselves by their number of genotypes with respect to the number of lesions collected. The percentage of different genotypes found in a total population was small in Western Australia (WA). Within the three populations of WA, the contribution of oosporic infections over the total infection varied between 4.5% (Caversham) and 12.5% (Pemberton). Genetic diversity of downy mildew populations were higher in the eastern states: in the Hunter Valley (NSW), 43 different genotypes were found in 135 lesions (32%). In the Yarra Valley (Vic), 79 different genotypes were detected in 125 lesions (63%). The only downy mildew infection in South Australia until mid-February 2005 was from an artificially inoculated vineyard in Lenswood. The genotypes, which were used for the artificial inoculation, were mainly clones (Wicks, personal communication). Therefore, a low genotypic diversity could be expected, and only 25 lesions were collected.

Shannon's equitability (E_H) shows low genotype diversity in Western Australian populations (0.28 for Caversham, 0.33 for Henley Brook, 0.34 for Pemberton). Populations from the east coast states are remarkably more diverse, with E_H values of 0.62 for the Hunter Valley (NSW) and 0.84 for the Yarra Valley (Vic), respectively (Table 8).

Table 8: Vineyard location (Plot and state), *P. viticola* population name (*P.vit* pop), sampling period, total number of samplings (N sampl), number of individuals collected (N indiv), number of genotypes identified (N Genotypes) and Shannon's equitability (E_H). States: WA=Western Australia, NSW=New South Wales, Vic=Victoria, SA=South Australia.

Plot	State	*P.vit* pop	Sampling period	N sampl	N indiv	N Genotypes	E_H
Caversham (Swan Valley)	WA	cav	12.11.2004-19.01.2005	6	709	32	0.28
Henley Brook (Swan Valley)	WA	heb	15.11.2004	1	103	12	0.33
Pemberton	WA	pem	23.12.2004	1	120	15	0.34
Lovedale/Pokolbin (Hunter Valley)	NSW	hun	09.12.2004	1	135	43	0.62
Yarra Glen (Yarra Valley)	Vic	yar	04.01.2005	1	125	79	0.84
Lenswood	SA	len	23.02.2005	1	25	3	0.10

Genetic distance estimators, e.g. F_{st} and R_{st} values, indicate a close degree of relationship between Western Australian populations, with values close to zero. East and west coast populations are not only separated by large distances, they also differ in their genetic appearance and seem to be far related to each other, e.g. the clone corrected populations of Pemberton (WA) and of the Hunter Valley (NSW): F_{st}=0.2, R_{st}=0.23 (Table 9). A pairwise test of differentiation (F_{st} values) unveiled mostly genotypic differences between Australian populations (P<0.05). Non-significant genotypic differences between clone corrected populations were only found comparing different regions in Western Australia: Caversham-Henley Brook and Henley Brook-Pemberton.

Table 9: Estimates of pairwise F_{st} (below diagonal) and R_{st} (above diagonal) over loci. F_{st} and R_{st} are calculated for five *P. viticola* populations (Pvp) of Australia, firstly without any clone correction (e.g. cav), secondly with clone corrected data (e.g. cavcc). *P. viticola* populations: cav=Caversham, heb=Henley Brook, hun=Hunter Valley, pem=Pemberton, yar=Yarra Glen. Significant F_{st} values are indicated by "*" (P<0.05).

Pvp	**cavall**	**heb**	**hun**	**pem**	**yar**	**cavcc**	**hebcc**	**huncc**	**pemcc**	**yarcc**
cavall		0.02	0.26	0.03	0.52					
heb	0.06*		0.11	0.00	0.32					
hun	0.25*	0.26*		0.12	0.02					
pem	0.12*	0.14*	0.19*		0.32					
yar	0.29*	0.29*	0.07*	0.25*						
cavcc							0.02	0.13	0.00	0.24
hebcc						0.00		0.12	0.00	0.24
huncc						0.22*	0.22*		0.12	0.08
pemcc						0.06*	0.04	0.20*		0.23
yarcc						0.21*	0.20*	0.05*	0.20*	

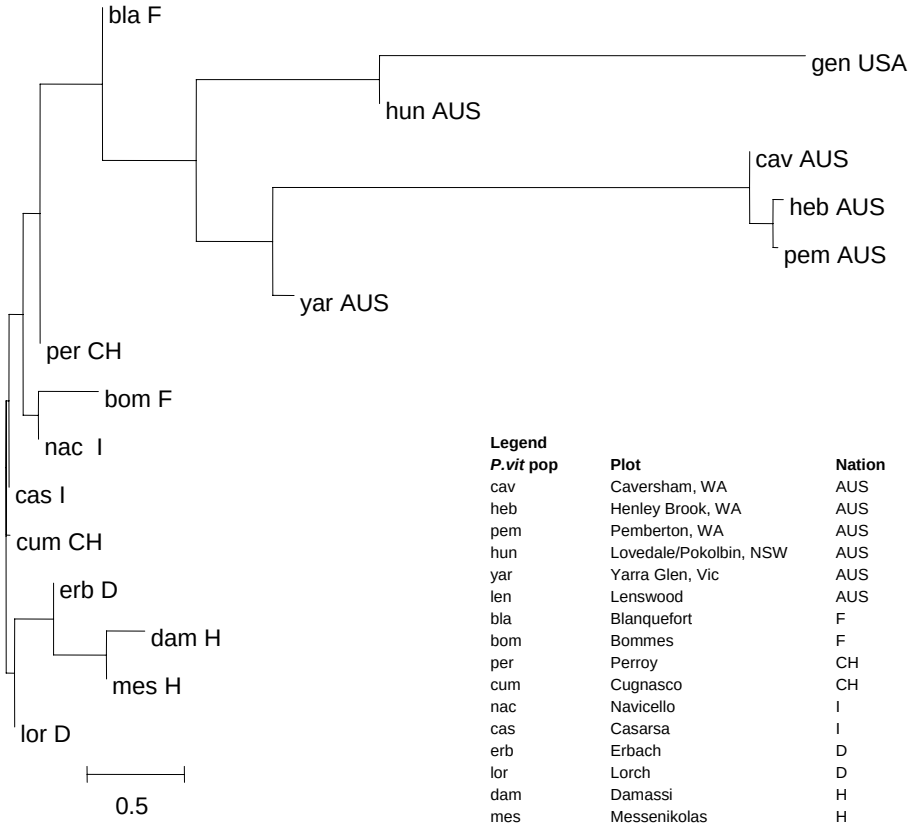
Genetic diversity of Australian populations compared to European and American populations

Eleven previously characterized European and American populations of *P. viticola* (Gobbin, personal communication; Eugster (2003)) were chosen according to their sample size. They were clone corrected and compared with Australian populations (Table 10).

Table 10: Nation, vineyard location (Plot), *P. viticola* population (*P.vit* pop), sampling period, number of samplings (N sampl), number of individuals collected (N indiv), number of genotypes identified (Genotypes), normalized Shannon Index (Shannon's Equitability, E_H).

Nation	Plot	*P.vit* pop	Sampling period	N sampl	N indiv	N Genotypes	E_H
AUS	Caversham, WA	cav	12.11.2004-19.01.2005	6	709	32	0.28
AUS	Henley Brook, WA	heb	15.11.2004	1	103	12	0.33
AUS	Pemberton, WA	pem	23.12.2004	1	120	15	0.34
AUS	Lovedale/Pokolbin, NSW	hun	09.12.2004	1	135	43	0.62
AUS	Yarra Glen, Vic	yar	04.01.2005	1	125	79	0.84
F	Blanquefort	bla	09.05.2000-07.08.2000	6	557	363	0.89
F	Bommes	bom	27.06.2000-27.08.2001	4	193	41	0.39
CH	Perroy	per	30.05.2000-11.08.2000	3	325	108	0.66
CH	Cugnasco	cum	07.06.2001-29.08.2001	4	484	193	0.73
I	Navicello	nac	15.05.2000-06.07.2000	5	572	464	0.94
I	Casarsa	cas	17.05.2000-04.07.2000	3	196	142	0.91
D	Erbach	erb	18.05.2000-01.08.2000	22	287	192	0.89
D	Lorch	lor	05.06.2000-27.06.2000	7	361	228	0.83
H	Damassi	dam	29.05.2001-15.10.2001	6	656	131	0.62
H	Messenikolas	mes	08.06.2001-19.10.2001	5	534	229	0.78
USA	Geneva	gen	12.06.2003-28.07.2003	10	869	386	0.75

Embedding all clone corrected Australian populations in the yet existing phylogenetic relations, some new branches could be found. The three populations from Western Australia form one group, whilst the populations of the Hunter Valley and Yarra Valley can be found individually in different branches of the unrooted Neighbour-joining tree (Fig. 10).



Legend		
***P.vit* pop**	**Plot**	**Nation**
cav	Caversham, WA	AUS
heb	Henley Brook, WA	AUS
pem	Pemberton, WA	AUS
hun	Lovedale/Pokolbin, NSW	AUS
yar	Yarra Glen, Vic	AUS
len	Lenswood	AUS
bla	Blanquefort	F
bom	Bommes	F
per	Perroy	CH
cum	Cugnasco	CH
nac	Navicello	I
cas	Casarsa	I
erb	Erbach	D
lor	Lorch	D
dam	Damassi	H
mes	Messenikolas	H
gen	Geneva	USA

Fig. 10: Unrooted Neighbour-joining tree based on D_{sw} (Shriver *et al*, 1995), representing phylogenetic relations amongst five Australian populations (AUS), one American population (USA), two Swiss (CH), two French (F), two Italian (I), two Greek (H) and two German populations (D) fo *Plasmopara viticola*. Note that all populations are clone corrected, cav stands for the clone corrected population of Caversham (cavcc in the rest of this diploma thesis), heb for hebcc, pem for pemcc etc.

Discussion

Epidemiology

Meteorological conditions and disease development

After two dry and hot seasons (2002/03 and 2003/04), the weather was unusual cold and rainy at the beginning of the season 2004/05, which resulted in conducive conditions for downy mildew infections, similar to 2001/02, the last “downy year”.

Two rules are used in Australia to determine whether infection events occur:

1. Rule 10-10-24 for primary downy mildew infections (10mm of rain at 10°C minimum temperature for 24 hours).
2. Rule 98-13-4 for secondary downy mildew infections (greater than 98% humidity, with above 13°C and at least four hours of darkness).

Between growth stages 12 (shoots 10 cm) and 19 (beginning of flowering), several rain events with up to 800 daily minutes of infection time occurred (Fig. 3), which may explain that downy mildew was already present in the field during the first visit at the Caversham site. During the sampling period, rule 10-10-24 was once more fulfilled with the rainfall on 25/11/04. For the rest of the time, only rule 98-13-4 applied, if ever. It seemed that morning dew or high humidity (sea-breeze) was enough to start other infection cycles (rule 98-13-4), but not for oosporic infections which occurred with minor extent throughout the whole sampling period (rule 10-10-24, see next section).

Downy mildew in the Swan Valley was resistant to heat, a quality which had been observed on Greek islands (Gobbin, personal communication; Rumbou & Gessler, 2004). Despite a temperature of 42°C in the shade (in 2m height; Stanicich, personal communication) in December 2004, downy mildew oil-spots seemed to fall into a dormant stage, and recommenced to grow with a subsequent temperature and humidity change. Although the effective temperature in the canopy was probably lower (cooling effect of water transpiration), it would still have been probably too hot for Central European genotypes to start any infection at all during the sampling period in Caversham. In Switzerland, relative humidity for primary infections needs to be more than 92% at darkness (ca. seven hours for a summer night), for secondary

infections, the product of temperature and time of leaf wetness needs to reach 50 degree-hours (Siegfried et al., 1997).

Relative contribution of oosporic vs. secondary inoculum to the epidemic - Primary inoculum

Oosporic contribution towards the epidemic was 10% in the first sampling and varied between five and seven percent after day 14 (Fig. 9b). In Europe, relative contribution of cumulative primary inoculum is usually over 70% (Gobbin *et al.*, 2005).

The constant detection of four up to six primary lesions per sampling over the course of the sampling period (68 days) gives room to the interpretation that oospores are released longer than assumed in presently used forecasting models.

There are three explanations for the recurrent detection of new genotypes.

1. A pool of oospores in the soil

From this pool, oospores might have germinated throughout the season, whenever environmental conditions were suitable. However, weather conditions were too atypical for oosporic germination in the second part of the survey period (Fig. 3). Weather phenomena like morning dew or high relative humidity were at the most suitable for the dispersal and germination of sporangia, but never enough to moisture the soil and allow the germination of oospores. With the given parameters for the time of infection [t_inf: (14<T<22; rH>96% in 10 m height)], no infection conditions could be observed after 25/11/04 (Fig. 3). The vineyard was not irrigated until the end of the year 2004, the drip irrigation in January 2005 did not seem to affect the contribution of primary and secondary inoculum in the overall epidemic.

2. Immigration of new genotypes from neighbor vineyards

Immigration from neighbor vineyards may be excluded, as they were all regularly treated against downy mildew pathogens. If there had been a directional flow of new genotypes into the epidemiological site, they would have been detected more than once due to their clonal stage. Immigrated new genotypes would have probably shown a clustered distribution pattern, with an intensity gradient starting from a

vineyard corner. Such a distribution pattern, reported from European vineyards by Gobbin *et al.* (2005), was not found. In this regard, found primary infections were supposed to have their origin in oospores from the soil of the survey vineyard.

3. The methodology might be queried

Working with very modern machines in the agrf (Australian Genome Research Facility), this hypothesis could be rejected. PCR-tests with the complete primer set (ISA, GOB, CES/BER) and following microsatellite analysis with six times repeated isolates showed highly reproducible results (data not shown). One minor concern arose with some weakly visible band patterns in the negative controls on agarose gels – however, subsequent microsatellite analysis uncovered atypical and weak peaks with unusual base pair lengths. As a consequence, "fake genotypes" could be easily separated from real genotypes.

Primary infections were possible even with low humidity. It seems that the rule 10-10-24 is too strict in the case of putatively caused primary infections in the Swan Valley. The biology of the pathogen in Western Australia might differ from what has been found elsewhere (Smith, 2004).

Relative contribution of oosporic vs. secondary inoculum to the epidemic - Secondary inoculum

Appearance and contribution of genotypes

Environmental conditions for secondary multiplication were more often fulfilled than for primary infections. Therefore, 90% of all lesions at the first sampling were clones (Fig. 9b). The four most frequent genotypes ("dgulosse", "dgulesse", "dgilesse", "dgilosse" in descending order) were present at the first sampling (12/11/04; grapevine growth stage 21/30% caps off (bloom), Table 2). Moreover, they had already reached a clonal stage at that moment. Genotypes found in the early season obviously had more time to spread and reach a high frequency. The conclusion that dominant genotypes are always found at the beginning, and not in the middle or in

the end of the season, is consistent with similar findings in the literature (Gobbin *et al.,* 2005).

Not all genotypes found in the first sampling reached a dominant level in the course of the infection. Certain genotypes of the group OG (other genotypes) were found at all sampling stages, but with a continuous and not increasing number of lesions. For instance, the occurence of the OG "dgugusse" in the cavall data set was the following: 1-1-2-1-2-3 (number of lesions in the order: sampling 1-6). Possible reasons for the low reproduction of certain genotypes may be low fitness, low reproduction advantage or an adverse microclimate. On the other hand, the most frequent genotypes probably had a general high fitness and reproduction advantage, took advantage of a good microclimate within the canopy, germinated quicker and spread more successfully. Studies in microclimate influence on oosporic/sporangial evolvement were not carried out.

Spatial dispersal of secondary inoculum

Both random and clustered distribution could be observed using data set cavmax3. The TMFG was the only genotype which showed an aggregated distribution in the research plot (Fig. 6c). All other most frequent genotypes, namely MFG, SMFG, FMFG (Fig. 6b, Appendix 3), were randomly distributed from the beginning, making it impossible to locate the place of the initial infection. Spatio-temporal dispersal diagrams of all genotypes (total disease, Fig. 6a) may be interpreted as an intermixed case: Random distribution at the beginning (sampling 1), with clusters around lesions found in the subsequent samplings 2-6. The random distribution of the total disease can be interpreted as a direct consequence of many secondary infection cycles.

As for the dispersal rate, no real assumption can be made, too advanced was the disease at the moment of the first sampling (12/11/04). The random distribution of a majority of the most frequent genotypes and the explosive appearance of downy mildew overnight (06/12/04) in all neighbouring vineyards indicate that no major border effects were present (at least in the direction "untreated research plot → adjacent (treated) vineyards"). The most plausible explanation for the random

distribution seems to be coherent with climatic conditions in the Swan Valley: due to the diurnal temperature difference between land and sea, it was usually very windy.

In Europe, the majority of the highly clonal downy mildew populations analysed showed a clustered distribution pattern, e.g. Bommes (France). Vineyards with a random distribution pattern of clonal genotypes like the one at the Caversham site were seldom found, e.g. in Geisenheim (Germany; Gobbin *et al.*, 2005).

Sampling size issues

Comparing the different sampling intensities (four data sets cavall, cavmax3, cavmax10, cavmdv), slight differences could be observed (Table 5a,b; Table 6; Appendix 4). The contribution of single genotypes (SGs) was higher in the data set cavmdv than in the other three data sets (4.1% compared to 2.4/2.5%). The intensive sampling intensity may explain this observation. With a higher sampling intensity in the whole vineyard (more than three lesions per sampling and vine), more single genotypes would have been found per sampling volume. In the different data sets, second, third and fourth most frequent genotypes (SMFG, TMFG, FMFG) changed their position in the descending contribution order in percentages (Table 5a,b; Table 6; Appendix 4). However, the overall contribution of the four most frequent genotypes (MFG, SMFG, TMFG, FMFG) remained at a high relative contribution level between 82.9% (cavmax3) and 87.7% (cavmdv). It can be concluded than different sampling intensities had little influence on the relative contribution of genotypes at the Caversham site, because the genotypes were spread everywhere and showed the same distribution at vine and vineyard level.

Conclusions

The downy mildew epidemic in the Caversham vineyard happened with few genotypes, starting putatively with a few simultaneously caused oosporic infections, followed by massive waves of asexual reproduction in the course of the epidemic. This observation, similar to the one in a Greek vineyard reported Rumbou & Gessler (2004), coincides with the general view of the present forecasting models. In the

Western Australian case, spring plant protection measures seem to be crucial to suppress few dominant genotypes before their explosive dispersal.

The second salient observation is the continuous detection of putative new oosporic infections throughout the sampling period (68 days). Clonal lineages found in sampling one (12/11/04) were the consequence of putatively caused oosporic infections occurring before the survey period. Adding up the (unknown) time between the moment of these first infections ("@" in Fig. 9a,b.) and the first sampling with the 68 days of the overall sampling period, a total time slot of up to three months for possible oosporic germination could be made out. Putatively caused oosporic infections at the last sampling (19/01/05, stage 35-37/veraison) are another clear indication that overwintering oospores may germinate much longer during the season and are not exhausted after grapevine bloom - an assumption commonly used in present forecasting models. A longer time frame for oosporic germination was already observed and reported by Eugster (2003) and Gobbin *et al.* (2005). In addition to spring plant protection, late season disease management programs aiming to avoid the formation of new recombined sexual propagules (oospores) should be equally taken into account, especially in "downy years" like 2004/05. In the case of the Swan Valley vineyards, plant protection after grape harvest may be especially important after first, heavy autumn rainfalls (70 mm within two days in early April 2005), because dormant downy lesions may re-flourish. If "downy years" repeat more in Caversham, late season oospore may become an important key factor in resistance management. More studies in other vineyards with different climatic conditions and plant protection history need to be done to further improve disease forecasting models for downy mildew disease management.

Population Genetics

Allelic and genetic diversity of Australian populations

Western Australia was with 929 isolates the most intensively analysed state in this diploma thesis. Allelic and genotypic diversity were very low in Western Australia, in comparison to the ones in Europe, America and on the east coast of Australia. Direct analysis of clone corrected populations showed non-significant deviations from

Hardy-Weinberg equilibrium for the Western Australian populations, indicating that genetic recombination occurs in populations.

32 genotypes and 20 alleles found in the Caversham population (data set cavall, 709 isolates; Table 7) may confirm the theory of fixed alleles and possible inbreeding. However, the Caversham population is only homozygote for the locus CES (one found allele). For ISA and BER, a certain allelic combination seems to be preferred: ISA shows 100% heterozygosity, BER 705 heterozygote in total 709 isolates (99%). This finding is even more surprising taking into account that only two alleles exist for both loci, ISA and BER. The large deviation from the Hardy-Weinberg equilibrium may be interpreted as a selection of clones with an evolutionary advantage, provided that the alleles are linked to genes that bring advantages. In Pemberton, at about 350 kilometres south-east of Perth, all loci were in the Hardy-Weinberg equilibrium (clone-corrected data set pemcc, Table 7). Pemberton lies in a cooler, higher and more humid region than Caversham and Henley Brook. Eventually the selection pressure was smaller in the south.

Pairwise comparison of all Australian populations showed considerably high values between east and west coast (Fst 0.2-0.22, Rst 0.12-0.24 for clone corrected populations, Table 9). Fst and Rst values found in Europe are usually much lower, indicating a higher degree of relationship amongst European populations (Gobbin, personal communication).

Possible explanations for the aforementioned findings may be:

1. Geographical distance between east and west

Caversham, which is part of the Perth metropolitan area, belongs to the most isolated state capital in the world. Between east and west, thousands of kilometres of desert work as a natural barrier. North, west and south are protected by the sea. Genetic exchange, unlike in Europe, is absolutely not possible under natural conditions (excluding human activities). Downy mildew populations are isolated from each other and develop independently.

2. Different introduction time of P. viticola into the states on the east and west coast.

P. viticola first appeared in Rutherglen, Victoria, in 1917, coming in with vine planting material being brought in from Europe (de Castella F & Brittlebank CC, 1917). It was found 'soon after' in the New South Wales (Hunter Valley), South Australia and Queensland (Laffer HE, 1918). Downy mildew had a much shorter evolution time in Western Australia, where it firstly was confirmed in 1998 (McKirdy *et al.*, 1999). In Western Australia, the pathogen had to go through a classic bottleneck to overcome the immense natural barrier. Population differentiation in the west was possibly connected with a founder effect.

3. Unfavourable climate in Western Australia

The Western Australian climate is adverse to the development of the pathogen. The fungus is forced to go through regular cycles of extinction and recolonization: After conducive weather conditions and an epidemical spread in the season 2001/02, two hot and dry seasons followed without any significant appearance of the pathogen in the same area, before it returned again in this year's season 2004/05 (Swan Valley table grape growers, personal communication). Even with temperatures of up to 42°C (in the shade, at 2m height), the oil-spots fell into a dormant state, but did not die. At the edge of the lesions, growing continued with a subsequent temperature and humidity change in December 2004. It seemed that during the two hot and dry years, especially heat overwintering oospores had been selected.

A correlation between (annual) rainfall and genetic diversity could not be found. Despite receiving 180 mm more annual rainfall than Yarra Glen (Vic), Pemberton in Western Australia shows much less genetic diversity (Table 1, Table 8).

South Australia

The last devastative downy mildew epidemic dates back to the season 1992/93 (Magarey, personal communication). Despite several conducive infection conditions in this year's season (2004/05), downy did not appear on a large scale in the state.

Probably the oosporic pool was exhausted by ten years of unsuited conditions. If colder and more humid weather conditions will repeat next season, it is possible that downy mildew returns to South Australian vineyards with a more destructive impact.

Relative diversity of Australian populations compared to European and American populations

The unrooted Neighbour-joining tree depicts all Australian populations in the same group (Fig. 10). Hence the five analysed Australian populations seem to be somehow related to each other. Within this group though, distances between populations are far bigger than those between European populations of other groups, e.g. of the Italian group. These distances are consistent with the hypothesis of isolated populations without exchange of genes (low gene and genotypic flow) mentioned above. Another explanation might be the human-mediated import of the pathogen from different grapevine growing areas.

In his population genetics studies, Eugster (2003) found a large genetic distance between the American (Geneva, NY) and any European population. Furthermore, he could show a direct connection between one French (Blanquefort) and the American (Geneva, NY) population, which reinforces the assumption that downy mildew was introduced into France in 1878.

Taking into account the conjectured genetic origin of the downy mildew pathogen, which lays in North America, it might be that infected vine stocks had been directly exported from the US (gen, Geneva, NY) to NSW (Hunter Valley), whilst downy mildew pathogens from the Victoria (Yarra Valley) were brought in from Europe with vine planting material (de Castella F & Brittlebank CC, 1917). This assumption is somehow confirmed in the unrooted Neighbour-joining tree, where the yar (Yarra Glen) population lays closer to bla (Blanquefort, France) than to gen (Geneva, NY, USA).

No information about the import of vine planting material from the US into New South Wales could be found in literature. It cannot be excluded that downy mildew in New South Wales (detected probably in 1918, at the latest in 1920; Laffer HE, 1918) had European origins as well.

Conclusions

Allelic and genetic diversity was much lower in the west coast populations than in the east coast populations. Populations from Victoria and New South Wales were similar to the ones for European and American populations.

Genetic distances within Australian populations are large, especially between east and west coast.

There are three possible explanations for the population differentiation in Western Australia, Victoria and New South Wales. Firstly, the populations seem to have had low genetic exchange between each other, thanks to natural barriers and interstate quarantine measures. Secondly, *P. viticola* was introduced at different times into the states on the east and west coast (Victora: 1917; New South Wales: 1918-1920; Western Australia: 1998). Thirdly, the Western Australian climate is adverse to the development of the pathogen. The fungus is forced to go through regular cycles of extinction and recolonization.

Outlook

For more precise phylogenetic info, a rooted neighbour-joining tree with its origin in Geneva (NY, USA) could be contemplated. The microsatellite method could be replaced by genetic sequencing with other molecular markers. The latter would exclude the bi-directional mutation possibility of alleles (microsatellite method). However, still much needs to be done to develop an appropriate method, not to mention the costs for such a project.

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Yarra Glen: Yering Station (Nathan Scarlett, Savaad Felich)

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Appendix 1 – Caversham genotypes listed after frequency and sampling

Caversham

Genotype/Sampling	1	2	3	4	5	6	7	Total
dbegusse							1	1
dbemasse						1		1
dfulisse					1			1
dgamasse				1				1
dgigissa						1		1
dgigisso						1		1
dgimisse					1			1
dgolisse	1							1
dgolosse					1			1
dgulesso						1		1
dgulossa			1					1
dlalosse			1					1
dlalusse						1		1
dlelesse			1					1
dlilisse			1					1
dlimesse	1							1
dlolosse	1							1
dgalesse					1	1		2
dnèlesse			2					2
dgigisse				1		2		3
dgagusse	1			4				5
dgalosse				2	2	2	1	7
dgumisse	2			1		1	3	7
dgigusse	3			2	1	2		8
dlelosse	1	2		2		3		8
dgugusse	1	1	2	1	2	3		10
dgimasse				9	1	7	3	20
dgumasse	2	2	2	7	4	4	1	22
dgilosse	16	5	2	21	7	12	6	69
dgilesse	18	9	7	22	14	20	5	95
dgulesse	36	21	9	24	9	14	6	119
dgulosse	44	28	28	77	34	57	47	315
Total	127	68	56	174	78	133	73	709
Genotypes	13	7	11	14	13	18	9	
Sampling Date	12.11.2004	26.11.2004	03.12.2004	08.12.2004	25.12.2004	19.01.2005	19.01.2005	
Days from S1	0	14	21	26	43	68	68	

Sampling 1 – 6: whole Caversham vineyard

Sampling 7: last intensive sampling on the most diseased vine (MDV)

Appendix 2 – Contribution of genotypes towards the epidemic

Data set: Sampling Area 1 (max. three lesions/sampling & vine)

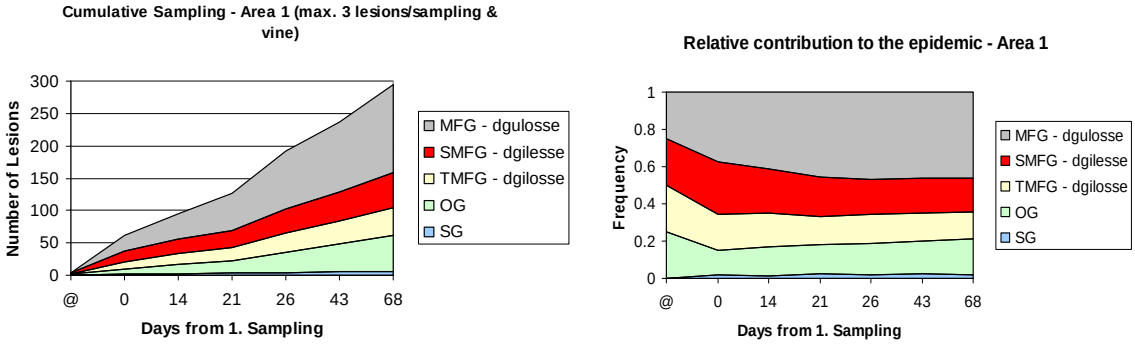


Fig. Aa,b: Cumulative number of lesions and percentage of number of lesions of the downy mildew infection in Caversham, caused by the most frequent genotype (MFG), the second (SMFG), third (TMFG) frequent genotype, other genotypes (OG*) and single genotypes (SG). Moderate sampling intensity for vineyard area 1 (max. three lesions/sampling and vine).

*OG: Genotypes which occurred between two and twenty times.

Data set: Sampling Area 2 (max. ten lesions/sampling & vine)

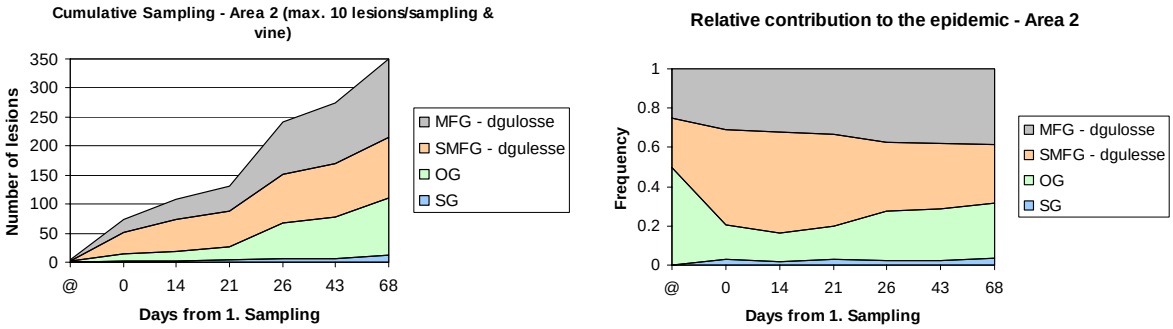
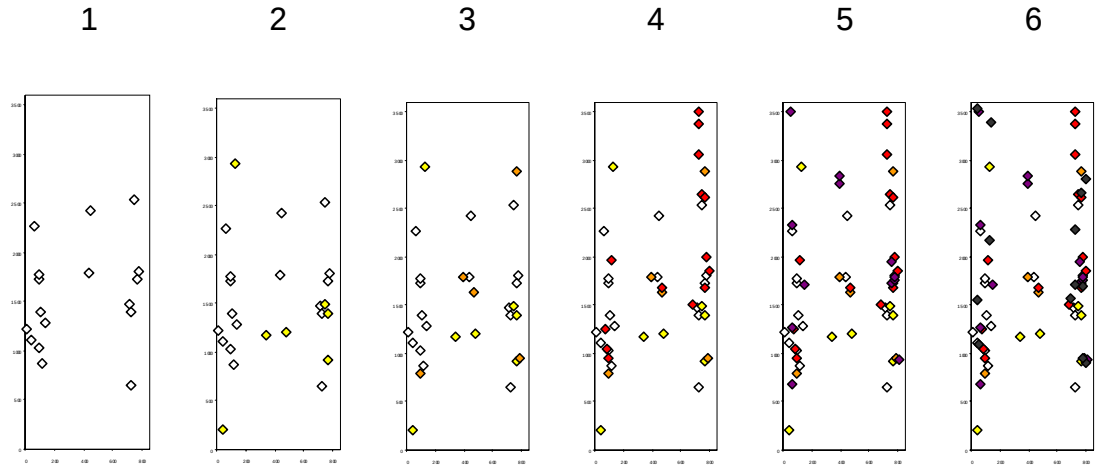


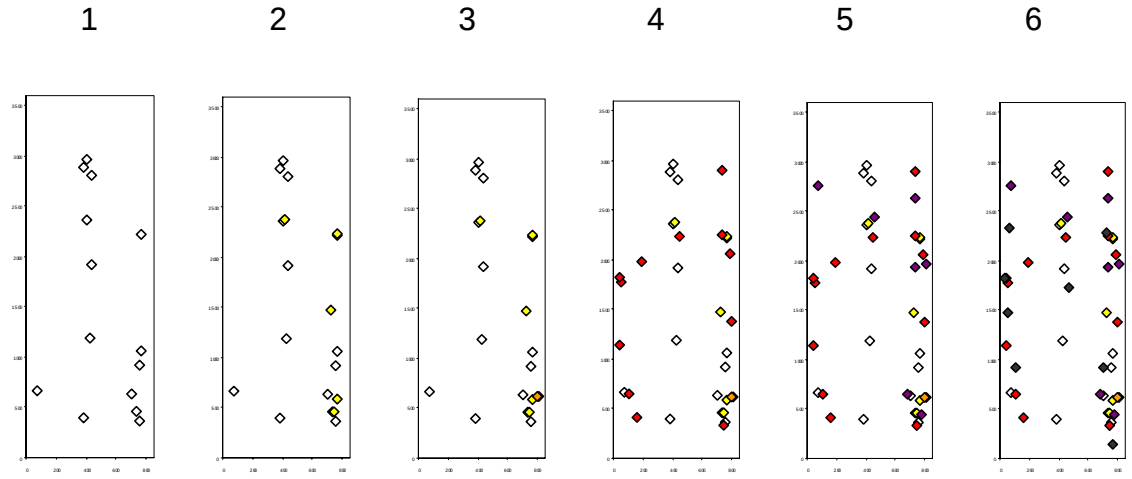
Fig. Ac,d: Cumulative number of lesions and percentage of number of lesions of the downy mildew infection in Caversham, caused by the most frequent genotype (MFG), the second (SMFG) frequent genotype, other genotypes (OG*) and single genotypes (SG). High sampling intensity for the vineyard area 2 (max. ten lesions/sampling and vine).

*OG: Genotypes which occurred between two and fourty times.

Appendix 3 – Spatial distribution of genotypes in the vineyard



second most frequent genotype (SMFG)



fourth most frequent genotype (FMFG)

Fig. Aa,b: Spatio-temporal dispersal of the second most frequent genotype (SMFG, Fig. Aa) and the fourth most frequent genotype (FMFG, Fig. Ab) in the Caversham vineyard. The vineyard is represented by a scaled rectangle. Top numbers represent the sampling dates. Lesions found at different sampling dates are shown in different colours, changing in the following order: white, yellow, orange, red, purple, and black. Sampling dates: 1=12/11/04, 2=26/11/04, 3=03/12/04, 4=08/12/04, 5=25/12/04, 6=19/01/05. Moderate sampling intensity for the whole vineyard (max. three lesions/sampling and vine). Data set used: cavmax3.

Appendix 4 - Genotype specific contribution to the epidemic – all non-clone corrected Caversham data sets at a glance

Table Ba,b,c,d: Genotype Group, number of times a genotype was collected (occurrence class, tN_{obs}), number of genotypes (N_{gen}), number of lesions (NL) and contribution to total sample size (%tNL) per genotype occurrence class with the following data sets used: cavall, cavmdv, cavmax10, cavmax3.

* Groups: SG = Single Genotype, found only once; OG = Other Genotypes, found between two and 25 times; FMFG = Fourth Most Frequent Genotype; TMFG = Third Most Frequent Genotype; SMFG = Second Most Frequent Genotype; MFG = Most Frequent Genotype.

Data set: cavall

Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	17	17	**2.4**
OG	2	2	4	
OG	3	1	3	
OG	5	1	5	
OG	7	2	14	
OG	8	2	16	
OG	10	1	10	
OG	20	1	20	
OG	22	1	22	**13.2**
FMFG - dgilosse	69	1	69	**9.7**
TMFG - dgilesse	95	1	95	**13.4**
SMFG - dgulesse	119	1	119	**16.8**
MFG - dgulosse	315	1	315	**44.4**
Total		32	709	100

Data set: cavmdv

Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	3	3	**4.1**
OG	3	2	6	
				8.2
FMFG - dgilesse	5	1	5	**3.8**
SMFG - dgilosse	6	1	6	**8.2**
SMFG - dgulesse	6	1	6	**8.2**
MFG - dgulosse	47	1	47	**64.4**
		9	73	100

Data set: cavmax10

Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	16	16	**2.5**
OG	2	2	4	
OG	3	1	3	
OG	4	1	4	
OG	5	1	5	
OG	6	1	6	
OG	8	2	16	
OG	10	1	10	
OG	17	1	17	
OG	21	1	21	**13.5**
FMFG - dgilosse	63	1	63	**9.9**
TMFG - dgilesse	90	1	90	**14.0**
SMFG - dgulesse	113	1	113	**17.8**
MFG - dgulosse	268	1	268	**42.1**
		31	636	100

Data set: cavmax3

Group*	tN_{obs}	N_{gen}	NL	% tNL
SG	1	11	11	**2.4**
OG	2	2	4	
OG	3	3	9	
OG	5	2	10	
OG	7	2	14	
OG	12	1	12	
OG	17	1	17	
				14.7
FMFG - dgilosse	48	1	48	**10.7**
TMFG - dgulesse	61	1	61	**13.6**
SMFG - dgilesse	68	1	68	**15.1**
MFG - dgulosse	196	1	196	**43.6**
		26	450	100

Appendix 5 – allele frequency of the remaining Australian populations (clone corrected)

Henley Brook, Swan Valley, WA

ISA	2*		GOB	8		CES	1		BER	2	
Repeat nr.	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**
7	0.5	110.6	116	0.04	285.3	33	1.00	137.4	11	0.5	175.3
16	0.5	129.7	118	0.21	289.3				12	0.5	177.3
			120	0.13	293.3						
			122	0.13	297.5						
			123	0.04	299.3						
			124	0.29	301.4						
			125	0.04	302.5						
			126	0.13	305.5						

Table Za: Repeat number, allele frequency (AF) and length in bp of the found alleles (clone corrected) in the Henley Brook population for the four SSR markers ISA, GOB, CES & BER.
*: number of found alleles

Pemberton, WA

ISA	3		GOB	7		CES	1		BER	2	
Repeat nr.	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**
7	0.47	110.6	75	0.03	202.5	33	1.00	137.4	11	0.33	175.3
8	0.03	112.6	115	0.03	283.1				12	0.67	177.3
16	0.50	129.7	120	0.03	293.3						
			122	0.40	297.5						
			124	0.43	301.4						
			125	0.03	302.5						
			126	0.03	305.5						

Table Zb: Repeat number, allele frequency (AF) and length in bp of the found alleles (clone corrected) in the Pemberton population for the four SSR markers ISA, GOB, CES & BER.
*: number of found alleles

Lenswood, SA

ISA	4		GOB	1		CES	1		BER	2	
Repeat nr.	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**
7	0.17	110.6	75	1.00	202.5	33	1.00	137.4	11	0.50	175.3
16	0.50	129.7							12	0.50	177.3
17	0.17	131.6									
18	0.17	132.9									

Table Zc: Repeat number, allele frequency (AF) and length in bp of the found alleles (clone corrected) in the Lenswood population for the four SSR markers ISA, GOB, CES & BER.
*: number of found alleles

Lovedale/Pokolbin, Hunter Valley, NSW

ISA	4		GOB	20		CES	5		BER	2	
Repeat nr.	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**	**Repeat nr.**	**AF**	**Length (bp)**
7	0.2	110.6	75	0.38	202.5	33	0.41	137.4	11	0.08	175.3
16	0.3	129.7	95	0.01	243.3	48	0.03	166.6	12	0.92	177.3
18	0.2	132.9	97	0.02	247.0	49	0.42	168.6			
20	0.2	135.8	103	0.01	259.3	50	0.12	170.5			
			104	0.01	261.1	51	0.02	172.5			
			105	0.01	263.0						
			107	0.02	267.0						
			108	0.02	268.8						
			109	0.01	270.8						
			114	0.01	281.0						
			115	0.01	283.1						
			118	0.02	289.3						
			120	0.07	293.3						
			122	0.12	297.5						
			124	0.06	301.4						
			126	0.01	305.5						
			151	0.01	353.9						
			155	0.02	361.0						
			161	0.08	373.4						
			163	0.07	377.5						

Table Zd: Repeat number, allele frequency (AF) and length in bp of the found alleles (clone corrected) in the Hunter Valley population for the four SSR markers ISA, GOB, CES & BER.
*: number of found alleles

Yarra Glen, Yarra Valley, Vic

ISA	5*		GOB	30		CES	6		BER	2	
Repeat nr.	AF	Length (bp)	Repeat nr.	AF	Length (bp)	Repeat nr.	AF	Length (bp)	Repeat nr.	AF	Length (bp)
7	0.03	110.6	75	0.03	202.5	33	0.42	137.4	11	0.13	175.3
16	0.25	129.7	104	0.01	261.1	48	0.09	166.6	12	0.87	177.3
17	0.01	131.6	105	0.01	263.0	49	0.22	168.6			
18	0.34	132.9	106	0.03	265.0	50	0.22	170.5			
20	0.38	135.8	107	0.03	267.0	51	0.06	172.5			
			108	0.01	268.8	52	0.01	174.4			
			109	0.04	270.8						
			110	0.01	272.9						
			111	0.03	275.1						
			113	0.04	278.9						
			114	0.10	281.0						
			115	0.06	283.1						
			116	0.10	285.3						
			117	0.05	287.1						
			118	0.08	289.3						
			119	0.03	291.3						
			120	0.06	293.3						
			121	0.01	295.2						
			122	0.09	297.5						
			123	0.01	299.3						
			124	0.08	301.4						
			126	0.01	305.5						
			128	0.01	309.6						
			129	0.03	310.4						
			135	0.01	322.3						
			137	0.01	326.2						
			155	0.01	361						
			157	0.03	365.2						
			158	0.01	367.3						
			159	0.01	369						

Table ZY: Repeat number, allele frequency (AF) and length in bp of the found alleles (clone corrected) in the Yarra Glen population for the four SSR markers ISA, GOB, CES & BER.
*: number of found alleles

Appendix 6 – The history of Yering Station Vineyards

The sampling site was in vineyards of the Yering Station, the oldest winery of Victoria. The first vines were planted in 1838, the first wine in Victoria was made at Yering in 1845. After purchasing the Yering Station in 1850, Swiss Paul de Castella established a sizeable vineyard, importing large quantities of cuttings from France. By 1860, his brother-in-law, Captain William Anderson, travelled to France to select cuttings from vineyards at Medoc, Burgundy and Cognac. No more information is available about further imports of cuttings from Europe after 1878, the year downy mildew supposedly was introduced to France and then spread over all Europe. Paul de Castella triumphed at the Paris Exhibition of 1889, when he was awarded a "Grand Prix". It is possible he purchased more cuttings from French vineyards in that year. By 1891, Yering was well down the list of Victoria's biggest vineyards. No information was found where other wineries purchased their cuttings. Phylloxera epidemics tore through the Victorian state in the late nineteenth and early twentieth century, which led to a rapid disappearance of viticulture in the Yarra Valley. Low yields and a decline in the fertility of the vines were other reasons why wineries got replaced by more prospective dairy farms. (Source: Sune N., "The history of Yering Station Vineyards")

Appendix 7 – Caversham vineyard

Fig. Ca,b: Sampling material (above left), remainder of sampled lesion (above right).

Fig. Cc: Caversham vineyard (rows 2&3, looking to direction east).

All pictures taken by Felix Hug.

Appendix 8 – Caversham A.Q.M.S.

Fig. Cd: Looking to direction Southeast. Whole site.

Fig. Ce: SSW. Main shed with 10 m mast behind (above left)

Fig. Cf: NW. Raingauge, 2m temp, Net Rad (above right)

All pictures taken by Peter Mountford

By courtesy of the Air Quality Management Branch, Department of Environment, WA