Monitoring of epidemics: disease, pathogen, insect, host

Simone Orlandini

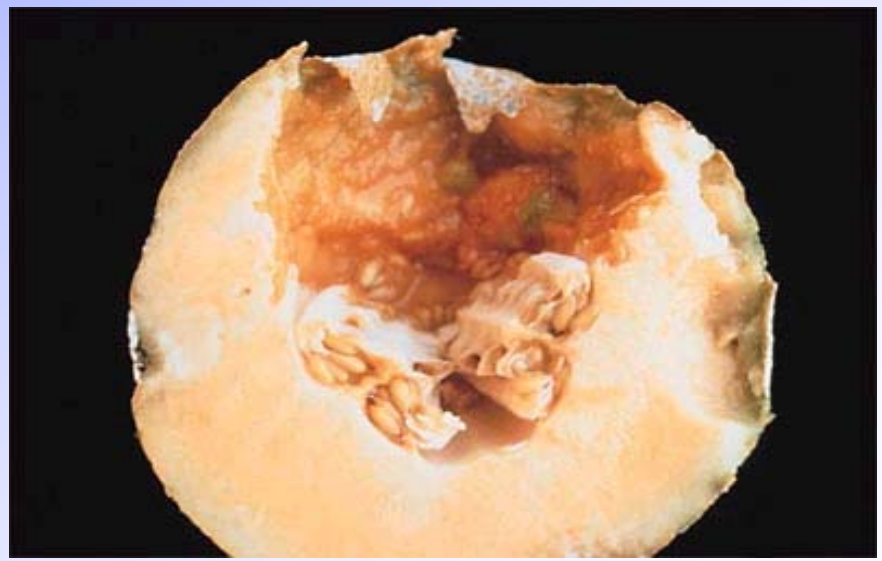
Department of Plant, Soil and Environmental Science - University of Florence

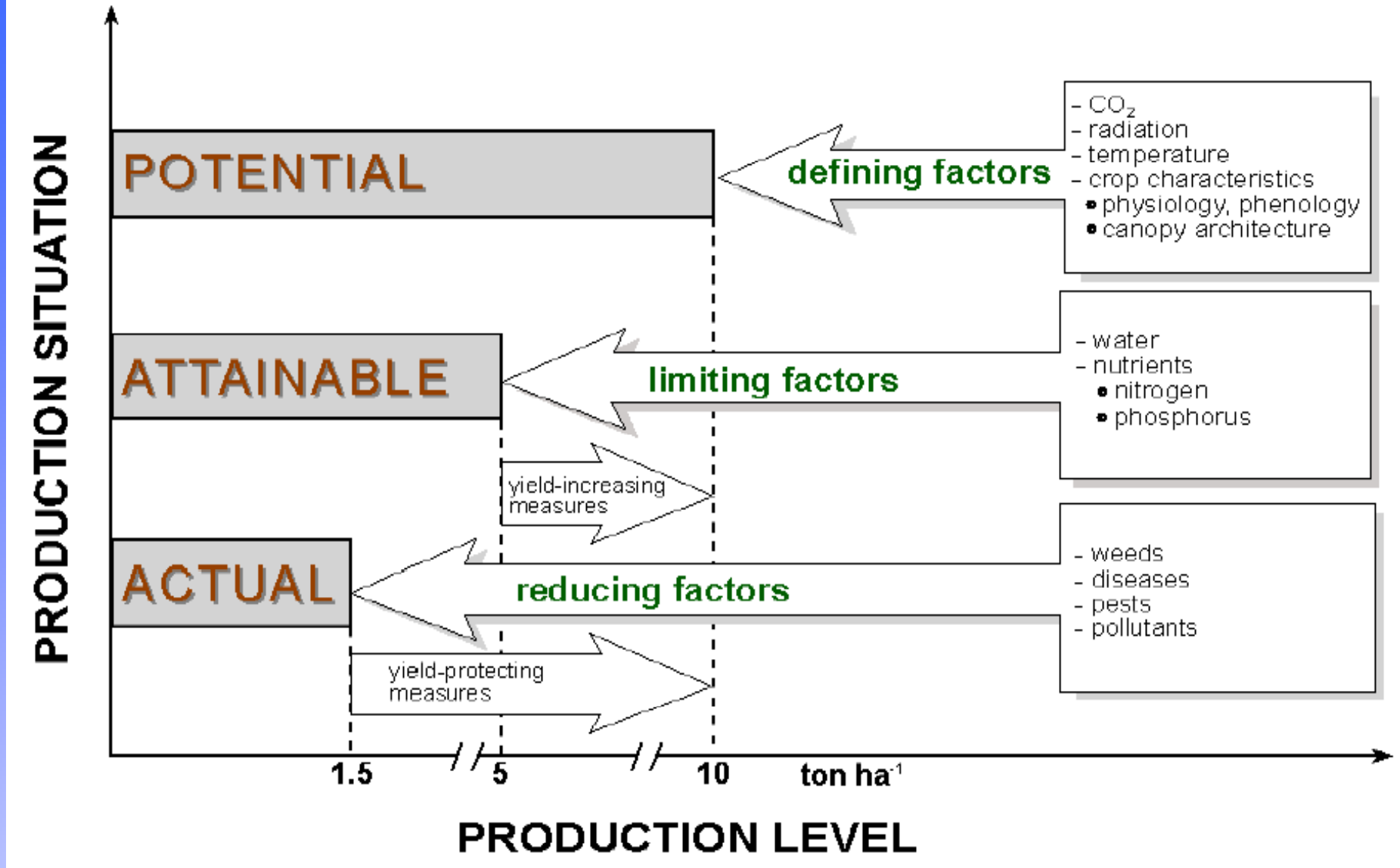
Structure of the lesson

- Plant disease measurements
- Pathogen measurements
- Insect measurements
- Host measurements
- Exercises

SIGNIFICANCE OF PLANT DISEASE

1. cause great economic losses to growers: increased price of product to consumers; some times cause direct and severe pathological affects on humans and animals that eat diseased plant products; destroy the environment by damaging plants and trees; trying to control the diseases, people release tons of toxic pesticides that pollute the water and the environment;
2. may limit the kinds of plant that can grow in a large geographic area; may also determine the kinds of agricultural industries and the level of employment in an area by affecting the amount and kind of yield available for local consumption or process;
3. reduce the quantity and quality of plant production;
4. may make plants dangerous to humans and animals (mycotoxins);
5. may cause financial losses;
6. the cost of controlling plant disease is also direct loss to disease.




PRODUCTION SITUATION
POTENTIAL
ATTAINABLE
ACTUAL
defining factors
limiting factors
reducing factors
yield-increasing measures
yield-protecting measures
- CO_2
- radiation
- temperature
- crop characteristics
• physiology, phenology
• canopy architecture
- water
- nutrients
• nitrogen
• phosphorus
- weeds
- diseases
- pests
- pollutants
1.5
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10
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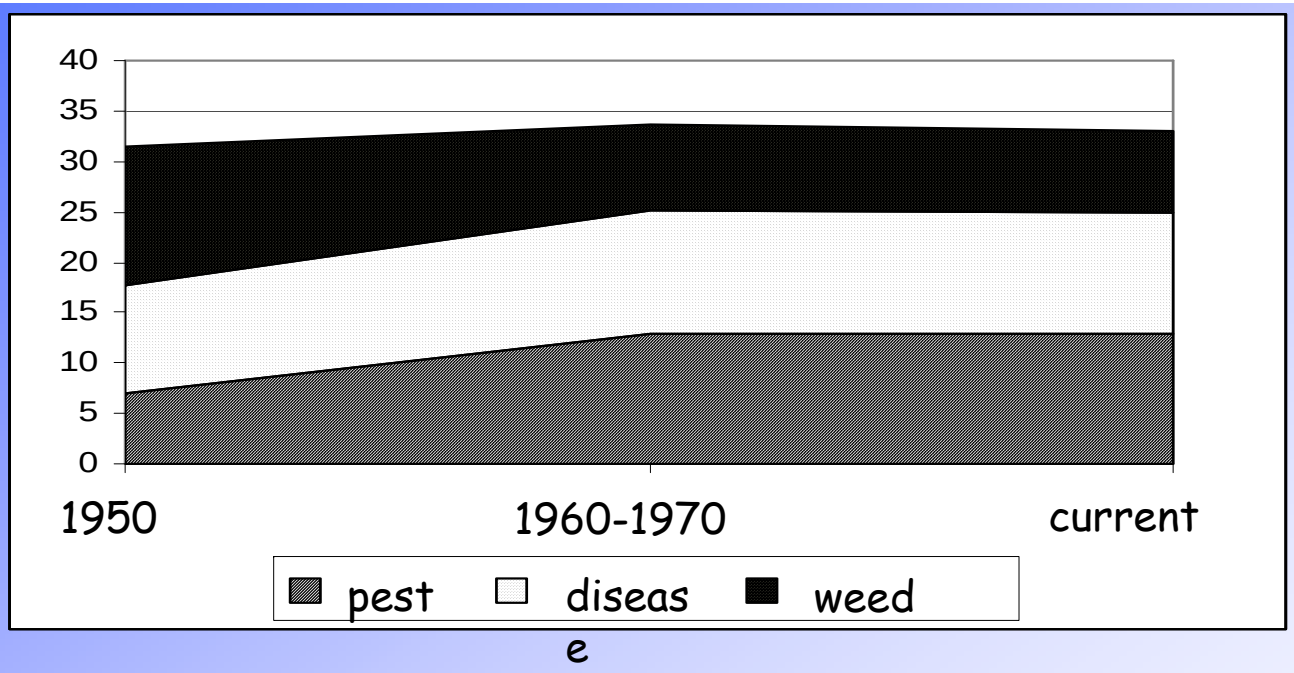
PRODUCTION LEVEL

Constant level of crop losses

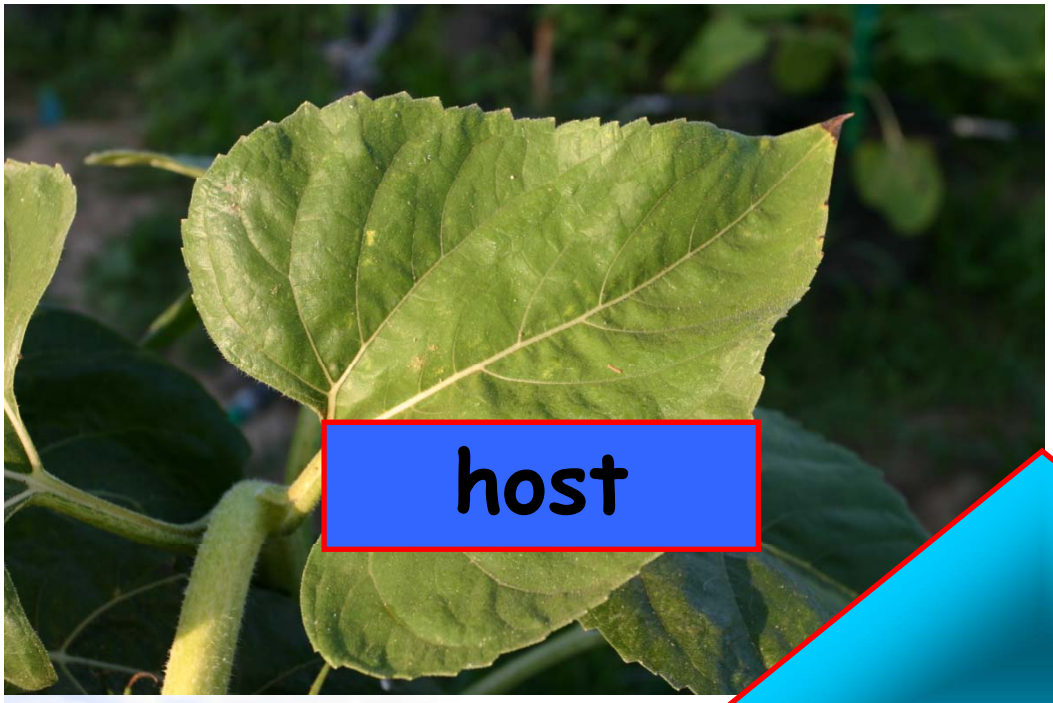


Most affected regions: tropics and developing countries

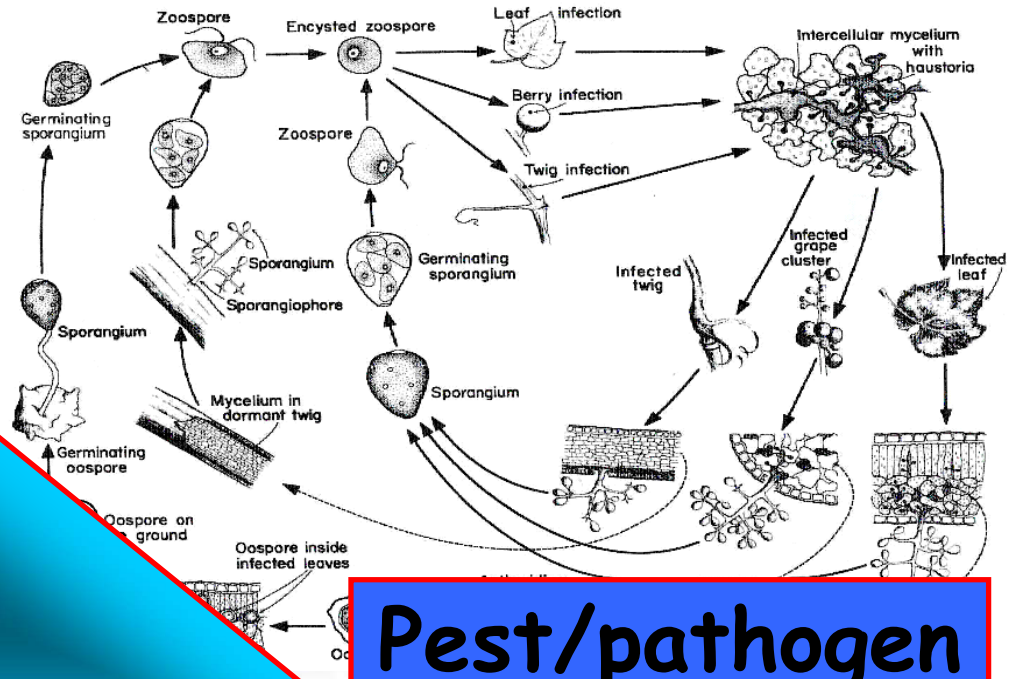
Causes:

- ✓ **Lack of technologies**
- ✓ **Crop successions**
- ✓ **High temperatures**
- ✓ **Possibility to have more than one cycle per year**

Area	Crop losses (%)
Europe	25
Oceania	28
North and central America	29
URSS e China	30
South America	33
Africa	42
Asia	43



host



Pest/pathogen

environment

T, RH, R, LWD, WS, WD

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Introduction

- The measurement of plant disease plays the same key role as diagnosis. It represents the basis for:
  - epidemiological studies,
  - assessment of crop losses,
  - plant disease survey,
  - development and application of models,
  - correct management of crop protection,
  - screening of resistance,
  - evaluation of protection methods and other experiment.

- Generally plant disease must be estimated by human activity, unless some equipment can ensure good measurements with different degree of precision and accuracy.

Introduction

- For field studies the assessment methods should be easy and quick in use for a wide range of conditions, but also adequately reliable and reproducible, accurate and precise. Generally this is not always possible.

- In plant disease measurements two different components have to be distinguished:
  - disease assessment (incidence and severity)
  - sampling techniques

Disease incidence

- It is the percentage of diseased plants or plant parts in the sample or population, irrespective of their severity.
- It is suitable for the early stages of the epidemic.
- It applies mainly to disease which affects entire plants (virus, wilts, smuts or spots on fruit) if one lesion makes the latter unfit for sale.
- It can be easily assessed by simple counting diseased plants vs healthy sampling unit. It is less affected by errors then disease severity.
- Sampling unit must be defined diseased only if the severity is beyond a certain level (physiological damage threshold).

Disease severity

- It is the the percentage of the relevant host tissues or organs covered by symptoms of the disease. This is the results of the combination from number and size of the lesions.
- It is appropriate for rust, downy and powdery mildew, leaf spots.
- It is essential to distinguish between symptom area directly caused by pathogen and the other indirect symptoms (chlorosis, necrosis, leaf shedding, etc.).
- Also the accelerated senescence must be taken into account.
- These differences between direct and indirect damages are important for epidemiological studies, but they are not important for crop loss assessment.

Other methods less used

- Disease indices (slight, moderate, severe) are often a nuisance because it is hard to understand what they mean in quantitative terms.
- Quantitatively defined grades (e.g. 1-9) are generally arbitrary and they are not applied.

Sources of error in disease assessment

- They are represented by the evaluator characteristics.
  - Perception is the human ability to distinguish by visual acuity the diseased proportion of plant surface.
  - The expectation based on experience or knowledge.
  - The personal optimism or pessimism.
- Characteristics of the sample:
  - Size, colour and shape of the lesions
  - light condition and leaf wetness
  - Three dimensional samples are particularly difficult to be estimate (the entire plant instead of the single leaf increase the overestimation)
  - The precision of disease assessment increased by choosing the smallest possible sampling unit
  - Error increases also in samples with more numerous lesions and with darker shades of colour

Sampling techniques

- Different methods (random, systematic, stratified, multiple-stage, etc.) can be applied to choose the sampled plants or organs.
- They can be affected by the spatial distribution of disease in the crop (random, clustered, regular or systematic) according to the soil, wind or vector-borne nature of the disease.
- Pilot studies can be performed to identify the spatial distribution characteristics of disease and their variability during the season.

Sampling units

- They can be defined in compliance with the objective and in some cases with sampling dates.
- They can be represented by single organs, by the entire plant, by a number of plants.
- Sometime also destructive measurements can be performed, to assess the weight of diseased plants or organs.

Sample size

- The adequate sample size ensures the desirable level of accuracy and precision.
- Also available funds, personnel and technology have to be considered, as well as the characteristics of disease and the period of the season.
- Specific methods have been proposed to allow a precise evaluation of sample size, both for disease incidence or severity evaluations.
- Generally the detection of very low disease incidence requires bigger sample size.

Frequency of sampling

- It depends on the course and time span of the disease progress curve.
- As general rule five points can be considered enough to establish a reliable curve at least.
- Sampling may be done at equidistant time intervals or at defined growth stages of the host.
- Yield loss assessment methods may require only one assessment in a particular growth stage of the host (generally when ripening is ended).

Techniques used in measuring plant disease

- In epidemiology disease intensity expresses the size of the population of lesions.
- The rate of their increase describes the dynamics of an epidemic as a reflection of past events.
- The age composition of lesions (i.e. infectious and no infectious lesions) is important to assess the possible future dynamics of an epidemic.

- Usually D.I. is computed as arithmetic means with 0 severity of healthy sampled included.

Actual measurements of disease intensity

- Counting of lesions and/or measurements of their size is practical only in research projects.
- If lesion size is regular, lesions per organs can be counted, their diameter measured and the total surface of lesions calculated. Then lesion surface is related to the total surface and expressed in percentage terms.
- If lesions differ in size, they can be assigned to different classes and computed accordingly. Field record forms can be provided with columns for these classes of size.

Use of keys, standard diagrams and classes of D.I.

- They shows typical disease symptoms for a range of severities, often up to 50% of total area, and they may also show the proportional distribution of the leaf area.
- Standard diagrams are a very useful tool for the calibration of the estimators and for training.
- A realistic range of severity values should be adopted.
- Coloured standard diagrams specific for a given disease would be useful particularly if the age of lesion indicates their sporulation intensity.
- Standard diagrams may be used for scaling disease intensity. Their values may be chosen as lower and upper limits of classes.

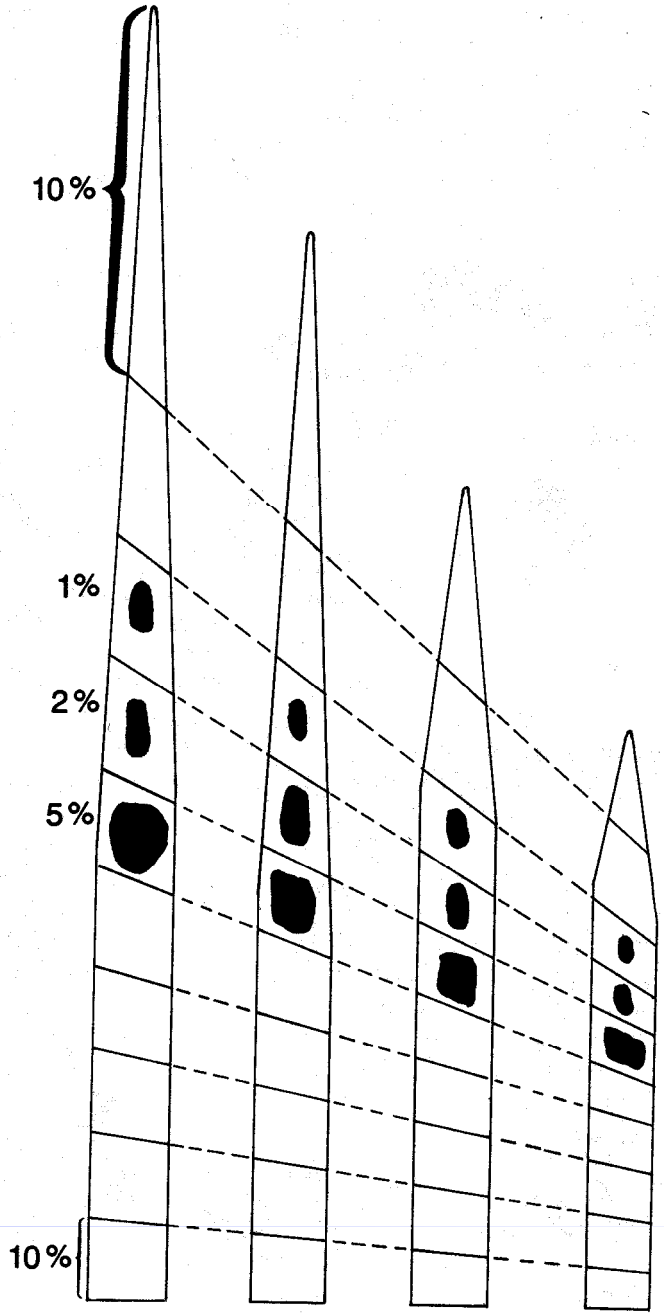


Figure 1. Example of a standard area diagram, with four leaf sizes and equivalent diseased areas (after Ref. 23).

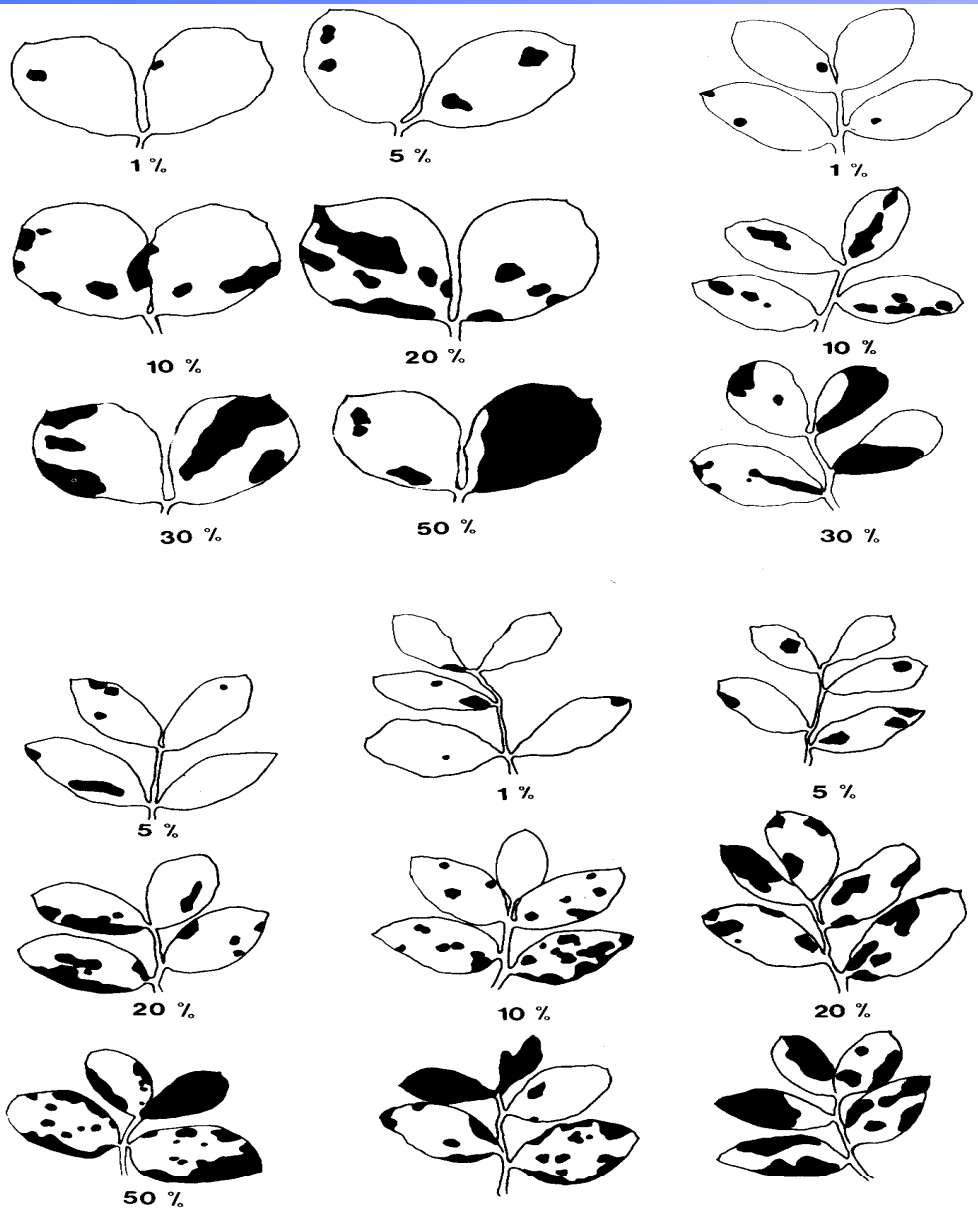


Figure 2. Example of a standard area diagram for compound leaves.

Number of classes

- Generally the number of classes should be higher at the lower levels of disease.
- For very discriminative work, a maximum of seven classes in addition to zero class seems to be optimum.
- Studies on inexperienced estimators indicate that their ability to assign the correct values is not great:
  - the 15.7% to the proper class
  - the 23.2% to the lower class
  - the 61.1% to the higher class

Field keys

- They can be used to improve field activity and they have to be particularly clear and simple.

Table 3.1 Field key for potato late blight *(Phytophthora infestans)*[a]

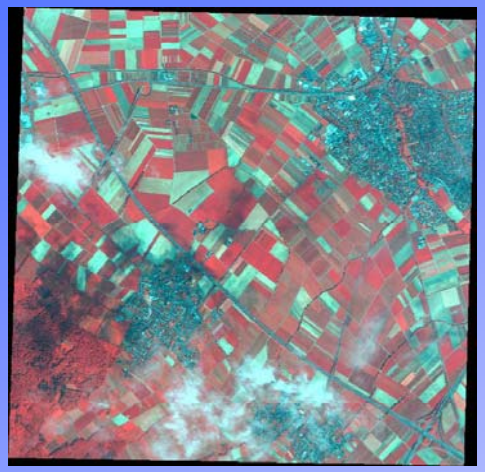
Percentage	description
0	No disease observed.
0.1	Only a few scattered plants affected, not more than 1 or 2 spots in 12-yd. radius.
1	Up to 10 spots per plant, or general, slight spotting.
5	About 50 spots per plant; up to 1 in 10 leaflets in 10 infected.
25	Nearly every leaflet with lesions, plants still retaining normal form; plants may smell of blight, field looks green although every plant is affected.
50	Every plant affected and about 50% of leaf area destroyed; field appears green-flecked with brown.
75	About 75% of leaf area destroyed by blight; field looks neither predominantly brown nor green.
95	Only a few leaves left green, but stems are green.
100	All leaves dead, stem dead or dying.

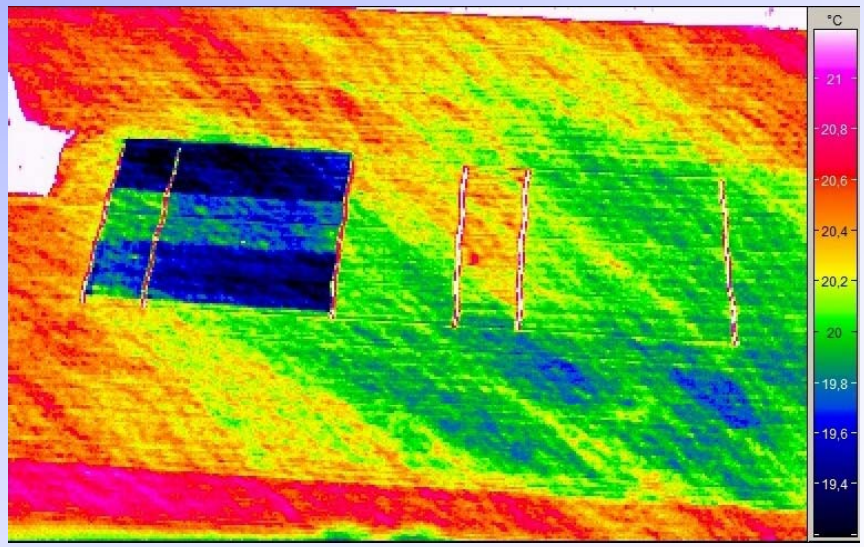
[a] Anon Trans Brit Mycological Soc 1947

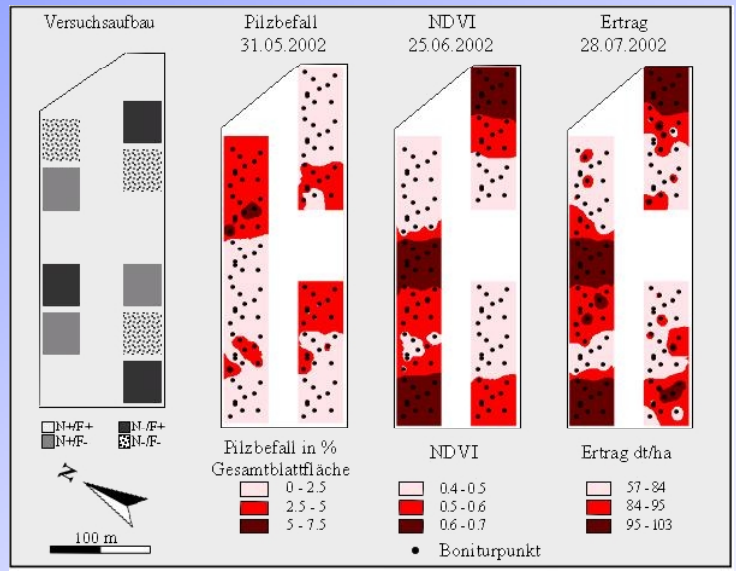
Automatic measurement and remote sensing

- Automatic measurements can be done using image analysis (700-950 nm), spectral radiometer or microdensiometer.
- Destructive and non destructive measurements can be done.
- On large areas, remote techniques can be applied by aircraft or satellite.
- The resolution of these techniques is higher at the medium or higher level of disease intensity, but it is inferior to visual assessment at the lower levels of intensity, which are more frequently encountered.

Remote sensing – identification of symptoms on crop canopies using multispectral images







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Assessment of several diseased in the same plot

- The first step consists in a reliable diagnosis.
- Then the single D.I. has to be assessed at each sampling date, to determine their effect and their interaction.
- Environmental conditions, such as leaf wetness or light, can change the appearance of the different symptoms, making difficult the correct estimation of D.I. for each disease.

Root disease assessments

- Two methods can be used:
  - The first uses additional plant in the experimental plot which, at given sampling date, are uprooted and examined.
  - The second assesses intensity of root disease according to features of the above ground plant parts. There relationship have to be experimentally determined.

PROCEDURES FOR DISEASE ASSESSMENT
EPIDEMIOLOGICAL FORMS
CE.S.I.A.

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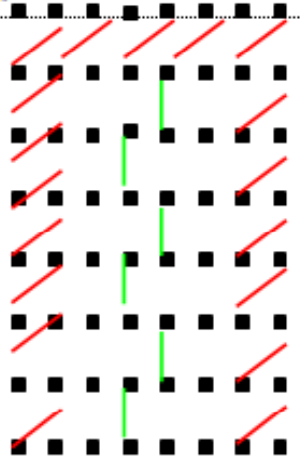
For the epidemiological monitoring it is necessary to choose into the vineyard an area which will not be treated against the considered pathogen (untreated area). For the treatment of the other vine diseases, it is necessary to spray selective products. To avoid into the untreated area the "drift effect" from neighbouring rows of the vineyard, it is advisable to close the nozzle of the spray nearest the plot that have to be examined.
Disease assessment will be performed every week starting from budbreak until grape maturation, to get 15 observations from April to August at least. Every time 400 leaves and 200 clusters on 100 plants will be evaluated.

CHOOSING THE PLOTS INTO THE NON TREATED AREA

The plots for the epidemiological evaluation must be sheltered on both sides by two rows, to minimize the "border effect". For the same reason, the plant at the top and at the bottom of the rows (see the Fig.1) will not be sampled.

Figure 1 The dots represent supported stakes for the vine. The rows are vertically disposed. The oblique line represent the boundary area around the effective plots that have to be evaluated. The vertical line represent six possible miniplots for the epidemiological assessment.



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Every plot will be marked with a symbol, for example A1, A2, A3,…., A10 to better identify the plot during the growing season. See in Table 1 and 2 an example of form for epidemiological measurement for clusters and leaves.

Table 1. Form for epidemiological measurements for leaves.

Data……Farm……Vineyard……
Variety……Note……

Leaves	A1	……	……	A10	
1					
2					
3					
4					
5					
6					
….					
….					
….					
….					
40					

Table 2. Form for epidemiological measurements for clusters.

Data……Farm……Vineyard……
Variety……Note……

Clusters	A1	……	……	A10	
1					
2					
3					
4					
5					
6					
….					
….					
….					
….					
20					

interruzione pagina

PLOT	1			2		
number	DM leaf	PM leaf	PM cluster	DM leaf	PM leaf	PM cluster
1	5	60	60	50	50	5
2	5	70	75	30	60	10
3	10	75	30	25	80	20
4	0	80	50	10	90	50
5	0	90	65	5	80	45
6	5	85	65	40	60	30
7	5	80	70	45	80	25
8	5	90	60	20	70	5
9	5	95	65	25	70	10
10	5	80	30	10	80	15
11	5	30	80	4	80	5
12	5	60	85	45	50	30
13	5	55	90	60	50	45
14	0	70	5	45	55	60
15	0	85	5	35	50	50
16	30	80	30	50	40	65
17	35	75	40	50	55	5
18	35	60	55	25	30	10
19	10	65	60	15	50	40
20	15	30	65	5	35	30

Classes

14/07/2010 **Leaves**

Class	Value (V)	Frequency (N)	N*V	Disease severity
0	0	23	0	
I	2.5	6	15	
II	5	20	100	
III	12.5	32	400	
IV	25	17	425	
V	50	1	50	
VI	75		0	
VII	87.5		0	
	∑N =	X 100		
		∑(N*V) =	Y 990	
			GDA =	Y/X 9.9

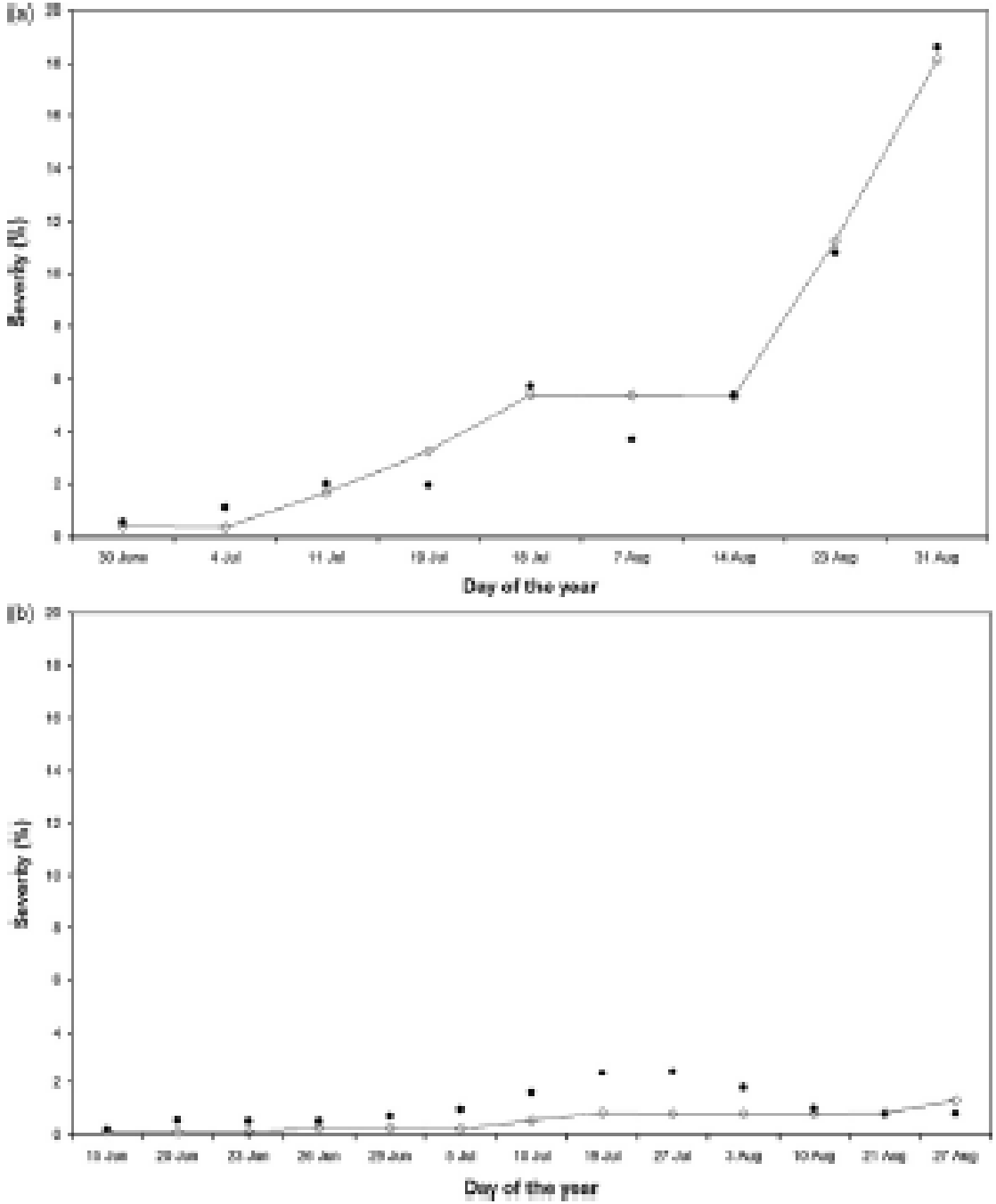

Table 3 – Simulation errors of downy mildew severity during the period 1998–2003

Year	RMSE	MAPE
1998	1.46	1.12
1999	3.89	0.80
2000	5.55	0.98
2001	3.73	0.76
2002	3.70	0.22
2003	0.74	0.24
AVG	3.18	0.69

RMSE = root mean square error; MAPE = mean absolute percentage error; AVG = average.

Table 4 – Simulation errors of downy mildew class of risk during the period 1998–2003

Year	Class obs	Class sim
1998	0	0
1999	1	0
2000	2	2
2001	1	0
2002	4	4
2003	0	0

Obs = observed in field; sim = simulated by PLASMO.

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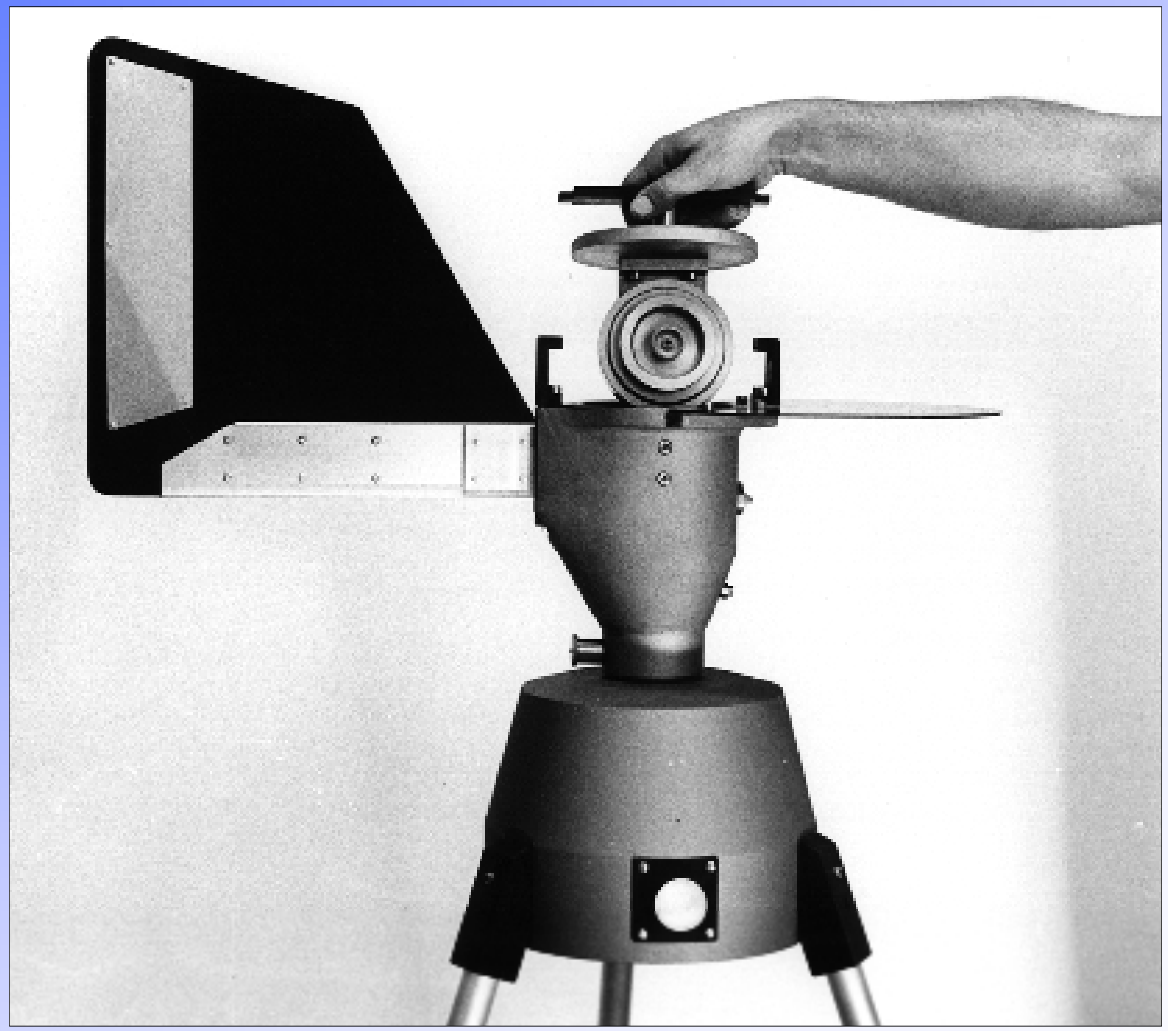
Pathogen measurements

- These measurements generally require specific equipments, such as, volumetric spore trap, small cyclone trap, washing method, etc. After pathogen organs are collected, they can be counted under a microscope.
- These method are not applied to filed studies, but particularly during experimental tests.
- They can be useful for a pre-season analysis of the risk in soil and seed borne disease.
- Also they allow to evaluate the dynamics of disease spatial variability inside a region.

Volumetric spore trap

Hirst-type sampler (VPPS2000, Lanzoni, Italy).



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Insect measurements

- Pest management depend on decisions, from the farmer to the national level. The measure of the population density is one of the most important point.
- Pest assessment is also important to understand the biology of the life-cycle stages, the distribution and migration, the effect of pesticide, to apply integrated protection method based on economical threshold, to apply simulation models, etc.
- The following principles can be also applied to mites, rodents and birds.

Basic principles of count methods

- ✓ Methods should be standardised
- ✓ They can be studied and tested
- ✓ They should be quick, cheap and simple, and clearly describes in survey manual
- ✓ The details of the method, the number, the size of sample, the time in the pest life-cycle and crop cycle should be explained
- ✓ A key to identify the pest and the crop growth stage is important

Direct counts

- They can be classified in two groups: those which can be based on a standard unit, such as area of ground or weight of crop, or which can be converted to such a unit from the number of leaves, stems, plants per area or yield of crop.
- There are a number of well-known methods:
  - Observation
  - Cutting open fruits, seeds, etc.
  - Knockdown with insecticide to remove the population onto a sheet
  - Sweep into a net
  - Washing off pests
  - Brushing
  - X-rays
  - Crushing

Counts in the environment

- In these cases an estimate of the population can be obtained, which must be corrected for the conditions of the trapping and for sampling error.
  - Chemical attraction, by using fruit or fruit extracts, fish meal, crop sections, pheromones (they are selective and simple to be used)
  - Attraction by colour (yellow during the day, red for fruit pest)
  - Sticky traps, with chemical of colour attractant
  - Water traps
  - Light traps
  - Suction traps
  - Sampling soil or debris
  - Pitfall traps (soil level containers)
  - Mark, release and recapture methods

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Physiological time

- A time scale where growth is repeatable in one that incorporates the effect of the environment is called physiological time. It standardised the time component of plant growth.
- The most typical is degree day, in which the effect of temperature, usually greater than some minimum values, is accumulated over time.
- Other unit can be considered, such as solar radiation time, etc.
- Therefore observation on disease development can be made during a particular physiological time, rather then a chronological time.
- Also the development of pathogens can be described as physiological time.

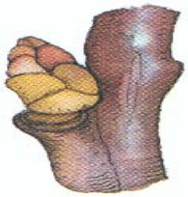
Degree days accumulation

Common name	Latin name	Number of growing degree days baseline 10 °C
Witch-hazel	*Hamamelis* spp.	begins flowering at <1 GDD
Red maple	*Acer rubrum*	begins flowering at 1-27 GDD
Forsythia	*Forsythia* spp.	begin flowering at 1-27 GDD
Sugar maple	*Acer saccharum*	begin flowering at 1-27 GDD
Norway maple	*Acer platanoides*	begins flowering at 30-50 GDD
White ash	*Fraxinus americana*	begins flowering at 30-50 GDD
Crabapple	*Malus* spp.	begins flowering at 50-80 GDD
Common Broom	*Cytissus scoparius*	begins flowering at 50-80 GDD
Corn (maize)	*Zea mays*	2700 GDD to crop maturity
Dry beans	*Phaseolus vulgaris*	1100-1300 GDD to maturity depending on cultivar and soil conditions
Sugar Beet	*Beta vulgaris*	130 GDD to emergence and 1400-1500 GDD to maturity
Barley	*Hordeum vulgare*	125-162 GDD to emergence and 1290-1540 GDD to maturity
Wheat (Hard Red)	*Triticum aestivum*	143-178 GDD to emergence and 1550-1680 GDD to maturity
Oats	*Avena sativa*	1500-1750 GDD to maturity
European Corn Borer		207 - Emergence of first spring moths

Host phenology

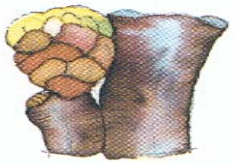
- This is a simple way to describe crop growth.
- Keys describing the phenological development of plants are available for many crops.
- They provide an estimate of the amount and type of plant organs which can be important for disease development.
- Also the timing of organs appearance can be described, related to senescence or susceptibility.
- The time of beginning and full phenological time can be recorded.

Phenological keys



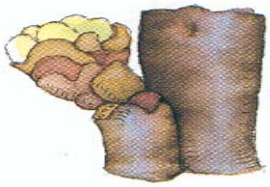
Gemma d'inverno

soglia critica –20 °C



Gemma gonfia

soglia critica –5 °C
10% di danni –8,3 °C
90% di danni –16,1 °C

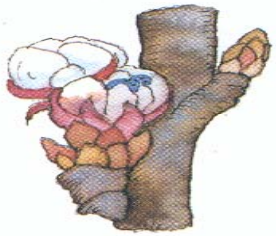


Bottoni visibili

soglia critica –4 °C
10% di danni –6,6 °C
90% di danni –13,9 °C

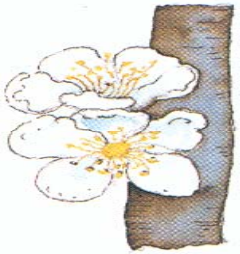
Bottoni bianchi

soglia critica –3 °C
10% di danni –3,3 °C
90% di danni –5,6 °C



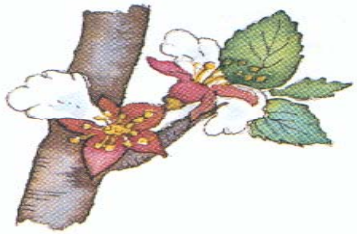
Inizio fioritura

soglia critica –2,8 °C
10% di danni –2,8 °C
90% di danni –5 °C



Piena fioritura

soglia critica 2 °C
10% di danni –2,2 °C
90% di danni –5 °C

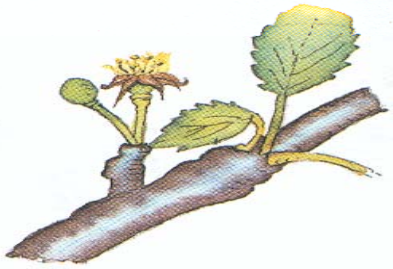


Caduta petali

soglia critica –1,5 °C
10% di danni –2,1 °C
90% di danni –5 °C

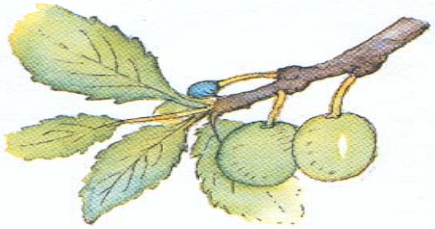
Allegagione

soglia critica –1 °C
10% di danni
90% di danni



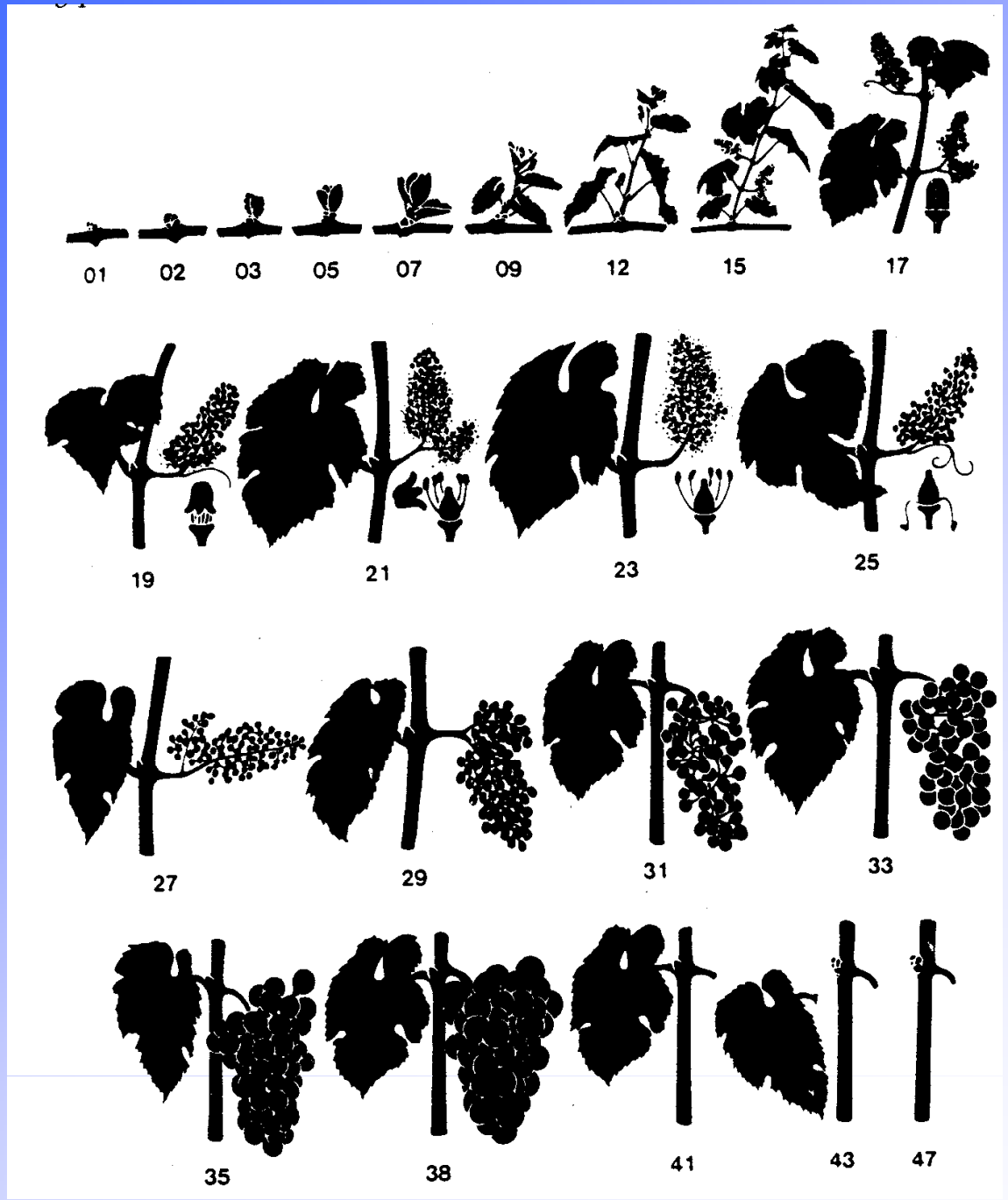
Scamiciatura

soglia critica –0,5 °C
10% di danni
90% di danni



Ingrossamento frutti

soglia critica
10% di danni
90% di danni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Growth analysis

- Two main parameters are generally considered:
  - the plant material present (total weight)
  - the magnitude of the assimilatory system (total leaf area)
- The leaf area index (LAI) can be also considered.
- Plant weight can be fresh or dry, partitioned into different components of plant (leaves, stems, roots, fruits, etc.), partitioned into susceptible and non-susceptible components.
- Leaf area can be easily measured using a leaf area digitiser, or traditional methods such as planimeter, length-width ratio, photographic techniques.
- Different analysis can be made using growth measures, including: growth rate, relative growth rate, net assimilation rate, leaf area ratio.

Stove

Scale

Leaf area meter

Structure of the lesson

- Plant disease measurements
- Pathogen measurements
- Insect measurements
- Host measurements
- Exercises

Exercises 1

- ✓ Determining the incidence and severity of downy mildew (leaves) and powdery mildew (leaves and clusters).
- ✓ Representing with a graphical format the average trend, with error bars (standard deviation divided by square root of number of observation), of the incidence and severity for both diseases.
- ✓ Assessing the relationship between incidence and severity.

Exercise 2

- The files contain disease assessments performed on grapevine downy and powdery mildew during the vegetative season. 50 leaves and clusters were observed in each plot per sampling date. 8 plots were used to reduce experimental variability.
- The average disease severity (DS) and incidence (DI) should be determined per each plot. Then the average value of all the plots should be determined and this represents DS and DI of this day.
- Then the seasonal trend can be plotted using the calculated values.