# Recent results in vine improvement regarding its resistance to downy and powdery mildews

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Summary: Resistance to mildew remains very important under Portuguese conditions and induced mutations present a great interest to obtain it.

$\gamma$-rays irradiation was applied to woody material and X-ray was used on *in vitro* cultures of stem apexes and leaf explants. After doses up to 2000 rad, two resistant mutants were selected: with $\gamma$-rays, the resistance being expressed as surrounding necrotic patches; with X-rays, the plant coming out from *in vitro* culture presented small necrotic patches.

Concerning powdery mildew, we are developing new techniques for laboratorial inoculation, including a technique for sowing isolated spores. By using these, we can conduct studies on morphology and biology of the isolated colony, variability of the resistance within the *Vitis* varieties and genetic variability of the parasite. Some results concerning these areas are presented.

Key words: Plasmopara, oidium, biotype, variety of vine, clone, resistance, mutagenesis, irradiation, bioassay, geography, Portugal.

## Introduction

Amongst the phytosanitary problems of the Portuguese vineyards, downy and powdery mildews still have great importance.

Last year, special weather conditions were favourable to the development of these diseases, particularly for *Plasmopara*, the damages caused being estimated at more than 30 % of the normal yield.

In spite of the higher efficacy of some new fungicides, it is still important to obtain cultivars showing resistance or semiresistance, due to the high cost of chemical treatments and the necessity of reducing to a minimum the pollution effects of pesticides.

So, we have been working on interspecific hybridization and, more recently, on irradiation mutagenesis in order to obtain semiresistant varieties (Coutinho 1977).

Concerning powdery mildew we are trying presently to develop new techniques for inoculations and evaluation of infections under laboratory conditions. By using these techniques we intend to clarify the morphology and the kinetics of the growing process of the isolated colony coming from a single spore. In the same way we search for clonal variability of resistance within vine varieties and for genetic variability of the fungus regarding some important traits.

## Material and methods

Concerning downy mildew, the experimental material chosen was cv. Touriga, largely grown both in Douro (Port wine region) and in Dao. At present this variety is being submitted to clonal selection through a national project.

Woody material, cuttings with 2 or 3 buds, and plants resulting from *in vitro* culture of stem apexes and leaf explants were utilized.

*In vitro* culture is, for several reasons, well suited for irradiation treatment, incrasing the mutagenic rate (Coutinho 1987).

For $\gamma$-ray irradiation we employed the $^{60}Co$ equipment of the Physical and Nuclear Engineering Laboratory at Lisbon. The X-ray treatments were performed in our laboratory by means of a Baltograph apparatus.

Selection criterion was based on leaf tissue reaction to the fungus after natural infections and artificial inoculations.

For the powdery mildew studies we perform artificial inoculations on detached leaf discs by two alternate methods: detachment and deposition of the spores by air flow (COUTINHO and MARTINS 1985) and by sowing isolated conidia. The second technique consists of obtaining single spores adherent to a very thin glass stylet ($\approx 30\,\mu$m diameter) and placing it by micromanipulation on the inoculation surface. This technique allows us to cultivate genetically homogeneous lines (clones) of powdery mildew, a very good way to study the biology of the isolated colony, the genetic variability of the fungus, etc. After inoculation, the spores are incubated in Petri dishes with humidified paper filter at 26 °C for periods from 5 to 10 d.

For the evaluations of artificial infections we estimate the density of the mycelium by counting the number of hyphae which cross a line segment (0.52 mm long) focused on the disc in a randomized way (COUTINHO and MARTINS 1985).

All the microscopic observations are made in a non-destructive way, allowing replicated measurements during the growth of fungus.

## Results and discussion

Concerning downy mildew program, we are presently studying plants originated from woody material that has been X-ray irradiated and planted in vegetation boxes under conditions suitable for the selection tests. No significant phenological differences have been found in the plants resulting from cuttings treated with 500 and 1000 rad (Fig. 1).

In material treated with $\gamma$-rays, one mutant was detected at 1000 rad. This mutant's resistance character shows leaf infection patches of 'ringspot' type, with an infection zone rapidly surrounded by necrotic tissue avoiding the spread of the parasite. These symptoms point out to a phytoalexins reaction, as described by POOL *et al.* (1980) around the leaf parenchyma at the site of the mycelium penetration.

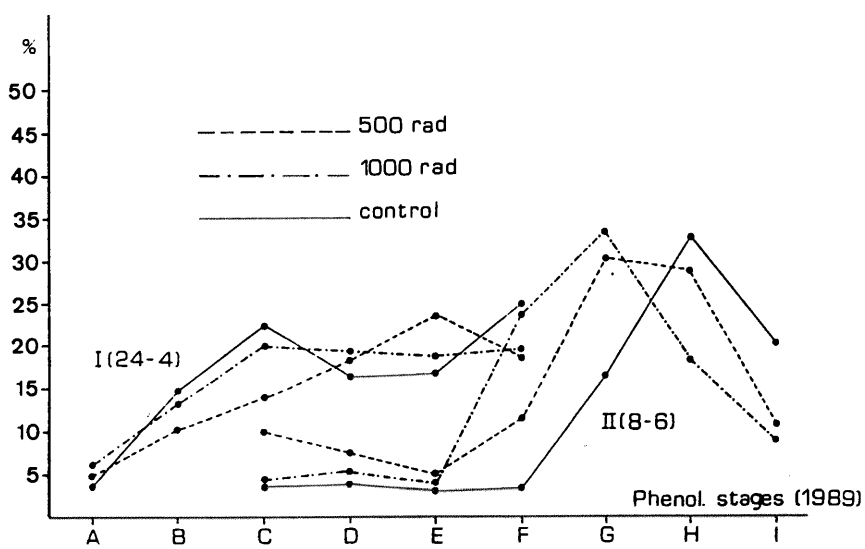


Fig. 1: Phenological stages of plants coming from cuttings treated with X-rays.

The speed of infection blocking by surrounding necrosis is clearly conditioned by weather conditions.

Plant regenerates from *in vitro* culture submitted to X-ray irradiation were transplanted to jiffypots. After inoculation, one plant of this modality showed resistance symptoms: limited and scattered small necrotic patches.

For the discussion regarding powdery mildew we shall follow the order of the objectives referred in the introduction.

## Biology of the isolated colony

After deposition of the spore on the disc surface, germination begins within 6 h. The first hypha appears at one end of the spore and when its length reaches around 20 $\mu$m it inflates and originates the primary appressorium. The appressorium penetrates the epidermal cell and produces a haustorium. After this, an elongating secondary hypha grows from the appressorium and one or two more from the conidia. The hyphae grow, ramificate and produce new appressoria and haustoria (Fig. 2).

The growing rate of the hyphae depends upon the host susceptibility and environmental factors. Studies conducted on an intermediate susceptible variety, Mourisco do Douro, gave the results shown in Table 1.

Results of other evaluated morphological and biological traits of the isolated colony are presented in Table 2.

Further studies on this subject will allow a better understanding of the biological behaviour of powdery mildew and host-parasite interactions. As an example, during another study we verified that sporulation occurs when mycelium density reaches a constant value around 10 hyphae/0.52 mm, independent of the incubation period (MARTINS 1984). Therefore, if we can identify races that differ in colony compactness, this may correspond to sporulation period differences, that is differences in pathogenicity.

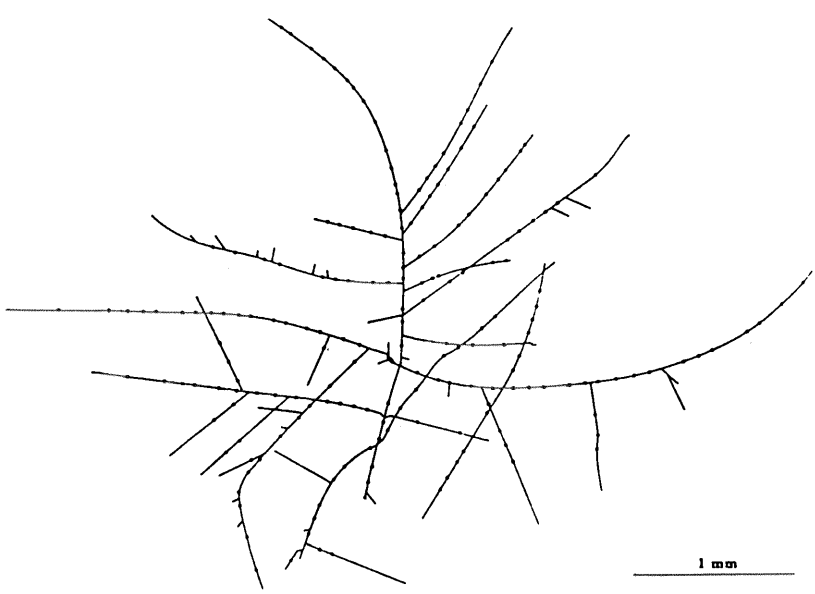


Fig. 2: Structure of the isolated colony of powdery mildew growing on detached leaf discs of cv. Mourisco do Douro during 11 d at 26 °C. • = appressoria.

Table 1: Growth rate of powdery mildew individual hyphae of isolated colonies on discs of cv. Mourisco at 26 °C

| classes of daily growth (mm) | 0-.09 | .1-.19 | .2-.29 | .3-.39 | .4-.49 | .5-.59 | .6-.69 |
|---|---|---|---|---|---|---|---|
| number of cases observed | 6 | 8 | 28 | 24 | 30 | 2 | 4 |

Table 2: Some biological and morphological traits of the isolated colony of powdery mildew growing on leaf discs of cv. Mourisco at 25 °C

| | |
|---|---|
| GROWTH OF THE INDIVIDUAL HYPHAE | .34mm/day |
| MEAN LENGHT BETWEN HAUSTORIA | .1 mm |
| DIAMETER OF THE COLONY | 5.0 mm |
| TOTAL AREA OF THE COLONY | 25 mm2 |
| NUMBER OF HYPHAE IN THE COLONY | 42 |
| TOTAL LENGHT OF THE HYPHAE | 38 mm |
| MEAN ANGLE OF THE RAMIFICATIONS | 66° |
| MEAN LENGHT BETWEEN RAMIFICATIONS | .47 mm |
| BEGINNING OF THE SPORULATION | 14 day |

Table 3: Mycelium growth rate of powdery mildew on discs of different clones of cvs Jaen and Trincadeira (inoculum density: 3.2 conidia/mm$^2$; incubation: 6 d at 26 °C)

| JAEN | | TRINCADEIRA | |
|---|---|---|---|
| clones | hyphae/.52mm | clones | hyphae/.52mm |
| J0566 | 9.4 | TR1 | 2.2 |
| J0609 | 4.1 | TR2 | 1.7 |
| J1612 | 15.3 | TR3 | 12.4 |
| J0656 | 2.4 | TR5 | 5.4 |
| J0771 | 6.6 | TR12 | 1.7 |
| J1402 | 11.1 | TR15 | 4.2 |
| J1417 | 6.7 | TA1 | 7.1 |
| J1602 | 11.4 | TA3 | 3.9 |
| J1626 | 10.3 | TA4 | 6.6 |
| J1636 | 5.6 | TA5 | 3.0 |

## Genetic variability of powdery mildew

Sowing isolated spores under the technique we described above enables us to cultivate genetically homogeneous lines of powdery mildew (clones) along several generations. These lines are observed in order to detect genetic differences regarding several traits: number of hyphae growing from the initial conidia of the colony; ramification angle between two hyphae; mean length between appressoria; mean length between contiguous ramifications; growing rate of the individual hyphae.

Our results point out differences in the angle of ramification and the morphology of the ramification point. These studies will be continued investigating powdery mildew strains of different geographic origin.

### Variability of resistance within varieties

By artificial inoculations performed over several years with conidia carried by air flow, we verified that differences of resistance between clones exist. The results of two comparative assays (amongst much others) regarding two ancient Portuguese varieties with good morphologic homogeneity, Jaen and Trincadeira, are presented in Table 3. By comparing these results with those of many other assays we verify that clones J0656 and TR2 show highest resistance, whereas clones J1612 and TR3 are more sensible.

At present we are applying the same tests to other varieties submitted to clonal selection in order to include the response to powdery mildew in selection criterium.

## References

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