CHAPTER 2

BACKGROUND AND OBJECTIVES

The present work is enclosed in:

- A four-years EU multidisciplinary project approved in 2001 (**Risk assessment and integrated ochratoxin A management in grape and wine; Quality of Life, Key Action 1, QLRT-2000-01761**), involving several European countries in the Mediterranean Basin, relevant for grape growing.

- A Spanish Project (**Evaluación del riesgo de contaminación por ocratoxinas y tricotecenos en alimentos y desarrollo de métodos de control, CICYT, Comisión Interministerial de Ciencia y Tecnología, AGL 2001 2974-C05-02**), which started in 2002.

Both projects appeared because of the importance of the OTA contamination in wine, the wide geographic area interested in it and the interest in protecting public health from OTA. The **overall objective** was the risk assessment of OTA presence in grapes and wine and protection of the consumer's health by decreasing the amount of this toxin with the aid of integrated management of production and processing. The projects embraced the whole aspects of the food chain, from field to the final processed product. Extension workers and producers involved in wine production were also included to guarantee the feedback of the results obtained to final end-users. The objectives will be followed by defining the critical control points for OTA synthesis during grape production and processing, and assessing possible preventive and corrective actions. Field data will be processed together in order to obtain risk maps, and to elaborate a Decision Support System to optimise preventive actions.

The contribution of the present thesis to both projects was focussed in a better understanding of the problematic related with OTA and in providing tools for preventive action to further reduce the human intake of OTA from grapes and wine. In particular, the **objectives**, grouped in the following sections, were:

- **Literature review:**

- To carry out a preliminary literature review regarding the occurrence of OTA in grapes and wine as well as the methodologies for its analysis.

- **OTA contamination in Spanish wines:**

- To assess OTA contamination in Spanish grape-based beverages, especially in wines, in order to increase Spanish data on OTA contamination and indirectly help in the decision of the establishment of legal limits of these products.

- To study the possible influence of several variables such as beverage categories, wine aging, wine colour, geographical origin, etc. in the OTA content of wine and related products.

- **Fungi responsible for OTA production:**

- To survey the mycoflora of several Spanish vineyards during four-year consecutive field samplings, in order to clarify where, when and to what extent OTA occurs in this country. The main aims of this section were:

  - to screen the mycobiota present in grapes.
  - to identify the ochratoxigenic species present in grapes.
  - to test the *in vitro* ability of the ochratoxigenic isolates to produce OTA.
  - to study the presence of OTA in natural grape musts.
  - to identify the key elements that influence OTA production in the field, with especial attention to meteorological parameters.

- **Ecophysiological studies**.

- To identify and study the effect of several ecophysiological factors and their interactions on potential ochratoxigenic fungi isolated from wine grapes, in order to find out the conditions that might minimize their growth and OTA contamination or might prevent its formation. Specifically:

  - to determine *in vitr*o the effect of a_w and temperature (marginal and optimum conditions) on several black aspergilli species growth and OTA production, especially on *A. carbonarius* species.
  - to study the influence of skin damage on visible fungal growth and OTA accumulation at different temperature and relative humidity on grapes.

- to determine the temporal OTA accumulation profile of *Aspergillus* section *Nigri* and in particular of *A. carbonarius*, at different a_w and temperature conditions.

- to model the effects of these ecological conditions.

- to establish the effect of photoperiod and alternation of temperatures, both on *A. carbonarius* growth and OTA synthesis.

- Complementary studies on fungal grape invasion were carried out in 2004 and 2005 in the Plant Pathology Department of the Royal Veterinary and Agriculture University of Copenhagen, Denmark, in order to:

  - transform *A. carbonarius* with the *Agrobacterium*-mediated transformation system by introducing the *green fluorescent protein* (*gfp*) gene into the fungal genome, and a hygB-resistance cassette as a selection marker.

  - study the stability of the transformants throughout one year.

  - visualize *A. carbonarius* grape colonization and early infection with epi-, stereo- and confocal fluorescence microscopy.

- Additional studies to investigate first the impact of application of fungicide treatments to grapes *in vitro*, and later the effect of different fungicides on grapes in the field were carried out.