Summary

Since the early 1990s, an important decrease in the survival rate of grafted grapevines in nurseries and young vineyards has been noted worldwide. Fungi involved in wood decay are among the most destructive pathogens either infecting grapevine propagation material, newly planted vines and mature established vineyards. Among the grapevine trunk diseases, a serious disease in most wine and grape-producing regions of the world, particularly in nurseries and young vineyards, is black-foot disease. The causal agents of this disease are included into the genera *Campylocarpon*, "*Cylindrocarpon*", *Cylindrocladiella* and *Ilyonectria*. It is well known that these pathogens are common in the soil and it has been demonstrated that nursery field sites can harbor them, causing infection of grafted vines after some months of growth in nursery soils. Nevertheless, the presence of black-foot disease pathogens in grapevine nurseries, as well as the potential inoculum sources of these pathogens in soils from nurseries and commercial vineyards, has not been explored in Spain. Thus, the main objective of this Thesis has been to study the epidemiology of black-foot disease in Spain.

Firstly, the different stages in the propagation of grapevine in Spanish nurseries were evaluated as potential sources of black-foot disease pathogens. To this end, samples were taken from four sources of the propagation process: pre-grafting hydration tanks, scissors used for cutting buds, omega-cut grafting machines and peat used for callusing. DNA from these samples was extracted and a multiplex nested-PCR with primers specific for "*Cylindrocarpon*" *pauciseptatum*, *Ilyonectria liriodendri* and *I. macrodidyma*-complex was used to identify the species present. *Ilyonectria liriodendri* and *I. macrodidyma*-complex were detected at different stages of the grapevine propagation process. Additionally, the detection of *Ilyonectria* spp. was also studied by multiplex, nested PCR and by isolation and genotyping in the grapevine planting material before and after the rooting phase in nursery fields. We confirmed that during the rooting phase in nursery fields the number of plants infected with black-foot pathogens increases markedly. By isolation on culture media, only one *I. torresensis* isolate was obtained from one of the cuttings sampled immediately after callusing. However, after one growing season in nursery fields, *I. liriodendri, I. novozelandica* and *I. torresensis* were more frequently isolated from rooted plants. Regarding the molecular detection of *Ilyonectria* spp. on grafted cuttings and plants, a greater number of positive samples were found before and after the rooting phase in nursery fields.

The soil of grapevine rootstock mother fields was evaluated as a potential inoculum source of black-foot disease pathogens by using four different techniques: fungal isolation from roots of grapevine seedlings used as bait plants, fungal isolation from roots of weeds, multiplex, nested PCR and qPCR. Four *Ilyonectria* spp., named *I. alcacerensis, I. macrodidyma, I. novozelandica* and *I. torresensis*, were isolated from the roots of bait plants grown in a rootstock mother field. "*Cylindrocarpon" macrodidymum* was also commonly isolated from weeds collected in rootstock mother fields showing a high rate of isolation. The analysis of soils collected from rootstock mother fields with the multiplex, nested PCR as well as with qPCR showed a high rate of detection of *I. macrodidyma*-complex from soil DNA samples, while the rate of detection of *I. liriodendri* was markedly lower in the same DNA samples.

Then, the contribution of soils of nursery fields as well as commercial vineyards in increasing the infections caused by black-foot pathogens on grapevine cuttings during the rooting phase in nurseries or in new plantations was also investigated. To this aim we used the same techniques described before. *Ilyonectria alcacerensis, I. macrodidyma, I. novozelandica* and *I. torresensis*, were isolated from the roots of bait plants grown in nursery fields. In addition, "*Cylindrocarpon" macrodidymum* was also commonly isolated from weeds collected in nursery fields showing a high rate of isolation. The results obtained with the multiplex, nested PCR as well as with the qPCR showed a high rate of detection of *I. macrodidyma*-complex from soil DNA samples collected in nursery fields, being the rate of detection of *I. liriodendri* markedly lower in the same DNA samples. Regarding the soil of commercial vineyards, three *Ilyonectria* spp., named *I. alcacerensis, I. novozelandica* and *I. torresensis*, were isolated from the roots of bait plants grown in pots filled with soils sampled from ten different commercial vineyards. "*Cylindrocarpon" macrodidymum* was also frequently isolated from weeds collected in several commercial vineyards.

It is interesting to note that in all soil types: roostock mother fields, nursery fields and commercial vineyards, species belonging to *I. macrodidyma*-complex were the most frequently detected.

Finally, the effects of temperature, pH and water potential (Ψ_s) on mycelial growth, sporulation and chlamydospore production of "*C.*" *liriodendri*, "*C.*" *macrodidymum* and "*C.*" *Pauciseptatum* were evaluated in order to provide further information on factors

affecting growth, reproductive and survival of these pathogens. All isolates were able to grow over a range of temperatures from 5 to 30ºC, with an optimum temperature between 20 to 25ºC, but they did not grow at 35ºC. Active mycelial growth was observed over a range of pHs, from 4 to 8. Regarding the effect of Ψ_s, in general, mycelial growth was greater on amended media at -0.5, -1.0 or/and -2.0 MPa compared with that obtained on nonamended PDA (-0.3 MPa), and was reduced at Ψ_s values lower than -2.0 MPa. Most of the *Cylindrocarpon* spp. isolates were sporulated at all temperatures, pHs and water potentials tested. In all studied conditions, "*C.*" *liriodendri* had the greatest sporulation capacity compared with "*C.*" *macrodidymum* and "*C.*" *pauciseptatum.* In general, chlamydospore production was not much affected by temperature, pH and Ψ_s. Chlamydospores were observed in PDA cultures of all isolates at all pH values studied, while some isolates did not produce chlamydospores at 5 and 10ºC or -4.0 and/or -5.0 MPa.

Additionally, in this Thesis, Petri disease pathogens were also detected on bait plants and weeds. *Cadophora luteo-olivacea, Phaeoacremonium aleophilum, Pm. parasiticum* and/or *Phaeomoniella chlamydospora* were isolated from xylem vessels of bait plants grown both in a rootstock mother field and a nursery field as well as from weeds collected in rootstock mother fields, nursery fields and commercial vineyards, confirming soil and weeds as inoculum sources of these pathogens.