ACREMONIUM-ENDOPHYTES OF PERENNIAL RYEGRASS (*Lolium perenne*) IN FRANCE

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ABSTRACT
Infection with endophytic fungi (*Acremonium* spp.) was detected in seeds of wild populations of perennial ryegrass (*Lolium perenne*) collected in France by private breeding companies and INRA. In total, 262 populations were examined and 70% contained *Acremonium*. It is worth noticing that most populations are endophyte infected but generally endophyte infection rates per population were low. Isolation of endophyte from each infected population is under progress and had already shown that the two taxonomic groups of endophytes of perennial ryegrass are present. This work lead to a view of the distribution of *Acremonium* endophytes from perennial ryegrass in France. The distribution of each taxonomic group is being established.

Such a work could be useful for plant breeders. If they decide to discard endophyte infected populations to avoid mammals toxicoses, they can see which are the populations free of endophyte. On another hand, they can decide to use it in their breeding programmes in order to improve host plants tolerance to abiotic and biotic stress. In this case, they must take into account the alkaloid context.

KEYWORDS
Acremonium endophytes, distribution, perennial ryegrass, taxonomic groups, wild populations

INTRODUCTION
Poaceae, particularly of subfamily Festucoideae, often harbour fungal mutualistic endophytes belonging to genus *Acremonium* (White, 1987). Perennial ryegrass is an economically important species of Festucoideae used as turf as well as forage. This species is native from Europe and can harbour two taxonomic groups of *Acremonium*-endophytes (Christensen *et al*., 1993). These endophytes could have an impact in breeding programmes, as the presence of endophytes could 1) lead to misinterpretation of plants evaluation data because it increases biotic and abiotic host plant tolerance, 2) cause toxicoses on grazing animals due to alkaloids. These effects due to the presence of endophyte are reviewed by Van Heeswijck and McDonald (1992).

Through a cooperative programme between private breeding companies and INRA to improve perennial ryegrass, 547 wild populations were collected in France (Charmet *et al*., 1990). As it is useful for forage breeders to know the endophyte status of his material and the alkaloids that each infected host plant could synthetize, endophyte was checked in 262 populations of this collection. Isolation of endophyte from each infected population is or will be realized to establish the distribution of each taxonomic group. This could be useful for the breeders due to a relationship between the taxonomic groups and the alkaloïds produced (Christensen *et al*., 1993).

MATERIAL AND METHOD
A sample of 262 French wild populations was selected for this study. It includes all the 114 populations belonging to the French core collection described by Charmet and Balfourier (1995). The other populations (148) were randomly sampled. Twenty seeds per population were observed using the method described by Latch *et al*., (1987), except that seeds were stained by soaking in anilin-blue for 12 hours instead of being heated for a few minutes.

Endophyte of each infected populations are being isolated on Potatoe Dextrose Agar (PDA). All isolates grow on PDA at 22°C in the dark. A piece of each strain removed from the margins was directly observed by microscopy for conidia. According to Christensen *et al*., (1993), strains not able to produce conidia were classified in *Acremonium lolii*, the other (able to sporulate) in the second taxonomic group.

RESULTS AND DISCUSSION
Only 74 populations were endophyte free. Most infected populations showed a low infection rate. These results are mapped on figure 1. Isolation and examination of conidia when they are produced are under progress. Preliminary results shows that most isolated strains are not able to produce conidia (20 strains among 25 observed for conidia production were not able to sporulate). So, sporulating strains seem to be rare. This could be explained by their hybrid origin (Schardl *et al*., 1995).

So, endophyte infection appeared to be frequent in French wild populations at low or intermediate levels (Figure 2). Low or intermediate infection rates were not expected because *Acremonium* endophytes are mutualistic symbionts (Clay, 1988). Therefore, endophyte-infected plants might be selected by natural selection, hence it was expected to find either high endophyte infection rates or no endophyte infection. Some models to explain such low or intermediate infection rates in the case of mutualistic relationships are under development.

Figure 2 shows that most populations highly infected were originating from areas with stressful climatic conditions (hot and dry summer like Mediterannean areas). This was confirmed by computing correlations between endophyte level of infection and some climatic variables (Ravel *et al*., submitted). So, the fungus seems to be more frequent in stressful environments; that may confirm that endophyte could improve adaptation of perennial ryegrass to stressful conditions. This suggests that breeders can use *Acremonium* endophytes to enhance *Lolium perenne* tolerance to stressful environmental conditions by using endophytes. This could lead to improved adaptation of perennial ryegrass to marginal areas.

Moreover, establishing the distribution of each taxonomic group could be interesting to see their relative importance and whether one of them is more frequent under certain environmental conditions than the other because each group might not confer to their hosts the same advantages.

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Figure 1
Location of collection sites of 262 populations of *Lolium perenne* in France

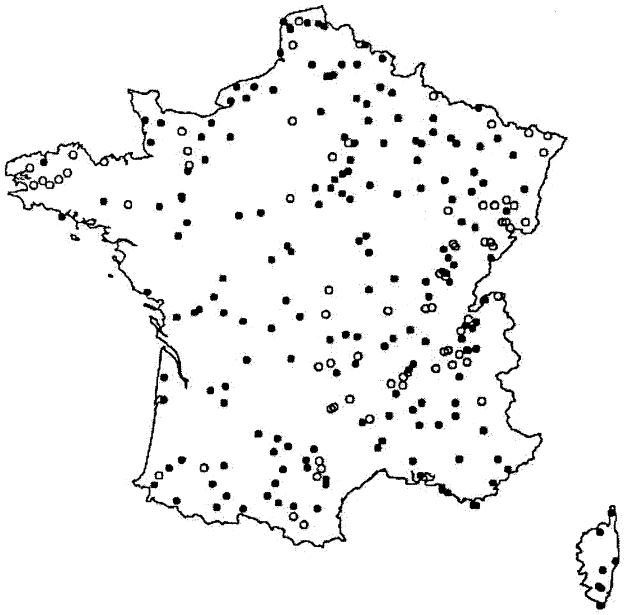

Figure 2
Locations in France of 188 wild populations of *Lolium perenne* i fected by *Acremonium* spp. The proportion of each circle shaded in black indicates the percentage of seeds infected.