TEAM WORKSHOP ON QTL DETECTION
FOR THE RESPONSE TO ROOT DISEASES IN MEDICAGO TRUNCATULA.

You will find in this section the data sets necessary for the detection of QTL by the analysis of RIL populations for the response of the model legume *Medicago truncatula* to various root pathogens. QTL detection analyzes will be performed by teams of 3-4 attendees.

A general discussion will follow on the genetic control of the quantitative resistances of *M. truncatula* to different root diseases. The perspectives to be given to this QTL detection analysis in terms of plant breeding and fundamental research on the interaction between *M. truncatula* and root pathogens will also be discussed.

Workshop 1. QTL detection for *Medicago truncatula* resistance to *Ralstonia solanacearum* on LR4 (A17xDZA315.16) RIL Population

A quantitative genetic analysis of the response towards *Ralstonia solanacearum* in the LR5 (A17 [susceptible] X F83005.5 [resistant]) RILs population of *Medicago truncatula* enabled us to identify a major QTL for resistance towards *R. solanacearum*.

To analyze a putative new genetic source for resistance to bacterial wilt, we used the A17 (susceptible) X DZA315.16 (resistant) RIL population that involves a different resistant parent from F83005.5.

The evaluation of the LR4 RIL population (A17 X DZA315.16) for bacterial wilt resistance was performed on 96 RILs in a randomized block design with four plants per block using three to four blocks per RIL.

The *R. solanacearum* GMI1000 strain was grown at 28°C in BGT medium. Plants were root-inoculated by cutting c. 1 cm from the bottom of the Jiffy pot, and the exposed roots were immersed for 20 min in a suspension containing 10_8 bacteria ml_1. The plants were then transferred to a growth chamber at 28°C (12 h of light) and 100 lmol m_2 s_1. The disease symptom assessment was performed

Mean disease scores, adjusted for block effect and combining F7 and F8 replicates, were computed along disease kinetics at 3, 5, 7, 10, 12, 14, 17, 19 and 21 dpi in 96 RILs.

You will detect QTL from mean disease scores 5 and 14 dpi as well as the genotypic data are available in this section.

Workshop 2. QTL detection for *Medicago truncatula* resistance to *Verticillium alfalfae* V31-2 strain in LR5 (A17xF83005.5) RIL population

The model legume *Medicago truncatula* was used as a host for studying resistance and susceptibility to *Verticillium alfalfae*. Quantitative trait loci were identifed using a multicross, multisite/multiyear design. Here, we focus on the analysis of the response to *Va* V31-2 strain in the LR5 RIL population from the cross between the two contrasted lines A17 (resistant) and F83005.5 (susceptible).

A total of 173 RILs of the A17 × F83005.5 (LR5) population in the F8 and/or F9 generations were evaluated in two different sites (ENSAT and Barenbrug-Tourneur). For each RIL, 8–12 plants were inoculated. Suspensions of conidia of *V. alfalfae* V31-2 strain were obtained and the concentration was adjusted to 10_6 spores ml_{-1} for all inoculations. Ten-day-old plants (first pair of leaves stage) grown in peat substrate were inoculated after cutting 1 cm of the bottom of the Jiffy pots which were then dipped in the conidial suspension during 30 minutes before being transferred to trays containing moist soil. All inoculated plants and respective controls treated with water were incubated in a growth chamber at 20 °C with 16 h photoperiod. Symptoms were scored regularly during 4 weeks on a scale from 0 to 4.

The area under the disease progress curve (AUDPC) was computed based on the disease scores from 0 to 4, using the 'agricolae' package of the R system. Model for disease progress empirical curves was based on a logistic model. The modelling allowed the description of the disease progress in terms of three functional parameters: (i) maximum disease index (Asym) at the end of the experiment (available here); (ii) time to reach 50% MDI (not available); and (iii) time to proceed from 50% MDI to (50% MDI)/(1 + e_{-1}) (c.75% MDI) (not available).

Phenotypic data for AUDPC and maximum disease index (Asym) at the end of the experiment as well as the genotypic data are available in this section.

Workshop 3. QTL detection for *Medicago truncatula* resistance to *Verticillium albo-atrum* LPP0323 in LR5 (A17xF83005.5) RIL population

In order to study the genetic control of resistance to a non-legume isolate of *Verticillium albo-atrum*, a population of recombinant inbred lines (RILs) from a cross between resistant line F83005.5 and susceptible line A17 was inoculated with a potato isolate of V. albo-atrum, LPP0323.

A set of 116 RILs (F_8) derived from the cross between M. truncatula lines A17 and F83005.5 (LR5) was used in this study. The parental lines exhibit contrasting responses in their resistance to *V. albo-atrum* LPP0323. The experiment was organized as randomized complete blocks with 116 RILs and their parents in three replications. Each replication contained 8–12 plants per genotype.

Verticillium albo-atrum LPP0323 was grown on potato dextrose agar in Petri dishes at 24°C. Spores were harvested after 21 days of mycelial growth by flooding the Petri dishes with sterile water. The concentration of spores was determined with a Malassez counting cell and adjusted to 10_6 spores mL_1 with distilled water.

Ten-day-old plants (first pair of leaves stage) grown in peat substrate were inoculated after cutting 1 cm of the bottom of the Jiffy pots which were then dipped in the conidial suspension during 30 minutes before being transferred to trays containing moist soil. All inoculated plants and respective controls treated with water were incubated in a growth chamber at 20 °C with 16 h photoperiod. Symptoms were scored regularly during 4 weeks on a scale from 0 to 4.

The symptom score at the end of the experiment (maximum symptom score, MSS) and the area under disease progress curve (AUDPC) were computed for each plant per line per replication, and mean values were calculated.

Phenotypic data for maximum symptom score (MSS) at the end of the experiment and area under disease progress curve (AUDPC) as well as the genotypic data are available in this section.

Workshop 4. QTL detection for *Medicago truncatula* resistance to *Verticillium alfalfae* V31-2 in LR4 (A17xDZA315.16) RIL population

The model legume *Medicago truncatula* was used as a host for studying resistance and susceptibility to *Verticillium alfalfae*. Quantitative trait loci were identifed using a multicross, multisite/multiyear design. Here, we focus on the analysis of the response to *Va* V31-2 strain in the LR4 RIL population from the cross between the two contrasted lines A17 (resistant) and DZA315.16 (susceptible).

137 RILs of the A17 × DZA315.16 (LR4) population in the F8 and/or F9 generations were evaluated in three sites (ENSAT, Barenbrug-Tourneur, and R2n). For each RIL, 8–12 plants were inoculated. Suspensions of conidia of *V. alfalfae* V31-2 strain were obtained and the concentration was adjusted to 10_6 spores ml_{-1} for all inoculations. Ten-day-old plants (first pair of leaves stage) grown in peat substrate were inoculated after cutting 1 cm of the bottom of the Jiffy pots which were then dipped in the conidial suspension during 30 minutes before being transferred to trays containing moist soil. All inoculated plants and respective controls treated with water were incubated in a growth chamber at 20 °C with 16 h photoperiod. Symptoms were scored regularly during 4 weeks on a scale from 0 to 4 and the proportion of dead plants (PDP) was determined at the end of the experiment. The area under the disease progress curve (AUDPC) was computed based on the disease scores from 0 to 4, using the 'agricolae' package of the R system.

Phenotypic data for AUDPC and percentage of dead plant (PDP) at the end of the experiment as well as the genotypic data are available in this section.