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Resource competitive interactions as mechanism of date palm Bayoud disease suppression

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In Morocco, soils that are naturally suppressive to date palm Bayoud disease have long been discovered. Although suppressiveness was attributed to biological activities of indigenous microbes, our knowledge on the specific mechanisms underpinning this property remains limited. In this study, we investigated nutrient competition between Fusarium oxysporum f. sp. albedinis, the causal agent of Bayoud disease, and saprophytic Fusarium as a factor of disease suppressiveness/conduciveness in suppressive and conducive soils. Growth of pathogenic and saprophytic Fusarium isolates from one suppressive and one conducive soils on 95 carbon sources was assessed. Fusarium isolates exhibited distinct nutrient use profiles and varied significantly with soil in carbon utilization. Isolates from the suppressive soil had significantly the greatest resource use efficiency, followed by the pathogenic isolates that grew significantly faster than the isolates from the conducive soil. Data on nutrient niche overlap showed that the pathogen is outcompeted by saprophytic Fusarium populations in the suppressive soil and outcompetes those in the conducive soil. Taken together, our results provide insight into the role of competition for carbon resources among pathogenic and saprophytic Fusarium communities as a driving factor in soil suppressiveness/conduciveness. This finding may also open novel research paths and offer opportunities for the development of biocontrol techniques against Bayoud disease in Moroccan date palm groves.

Keywords: Date palm, Bayoud disease, nutrient competition, disease suppression

INTRODUCTION

Date palm (Phoenix dactylifera L.) has long been a major fruit crop in the semiarid regions of southern Morocco. In these areas, date production is an important source of food and income for local populations and plays key economic and environmental roles by creating a suitable microclimate for growing other food crops and protecting oases from silting and desertification. Up to 10 years ago, date palm had been grown in traditional oases under agroforestry systems over an area of 48 000 Ha, encompassing more than 5 million palm trees that are genetically heterogeneous. In 2008, Morocco launched the Green Morocco Plan to leverage agriculture into business opportunities and improve production of many crops including date palm. Significant efforts have been made ever since to encourage establishment of new and modern date palm plantations outside traditional orchards through financial incentives to investors. As a result, more than 10 000 Ha of date palm plantations with modern management technologies have been established to date in expansion areas. Other similar investments are in progress for an expected total acreage of more than 17 000 Ha of modern date palm plantations by 2023. Notably, more than 70% of these orchards are, or will be, cultivating “Mejhool”, a variety that is highly prized in local and international markets for the large size, the sweet taste and the juicy flesh of its dates. A negative consequence of this intensification of date production, coupled with genetic uniformity in

cultivars, is an anticipated increase in vulnerability to diseases caused by microbial pathogens.

Specifically, “Bayoud” disease is the principal enemy of palm trees that puts at stake the future of the date industry in Morocco. “Bayoud” is a vascular disease caused by the soilborne fungal pathogen Fusarium oxysporum f. sp. albedinis (Foa). The parasite attacks palms through the roots and ends up colonizing the entire vascular system, leading to wilt and ultimately to palm death. Since it first appeared in Morocco in the late 19th century, the disease has spread throughout all date-producing areas in Morocco and killed more than 10 million palm trees (Sedra, 2003). In addition, the disease has been reported in neighboring countries, Algeria and Northern Mauritania (Sedra, 2003). Attempts to control “Bayoud” in early years following disease identification using systemic fungicides were unsuccessful (Selvaraj, 1978). Currently, genetic resistance is the only effective method to control the disease. However, although breeding programs have been carried out in Morocco, only a few Bayoud-resistant varieties producing high quality dates have been identified. Most of commercial varieties, including the “Mejhool” cultivar, are highly sensitive and require intensive management to control the disease.

Soils that are naturally suppressive to a number of soilborne plant diseases, particularly those caused by formae specialies of Fusarium oxysporum, have long been described worldwide (Mendes et al., 2011; Alabouvette et al., 2009; Weller et al., 2002; Alabouvette, 1999, 1986; Louvet et al., 1976). Similarly, “Bayoud” suppressive soils have been discovered in Morocco and the involvement of microbial activities in disease suppression has been experimentally elucidated (Sedra et Rouxel, 1989). This has opened novel research paths for the development of biological control to protect susceptible varieties against this disease. For example, a better understanding of how Bayoud disease is suppressed by cohabitating microbes can guide intensive soil management that promotes beneficial microflora, and/or soil inoculation with select microbes to mitigate disease impact. However, the specific mechanisms by which microbial communities repress Foa in the suppressive soils remain poorly understood. Based on findings from other similar systems, one possible factor that could underpin Bayoud suppression/induction is competition over resources between Foa and non-pathogenic (saprophytic) Fusarium in soil. The objective of this study is to characterize resource use profiles and contrast competitive resource interactions between Foa and saprophytic Fusarium in suppressive versus conducive soils.

MATERIALS AND METHODS

Soil sampling

Samples were collected from 2 plots planted to date palm for more than 15 years in Zagora and Marrakech regions, Morocco. Soil from Zagora is conducive and that from Marrakech is suppressive to Bayoud disease (Sedra and Rouxel, 1989). In each location, 3 soil samples were taken 1m apart from 3 palms showing severe symptoms of Bayoud in Zagora and randomly chosen in Marrakech. Soils were sampled at a depth of 0-20 cm. Samples from the same location were bulked and homogenized in plastic sample bags. The soil samples were stored at -20°C prior to processing.

Pathogenic and saprophytic Fusarium isolates

Strains of pathogenic Fusarium oxysporum f. sp. albedinis (Foa) were isolated from the three soil-sampled diseased palms in Zagora. Additionally, 10 saprophytic (non-pathogenic) Fusarium sp. (Fus) isolates were isolated from each bulked soil sample taken in each location following a method described in Essarioui et al., (2017). Briefly, for each sample, a single 5 g sub-sample was dried overnight under sterile cheesecloth. Dried soil samples were dispersed in 50 mL of sterile deionized water on a reciprocal shaker (175 rpm, 60 min, 4°C). Soil suspensions were serially diluted to 10-3. The soil diluents were used to isolate Fusarium spp. using peptone pentachloronitrobenzene (PCNB) Agar Medium (PPA; peptone 15 g, KH_2PO_4 1g, $MgSO_4.7H_2O$ 0.5 g, PCNB (75%) 750 mg,

agar 20 g, H2O 1L), a medium that is highly inhibitory to most other fungi and bacteria but allows slow growth of Fusarium (Nelson et al., 1983). Following autoclaving, streptomycin and neomycin were added to the cooled medium to inhibit the growth of Gram-negative and Gram-positive bacteria, respectively, and the pH was adjusted to 5.5-6.5. The streptomycin stock solution was 5 g of streptomycin in 100 ml distilled H2O, and was used at the rate of 20 ml/L of medium. The neomycin stock solution was 1 g of neomycin sulfate in 100 ml distilled H2O, and was used at the rate of 12 ml/L. For each soil suspension, individual aliquots of 100 µl were spread onto 15 ml plates of PPA and incubated at 28°C for 3-4 days. Subsequently, 10 to 15 randomly chosen single fungal colonies from each plate were transferred to plates containing 15 ml of potato dextrose agar medium (PDA) and allowed to grow for 3 days and Fusarium isolates were identified based on the morphology and pigmentation of the colonies and the shape of macroconidia (Leslie and Summerel, 2006). Finally, 2 sub-sets of 10 morphologically distinct isolates were selected from each collection (location). All pathogenic and saprophytic Fusarium isolates were single-spored prior to further use. Thus we had a total of 23 Fusarium isolates; 3 pathogenic strains (Foa), 10 saprophytic Fusarium sp. from the Marrakech suppressive soil (Fus_M), and 10 saprophytic Fusarium sp. from the Zagora conducive soil (Fus_Z).

Fusarium resource use characterization

Utilization of carbon sources by every individual Fusarium isolate (n = 22) was determined using Biolog SF-P2 plates (Biolog, Inc. Hayward, CA). Biolog SF-P2 microplates measure the growth of an isolate on a single source of carbon by comparing the turbidity of each well to a water control. The 95 carbon substrates in the SF-P2 panel belong to 11 carbon groups (the numbers of substrates for each group are indicated between parentheses): alcohols (3), amides (3), amines (1), amino acids (9), aromatic compounds (4), carbohydrates (41), carboxylic acids (15), esters (3), phosphorylated compounds (8) and polymers (8) (http://www.biolog.com/pdf/milit/00A_008rA_SFN2_SFP2.pdf). Spore suspensions of each isolate were made by swabbing spores from a pure culture grown on PDA for 10 days into 1.5 ml of 0.2% carrageenan. Suspensions were adjusted to an optical density of 0.20–0.24 at 590 nm and then diluted in 13.5 ml of 0.2% carrageenan. One hundred microliters of the new suspension were pipetted into each well of the Biolog plate. Plates were incubated at 28 °C. Utilization of each of the 95 sole carbon compounds on the Biolog SF-P2 plate was assessed by recording the absorbance of each well at 590 nm 96 h post-inoculation. The absorbance of the well containing only water was subtracted from the absorbance of every other well to standardize absorbance values. Each isolate was tested on 3 replicates.

“Used nutrients” were defined to be those on which an individual Fusarium isolate grew to an absorbance value greater than 0.5 (Sammar and Alsanius, 2009). Using this definition, niche width (NW), average growth (AG), and total growth (TG) were determined for each isolate. NW was defined as the number of used nutrients for an isolate; and AG and TG were defined as the average and the sum of OD over all used nutrients, respectively.

Resource competition between pathogenic and saprophytic Fusarium isolates

For each soil, the potential for resource competition between Foa and Fus was indexed. As the significance of the shared niche to each isolate’s growth might differ between Foa and Fus (Figure 1), asymmetrical pairwise niche overlap values were determined for each Foa isolate and each Fus isolate in every pairwise Foa-Fus combination. Pairwise niche overlap of Fus with Foa was defined as in Essarioui et al., (2017):

and pairwise niche overlap of Foa with Fus as:

Mean niche overlap was computed for each soil by averaging pairwise niche overlap values among all isolate pairs.

RESULTS AND DISCUSSION

Nutrient use profiles of Fusarium isolates

Saprophytic Fusarium isolates from the same soil were more similar to each other and different in terms of nutrient use profiles from the pathogenic isolates (Figure 2). Isolates from the suppressive soil form a distinct cluster, exhibiting distant metabolic relatedness with those from the conducive soil that constitute a more similar group. Additionally, all Fusarium individuals from the suppressive and the conducive soils showed strong differences in carbon utilization profiles with the pathogenic isolates. In accordance with previous work (Tang and Hartman, 2010; Sammar and Alsanius, 2009), these results suggest that geographic location and pathogenicity are important determinant of metabolic profiles in Fusarium.

Regardless of their geographic origin or pathogenicity, both saprophytic and pathogenic Fusarium grew on a wide myriad of carbon sources. Overall, isolates used all 11 carbon groups, ranging from labile carbon substrates such as carbohydrates, amino acids, and amines to recalcitrant carbon sources including esters, phosphorylated compounds, and polymers, illustrating the diverse metabolic capacities among Fusarium populations. This suggests that Fusarium species are often generalist. The ability to degrade diverse carbon molecules with different levels of chemical complexity reflects significant metabolic capacities among Fusarium (González-Márquez et al., 2019; Vergara-Fernández et al., 2019; Shi et al., 2018) and rest upon their capacity to produce a vast array of degradative enzymes that enable them to use these substrates as source of carbon and energy in oligotrophic soil environments (Chater et al., 2010; Schrempf et al., 2011). It also corresponds to their active role in biogeochemical nutrient-cycling processes. Additionally, Fusarium possess large genomes, ~35-50 Mb (King et al., 2015), rich in transposable and horizontally-transferred elements (Ma et al., 2010) allowing for widespread exchange of genes. This offers flexibility in fine-tuning of metabolic machinery and is likely to foster Fusarium adaptation to local conditions created by variation in soil physical and chemical properties.

Fusarium niche width, average growth and total growth

The effect of isolate origin (pathogenic, from suppressive or conducive soil) was investigated using unbalanced 1-way ANOVA. Isolate origin had a significant effect on Fusarium growth characteristics. Indeed, mean niche width of FusM (from the suppressive soil) was significantly broader than that of Foa, which in turn was significantly greater than mean niche width of FusZ (from the conducive soil) (Figure 3A; 1-way-ANOVA, $p<0.001$). Similarly mean growth per used nutrient among FusM was modestly, but significantly, greater than that of Foa and FusZ (Figure 3B, 1way-ANOVA, $p=0.002$). As a result of their greater niche widths and mean growths, FusM isolates had significantly greater total growth than Foa isolates and FusZ isolates which had the smallest total growth (Figure 3C, 1way-ANOVA, $p=0.001$). Different patterns of growth ability among saprophytic and pathogenic Fusarium in the suppressive and conducive soils suggest that carbon sources might be a focal point of competition between saprophytic Fusarium and Bayoud pathogen populations in the soil and are central to mediating soil conduciveness or suppressiveness to Bayoud disease in Moroccan palm groves.

Niche overlap between saprophytic and pathogenic Fusarium

Niche overlap (NO) between saprophytic (FusM and FusZ) and pathogenic (Foa) Fusarium was asymmetrical and its patterns varied significantly with soil (Figure 4). Indeed, NO of FusM with Foa was significantly greater than NO of Foa with FusM, suggesting intense resource competition over nutrient imposed by saprophytic Fusarium on the causal agent of Bayoud disease (Foa) in the suppressive soil. In contrast, NO of FusZ with Foa was significantly smaller than NO of Foa with FusZ, implying that the Bayoud pathogen (Foa) has larger competition-free niche space in the conducive than the suppressive soil.

Results on NO and isolate niche width and total growth indicate that, in terms of nutrient acquisition, saprophytic FusM in the suppressive can utilize greater numbers of carbon substrate for which they do need to compete with Foa and have faster access to their shared nutrients. Conversely, in the conducive soil, Foa has greater resource competitive advantage over saprophytic FusZ. The variation in the capacity of saprophytic Fusarium to utilize carbon resources in the two soils may partly be a mechanism by which the Bayoud pathogen is suppressed in the Marrakech suppressive and not the Zagora conducive soil. The capacity of members of Fusarium genus to degrade a surfeit of carbon substrates and overcompete soil microbes have been recently described (Essarioui et al., 2017) and the role of populations of non-pathogenic Fusarium as potent competitors for carbon resources in Fusarium wilt suppression have long been speculated on (Alabouvette, 1986, 1999). Our study took a different approach by increasing the resolution of Fusarium resource use efficiency and competition and demonstrates that in the suppressive soil, saprophytic Fusarium compete more with the pathogen than the opposite, whereas in the conducive soil the pathogen have greater niche escape to grow and potentially infect palms. Saprophytic Fusarium greater resource competition may also result in faster occupation of infection site in the suppressive versus the conducive soil, preventing the pathogen from causing disease. In addition to resource competition, we postulate that members of soil Fusarium in the suppressive soil may have greater potential of producing antimicrobial compounds that can inhibit Foa as well. Antimicrobial properties of many substances produced by diverse non-pathogenic Fusarium have long been reported (Tirunarayan and Sirsi, 1961; Liu et al., 2012) and may underlie essential functions in disease suppression in the soil and in plants (Essarioui et al., 2017).

CONCLUSION

Evidence from our study suggests that competition for carbon sources is a critical determinant of suppressiveness or conduciveness to Bayoud disease in date palm of Moroccan soils. Consistent with previous work (Essarioui et al., 2017; Tang and Hartman, 2010; Sammar and Alsanius, 2009), the data generated from the research presented here demonstrate that both saprophytic and pathogenic Fusarium in Moroccan oases soils possess diverse metabolic capacities, suggesting key roles in organic matter decomposition and nutrient cycling. Additionally, our data show that niche width, mean growth, and total growth are greater among Fusarium populations in the suppressive than the conducive soil, purporting the hypothesis of greater competition over resources in the suppressive soil. Indeed, niche overlap of saprophytic Fusarium with the Bayoud pathogen was greater in the suppressive than in the conducive soil. In fact, the pathogen imposes more intense competition pressure over non-pathogenic Fusarium in the conducive than in the suppressive soil, suggesting that saprophytic Fusarium have a greater niche escape allowing for more important competition-free growth niche in the suppressive soil. Taken together, our findings suggests that variation in Fusarium resource use creates different levels of resource competition among saprophytic and pathogenic populations and may be central to mediating Bayoud disease suppression in the Marrakech soils.

A long-term goal for date palm cultivation in Morocco is the ability to effectively manage microbial communities to control the soil-dwelling Bayoud pathogen in both traditional and modern palm groves without pesticides. Our findings may have direct implications for sustainable and environmentally-friendly management of this disease. Further studies based on periodic soil inoculations with highly competitive non-pathogenic Fusarium, and/or soil management practices that facilitate selection for saprophytic Fusarium populations with greater efficiency in carbon resource use may offer a promising pathway to Bayoud suppression and date palm protection.

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