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Water Deficit as a Driver of the Mutualistic Relationship between the Fungus *Trichoderma harzianum* and Two Wheat Genotypes[▽]

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The aim of this study was to assess the occurrence of mutualistic interactions between the fungus *Trichoderma harzianum* and two wheat genotypes, *Triticum aestivum* cv. Talhuén and *T. turgidum* subsp. *durum* cv. Alifén, and the extent to which water deficit affected these interactions. Two wheat genotypes were cultivated in the presence or absence of *T. harzianum* and in the presence or absence of water deficit. *T. harzianum* was in turn cultivated in the presence or absence of wheat plants and in the presence or absence of water deficit. To evaluate the plant-fungus interactions, the root volume, dry biomass, and fecundity of wheat were determined, as was the population growth rate of the fungus. *Trichoderma harzianum* exerted a positive effect only on plants subjected to water deficit. The population growth rate of *T. harzianum* was negative in the absence of wheat plants and reached its highest level in the presence of plants under conditions of water deficit. These results confirm the occurrence of a mutualistic interaction between wheat and *T. harzianum* and show that it is asymmetric and context dependent.

The study of mutualistic interactions is becoming an important research avenue to elucidate the ecology and evolution of species in natural communities (23). A deeper understanding of the extent to which these interactions affect population and community dynamics requires the assessment of the role played by the ecological context (biotic/abiotic) under which the interactions occur (8, 9, 32). During the last few years, conceptual models examining biological interactions have changed their focus from competition and predation to mutualistic interactions, particularly among plants (22).

Trichoderma harzianum (Hyphomycetes) has been isolated from a wide range of environments, such as crops, native prairies, forests, salt marshes, and desert soils of all climatic zones, as well as from lake water, dead plant material, and living roots of virtually every plant species examined. It is fast growing and a prolific producer of antibiotic substances (14). *Trichoderma harzianum* is known to benefit many plant species by increasing shoot and foliar area, plant biomass, fruit production, the exploration capacity of the root system, and tolerance to water deficit and to low levels of photosynthetically active radiation (14).

Trichoderma harzianum has been reported to act as a biological control agent for several diseases of wheat. The biocontrol capacity depends on environmental factors such as soil water content and pH (10, 20). The fungus also has been shown to increase the root length, total biomass, and seed production of wheat, probably as a consequence of an increased efficiency in the absorption of phosphorus and nitrogen from the soil (5, 13). Curiously, the effect of the plant on the fungus is an issue that remains to be addressed. Thus, the system composed by *T. harzianum* and wheat is an appropriate model to explore the nature of mutualistic interactions under controlled conditions as well as to analyze the role of the abiotic environment on the outcome of the interaction.

Wheat is among the cereals that accumulate hydroxamic acids (Hx) in their tissues. Hx are a family of secondary metabolites found mostly in the Gramineae (Poaceae), particularly in the tribe Triticeae (25, 26), which are involved in the defense of the plant against a wide range of organisms (27). Hx are absent from the seed but are present in all tissues of growing plants, including the roots, and also are found in root exudates (17, 29, 40).

The aim of the present study was to experimentally examine the existence of a mutualistic interaction between *T. harzianum* and wheat and to evaluate the role of water limitation on the strength and direction of this ecological interaction. Two factorial design experiments were set up. The first involved the effect of the fungus on two wheat genotypes in the absence or presence of water deficit, and the second involved the effect of the plant on the fungus in the absence or presence of water deficit. The wheat genotypes used were *Triticum aestivum* cv. Talhuén and *T. turgidum* subsp. *durum* cv. Alifén. The first genotype accumulates low levels of Hx (11) and is irrigation dependent, while the second accumulates high levels of Hx (11) and usually is sown in dry farming conditions. We hypothesized that (i) given the deleterious effects of Hx on fungi (38), Hx will negatively affect the in vitro growth of *T. harzianum*; (ii) if *Trichoderma* is negatively affected by Hx in vitro, then the growth rate of the fungus and the wheat-*Trichoderma* interaction will be lower in *T. turgidum* subsp. *durum* cv. Alifén (high Hx) than in *T. aestivum* cv. Talhuén (low Hx); (iii) given the differences in the irrigation dependence of the cultivars studied, the strength of the mutualistic relationship between wheat and *Trichoderma* depends on the irrigation regimen under which the interaction occurs; and (iv) if the interaction between wheat and *Trichoderma* is cultivar dependent, then the mutualistic relationship involving *T. aestivum* cv. Talhuén is

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stronger than that involving *T. turgidum* subsp. *durum* cv. Alifén on account of the higher level of dependence of the former on water for growth.

MATERIALS AND METHODS

Plants and fungi. Seeds of *T. turgidum* subsp. *durum* cv. Alifén and *T. aestivum* cv. Talhuén were obtained from the Instituto de Investigaciones Agropecuarias, Chile. The Hx concentrations were determined in 5-day-old seedlings ($n = 10$ of each cultivar) grown in a cabinet at 25 ± 1°C and a 10-h light/14-h dark photoperiod. The mean Hx concentrations (± standard errors [SE]) were 9.55 ± 0.16 and 1.83 ± 0.09 mmol kg fresh wt^{-1} for *T. turgidum* subsp. *durum* cv. Alifén and *T. aestivum* cv. Talhuén, respectively; a *t* test showed that these values were significantly different ($t = 41.81$; df = 18; $P < 0.001$).

Trichoderma harzianum strain Queule was collected by E. P. Donoso at the Los Queules National Reserve (35°59′.18″S; 72°41′60′W; 500 meters above sea level) and deposited at the Phytopathology Laboratory, Universidad de Talca. Its identification was performed by Eduardo Piontelli (Mycology Laboratory, University of Valparaíso) by following Rifai's taxonomic key (33). This strain is widely used for biological control in Chile.

Cultivation of the fungus. *Trichoderma harzianum* from pure cultures in malt extract agar was inoculated on 100 g of a mixture of dry cereal seeds (wheat, oat, and corn in a 1:1:1 proportion) contained in 500-ml glass vials and was incubated at 25°C for 5 days. The seeds previously were sterilized by being autoclaved at 121°C for 30 min, thus achieving an adequate degree of humidity. Ten grams of incubated seeds was agitated at 250 rpm for 30 min in 1 liter of sterile water with 50 μl/liter of Tween 20 and then filtered through a 140-mesh sieve to separate the fungus from the grains. This conidial suspension was diluted 1:1,000 to achieve a final concentration of 1.7×10^8 conidia ml^{-1}, as determined in a Neubauer chamber.

Effect of *T. harzianum* on wheat. A two-by-two-by-two factorial design was used in which the factors were the wheat genotype (cv. Talhuén and Alifén), the presence or absence of *T. harzianum*, and the presence or absence of water deficit. Wheat seeds were individually sown in 125-ml polyethylene pots (Termomatrices, Santiago, Chile) containing a sterile substratum (sterilized by two heat treatments to 121°C for 30 min) containing a 1:1:1 mixture of peat, sand, and perlite. In the treatments with fungus, 10 ml of conidial suspension at a concentration of 1.7×10^8 conidia ml^{-1} was added immediately after sowing, thus producing a final concentration of 2.5×10^6 conidia g^{-1} of soil. Pots were grown in a greenhouse at 25 ± 4°C. Each treatment was replicated 27 times, with each replicate consisting of a pot with a single seed in order to exclude possible intraspecific competition.

Two contrasting experimental conditions of water supply were created. Immediately after being sown, pots were irrigated to 100% of field capacity; when the soil water content decreased to 40% of field capacity, the pots were again irrigated to 100% of field capacity. When the first leaf appeared, irrigation was continued as described above in the treatments without water deficit, whereas in the treatments with water deficit the irrigation to 100% of field capacity was performed when the soil water content had decreased to 20% of field capacity. Water irrigation was conducted by using independent irrigation systems that delivered the appropriate water supply to every individual plant. An evaporation plate was set up in the greenhouse as an indicator of the water evaporation rate. The timing of irrigation was decided on the basis of data from the evaporation plate and the corresponding adjustments for wheat crop and plate coefficients (1). Nutrients were added to all pots in a standard Wyre solution, first with the original substratum and subsequently through the irrigation system.

Soil-borne plant pathogens were avoided by sterilizing the substratum and using potable water for irrigation and fertilization; the absence of pathogens in soil samples was corroborated by incubating five randomly chosen soil samples from each treatment in petri dishes containing potato dextrose agar medium for 5 days at 25°C. Foliar pests (principally aphids) were excluded by using mesh around the pots; foliar diseases were avoided by eliminating grasses around the greenhouse.

The following parameters of plant performance were determined: (i) root volume, estimated by measuring the volume of water displaced after the introduction of the roots into a graduated cylinder filled with water; roots were previously cleaned by immersing them in water and gently shaking them (at 50 rpm) for 30 min; (ii) total dry biomass, excluding seeds; and (iii) fecundity, estimated as the number of seeds produced by each individual plant. All parameters were assessed in 120-day old plants. Data were $\ln(x + 1)$ transformed to satisfy analysis of variance (ANOVA) assumptions.

Effect of wheat on *T. harzianum*. To evaluate the effect of wheat on the fungus, a three-by-two factorial design was used in which the factors were the nature of the fungal growth substrate (the absence of wheat plants or the presence of wheat cultivars Alifén and Talhuén) and the presence or absence of water deficit. The water regimens were the same as those for the experiment described above. Nutrients were added to all pots, with and without plants, first with the original substratum and subsequently through the irrigation system using a standard Wyre solution. Each treatment was replicated 40 times. The population growth rate of the fungus in each replicate pot was determined on the basis of two 1-g soil samples collected from the rhizosphere, 1.5 cm away from the plant crown and 3 cm deep. The first sample was withdrawn 15 days after the seed was sown and the fungus was inoculated in order to allow for stability in the fungus population, and the second sample was withdrawn 150 days after the seed was sown. Soil samples were suspended in 200 ml sterile water, homogenized by agitation at 150 rpm for 30 min, and then subjected to serial dilutions up to 1:1,000. From these solutions, 500-μl samples were sown in 8.5-cm-diameter petri dishes (Phoenix) containing medium modified from one previously described (34) and composed of (in grams $liter^{-1}$) 1.0 $Ca(NO_3)_2$, 0.26 KNO_3, 0.26 $MgSO_4 \cdot 7H_2O$, 0.12 KH_2PO_4, 1.0 $CaCl_2 \cdot 2H_2O$, 0.05 citric acid, 2.0 sucrose, 20.0 agar, 1.0 50% Flint WG (trifloxystrobin; Bayer, Germany), 0.005 chlortetracycline, 0.004 80% Captan WG (Arvesta Corporation), 0.0025 Previcur N (propamocarb HCl; Aventis Crop Science, Germany). The petri dishes were incubated at 25 ± 0.5°C for 7 days. After the incubation period, the number of CFU, i.e., the number of all colonies originating from mycelia, conidia, and chlamydospores, in each sample was determined (34). The population growth rate of the fungus was estimated as $r = \ln(N_f/N_o)$, where N_o is the number of CFU at the first programmed assessment after the water deficit was initiated (15 days after sowing) and N_f is the number of CFU at the end of the experiment (150 days after sowing). The population growth rate of the fungus was analyzed with a two-way ANOVA in which the factors were the substrate (no plant, wheat cv. Alifén, or wheat cv. Talhuén) and irrigation regimen (with or without water deficit). Additionally, 10 randomly chosen colonies per treatment were replicated in petri dishes containing potato dextrose agar medium until conidiophores developed. The morphological characteristics of conidiophores observed under the microscope were used to confirm the identity of *T. harzianum* (33).

Physical interaction of wheat with *T. harzianum*. To assess the interaction between the fungus and the plant root, wheat seeds were sown in petri dishes with water agar. The fungus was inoculated (500 μl per petri dish of a conidial suspension containing 2.5×10^6 conidia ml^{-1}), and the dishes were incubated in the dark for 48 h at 25°C until seedling emergence, and thereafter they were grown in a growth chamber with a 12-h light/12-h dark photoperiod for 5 days. At this time, the roots were directly extracted from the petri dishes. Transversal sections of the roots were stained with lactophenol cotton blue (Sigma, Spain) and observed with a phase-contrast microscope (magnification, ×400) to determine the degree of fungus-root interaction.

Effect of Hx on *T. harzianum*. One milliliter of conidial suspension at a concentration of 1.7×10^8 conidia ml^{-1} was added to 250 ml of malt extract liquid medium containing hydroxamic acid, 2,4 dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA), the main Hx aglucone from wheat extracts, at concentrations of 0 (control), 0.25, 0.5, and 1 mM. After 5 days at 25°C and constant agitation (180 rpm), the fungal dry biomass was obtained by filtering the liquid medium, drying the solid residue in an oven at 40°C for 24 h, and weighing the sample.

RESULTS

Effects of *T. harzianum* on wheat. The irrigation regimen and the presence of fungus significantly affected the three wheat performance parameters measured (Table 1). The nature of the cultivar significantly affected the dry biomass of the plant but not the root volume or fecundity (Table 1). Given the absence of significant interactions between wheat genotype, fungus, and irrigation regimen for all of the performance variables studied (Table 1), the effects of the fungus and irrigation regimen were assessed for each wheat genotype independently.

The root volume increased significantly in the presence of fungi only in the presence of water deficit (ANOVA for cv. Alifén, $F_{1,91} = 13.53$ and $P = 0.0004$; ANOVA for cv. Talhuén, $F_{1,55} = 8.63$ and $P = 0.005$). Water deficit significantly affected the root volume of cv. Talhuén ($F_{1,55} = 45.7$ and $P < 0.001$) but not that of cv. Alifén ($F_{1,91} = 1.13$ and $P = 0.29$) (Fig. 1A).

TABLE 1. ANOVA of the effect of *T. harzianum* on various performance parameters of wheat plants

Performance parameter[a]	df	MS[b]	*F*	*P*
Fecundity (no. of seeds per plant)				
Fungus	1	4,388.03	17.05	<0.001
Irrigation regimen	1	2,760.29	10.73	0.006
Cultivar	1	80.46	0.31	0.577
Fungus and irrigation regimen	1	3,565.79	13.86	<0.001
Fungus and cultivar	1	7.03	0.03	0.869
Irrigation regimen and cultivar	1	979.13	3.81	0.053
Fungus, irrigation regimen, and cultivar	1	4.34	0.02	0.897
Error	144	257.29		
Biomass (g dry wt)				
Fungus	1	36.84	8.51	0.004
Irrigation regimen	1	124.79	28.83	<0.001
Cultivar	1	51.92	11.99	0.001
Fungus and irrigation regimen	1	3.66	0.85	0.359
Fungus and cultivar	1	1.60	0.37	0.545
Irrigation regimen and cultivar	1	2.82	0.65	0.421
Fungus, irrigation regimen, and cultivar	1	0.89	0.21	0.650
Error	144	4.33		
Root vol (ml)				
Fungus	1	107.76	19.55	<0.001
Irrigation regimen	1	160.13	29.05	<0.001
Cultivar	1	<0.001	<0.001	0.981
Fungus and irrigation regimen	1	75.53	13.70	<0.001
Fungus and cultivar	1	1.23	0.22	0.637
Irrigation regimen and cultivar	1	75.50	13.70	<0.001
Fungus, irrigation regimen, and cultivar	1	0.82	0.15	0.699
Error	144	5.51		

[a] Factors examined include fungus (presence or absence of *T. hartzianum*), irrigation regimen (occurrence or absence of water deficit), and cultivar (wheat genotype *T. turgidum* subsp. *durum* cv. Alifén or *T. aestivum* cv. Talhuén) (n = 27).

[b] MS, mean square.

The presence or absence of fungus and the irrigation regimen showed a significant interaction for both cultivars (ANOVA for cv. Alifén, $F_{1,91} = 6.21$ and $P = 0.01$; ANOVA for cv. Talhuén, $F_{1,55} = 9.25$ and $P < 0.001$). No differences were detected between the two wheat genotypes in the presence or absence of fungus ($P = 0.58$ and 0.81, respectively; by Tukey's honestly significant difference [HSD] test).

The presence of fungus affected the total dry plant biomass of cv. Talhuén ($F_{1,55} = 8.91$ and $P = 0.004$) but not that of cv. Alifén ($F_{1,91} = 2.73$ and $P = 0.102$). The irrigation regimen negatively affected the dry plant biomass of both cultivars (ANOVA for cv. Alifén, $F_{1,91} = 10.66$ and $P = 0.002$; ANOVA for cv. Talhuén, $F_{1,55} = 27.37$ and $P < 0.001$). No significant interaction was detected between the presence or absence of fungus and the irrigation regimen (ANOVA for cv. Alifén, $F_{1,91} = 0.111$ and $P = 0.740$; ANOVA for cv. Talhuén, $F_{1,55} = 1.353$ and $P = 0.250$) (Fig. 1B), and no differences in total plant biomass were detected between the two wheat genotypes in the

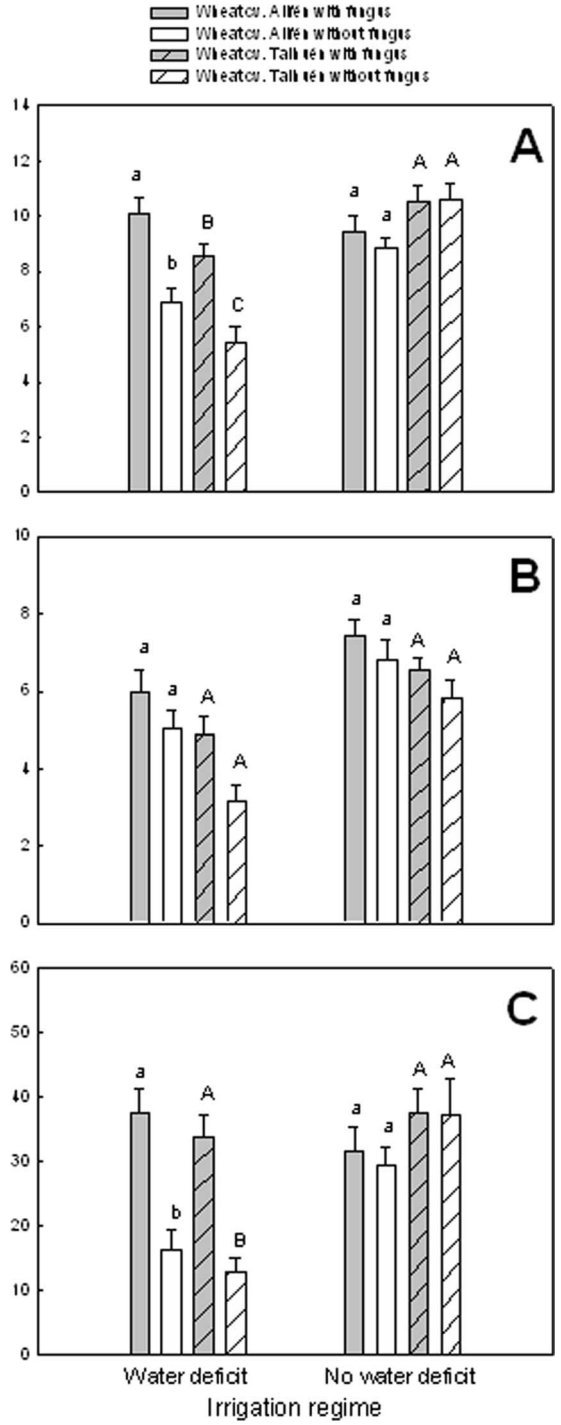

FIG. 1. Variation of performance parameters (means ± SE) of two wheat genotypes (*Triticum turgidum* subsp. *durum* cv. Alifén and. *T. aestivum* cv. Talhuén) in response to two contrasting water regimens and with or without *Trichoderma harzianum* strain Queule. (A) Root volume; (B) dry biomass; and (C) fecundity. Letters reveal the results of Tukey's HSD tests; uppercase letters are for comparisons involving *T. aestivum* cv. Talhuén, and lowercase letters are for comparisons involving *Triticum turgidum* subsp. *durum* cv. Alifén. Each treatment was replicated 27 times.

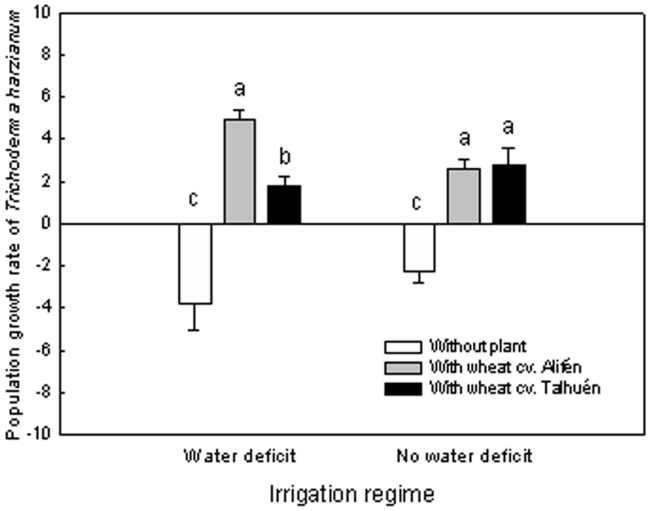

FIG. 2. Population growth rate (r) (means ± SE) of *Trichoderma harzianum* strain Queule in response to the absence or presence of wheat plants (*Triticum turgidum* subsp. *durum* cv. Alifén and *T. aestivum* cv. Talhuén) in the presence or absence of hydric stress. Letters indicate the results of Tukey's HSD tests. Each treatment was replicated 40 times.

presence or absence of the fungus ($P = 0.77$ and 0.36, respectively; Tukey's HSD test).

The number of seeds per plant increased significantly in the presence of fungus (ANOVA for cv. Alifén, $F_{1,91} = 11.83$ and $P = 0.001$; ANOVA for cv. Talhuén, $F_{1,55} = 6.62$ and $P = 0.013$). The irrigation regimen significantly affected the number of seeds per plant of cv. Talhuén ($F_{1,55} = 11.50$ and $P = 0.001$) but not of cv. Alifén ($F_{1,91} = 1.13$ and $P = 0.292$). A significant interaction was detected between the presence or absence of fungus and the irrigation regimen (ANOVA for Alifén, $F_{1,91} = 8.28$ and $P = 0.005$; ANOVA for Talhuén, $F_{1,55} = 6.25$ and $P = 0.015$). The presence of fungus increased the number of seeds per plant only under conditions of water deficit ($P = 0.03$; Tukey's HSD test) (Fig. 1C). No differences were detected between the two wheat genotypes in the presence or absence of fungus ($P = 0.99$ and 0.99, respectively; Tukey's HSD test). No plant disease symptoms were observed in the trial, and no pathogens were detected in the soil samples.

Effects of growth substrate on *T. harzianum*. The population growth rate of the fungus was significantly affected by the nature of the substrate in which it grew ($F_{2,24} = 46.71$ and $P < 0.001$) and by the interaction between the irrigation regimen and the growth substrate ($F_{2,24} = 7.18$ and $P = 0.03$), but not by the irrigation regimen alone ($F_{1,24} = 0.02$ and $P = 0.90$). Significant differences occurred between the treatment without a plant and the treatments involving wheat genotypes, with the level of growth of the fungus being higher in the presence of plants ($P < 0.001$; Tukey's HSD test); additionally, the level of fungal growth in plants under conditions of water deficit differed between the wheat genotypes ($P = 0.05$; Tukey's HSD test) (Fig. 2).

Physical interaction of wheat with *T. harzianum*. Fungal growth was observed only in the root epidermis, and no hyphal growth was observed within the internal root tissues.

Effect of Hx on *T. harzianum*. The range of variation of mean fungal biomass was 1.20 to 1.22 g. An ANOVA showed that the presence of DIMBOA in the culture medium of *T. harzianum* did not affect the fungal biomass ($F_{3,8} = 0.48$ and $P = 0.702$).

DISCUSSION

The results of this study provided unequivocal experimental evidence of a mutualistic relationship between *T. harzianum* and wheat. The results also showed that under the conditions of the study, the mutualism between *T. harzianum* and wheat was asymmetrical: wheat plants thrived in the presence or absence of the fungus, whereas *T. harzianum* showed a negative growth ratio when it grew in the absence of wheat plants, probably due to the depletion of organic matter from the soil. Moreover, the growth stimulation effect on plants occurred only under conditions of water deficit, thus supporting the general assertion that mutualistic relationships depend on the environmental context (12, 36).

Both wheat genotypes responded in the same manner to the two factors analyzed, i.e., the presence or absence of fungus and irrigation regimen (Fig. 1), and under conditions of water deficit the fungus grew at a higher rate in the presence of the water deficit-tolerant wheat genotype Alifén than in the presence of the irrigation-dependent wheat genotype Talhuén (Fig. 2). These results indicate that the mechanism by which a wheat plant is tolerant to water deficit is different from the mechanism by which a wheat plant compensates for water deficit in the presence of *T. harzianum* and that Hx are not of importance for the wheat-*Trichoderma* interaction.

While wheat tolerance to water deficit is related to dehydration avoidance, mainly by osmotic adjustments, and escape through early flowering (6), little is known about the mechanism by which the fungus enhances plant tolerance to water deficit; however, given that the interaction between the plant and the fungus occurs mainly at the rhizosphere, such a mechanism likely is related to an increase in the water absorption efficiency, which presumably is related to the increased root volume leading to increased water absorption.

Our hypotheses on the effects of Hx were based on the presumptions that (i) the roots of wheat plants having Hx in their aboveground tissues also contained Hx (39), (ii) Hx has a potentially deleterious effect on the fungus (38), and (iii) the fungus interacts with internal root tissues of the wheat plant. Observations of root cross sections of the two wheat genotypes grown in the presence of the fungus showed that *T. harzianum* grew only on the surface of the wheat roots, i.e., it did not penetrate the roots. Hence, Hx can affect the fungus only if they are present in root exudates. The presence of Hx in root exudates has been shown to be genotype dependent (30, 39, 40). In a large screening of wheat genotypes, it was found that only 19% of them exuded Hx to the growth medium (40); additionally, Hx were absent from *T. turgidum* subsp. *durum* cv. Alifén and also from *T. aestivum* cv. Andifén (29), a cultivar that shares a proximal ancestor with *T. aestivum* cv. Talhuén (24). Finally, we showed that Hx did not affect the fungal biomass in vitro. Hence, under these conditions, an effect of the wheat genotype on the growth of *T. harzianum* should not have been expected.

The strength of the mutualistic interaction detected in this

study was remarkable. For example, in wheat under conditions of water deficit, the presence of the fungus increased the plant fecundity and root volume to levels similar to those observed under conditions of no abiotic constraints. Moreover, this effect occurred in two wheat genotypes that differ in their tolerances to water deficit. Thus, this interaction may help the plant endure in habitats that take it beyond its physiological limits in the absence of the fungus. In fact, the ecological range of organisms involved in mutualism usually appears to be greater than that of the organisms living alone (i.e., a niche expansion occurs) (4). It is possible that historical associations with microorganisms that facilitate water absorption (21, 35) and/or a more efficient uptake of nutrients, as is the case for mycorrhizal fungi (31) or rhizobia (4), allows the expansion of wheat crops to more extreme xeric regions of the world. These findings may be locally relevant, as in Chile a large proportion of wheat (over 20%) is sown in unirrigated areas with relatively little rainfall (28). However, further field research is needed to elucidate the mutualistic relationship between the fungus and wheat across a range of fungus and wheat genotypes and across biogeographical gradients.

As the sign and magnitude of plant-microbe interactions are context dependent, the picture that emerges of the interactions in which these species are involved is quite complex (19). For instance, assuming that water availability is heterogeneously distributed across space (15), we expect a situation in which mutualism between the plant and the fungus will occur only in patches with water deficit; in patches without water deficit, this mutualistic relationship would not occur and the plant would not benefit from the presence of the fungus. This supports the general assertion that mutualisms occur and evolve in a geographic mosaic (37).

Mechanisms explaining how *T. harzianum* stimulates plant growth and increases plant fitness are not fully understood. Studies on induced systemic resistance to plant pathogens in response to the plant-*Trichoderma* interactions have revealed the accumulation of mRNAs associated with defense genes involved in the production and accumulation of phytoalexins (41). The growth-stimulating capacity of *Trichoderma* can result from the decreased activity of deleterious microflora, the inactivation of toxic compounds, the increase of nutrient uptake, the increased efficiency in nitrogen use, and the solubilization in the soil of nutrients such as phosphate, Fe^{3+}, Cu^{2+}, Mn^{4+}, and Zn^{0} (2); however, the genetic and molecular nature of these effects remains unknown (14).

Other plant-microorganism interactions have been described in which the ecological context is of importance for their occurrence. In leguminous plants interacting with bacteria of the genus *Rhizobium*, the formation of nodules in the roots of plants (a condition required to initiate mutualism) is possible only if the nitrogen content in the soil is low (3, 4). In plant-fungus interactions, the plant *Festuca arundinacea* (Poaceae) and endophytic fungi generate a protection mutualism only under conditions of water deficit or under extremely high herbivore pressure (8). In soils of Indian native forests, the temporal peaks of population abundance of *T. harzianum* coincide with the peaks of maximal growth of tree roots, suggesting that roots provide better conditions and resources for fungal development and growth (18). The above examples provide only correlative evidence of mutualism. To our knowledge, our study is the first to evaluate experimentally the mutualistic effect of a plant on the fungus *T. harzianum* and vice versa. The results of our study reinforce the concept of conditional mutualism (7, 16), i.e., in order for mutualism to occur, some ecological conditions must be satisfied. Under a scenario of environmental heterogeneity, mutualisms will evolve in patches of habitats that constrain the fitness of interacting species. The fitness of the species living independently will be improved by the mutualistic relationships it establishes.

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