NOTE

Nutrients and Abiotic Stress Interact to Control Ergot Plant Disease in a SW Atlantic Salt Marsh

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Abstract Over the last decades, human activities have strongly affected ecosystems, with pervasive increases in nutrient loadings, abiotic stress, and altered herbivore pressure. The evaluation of how those environmental factors interact to influence plant–pathogen interactions under natural conditions becomes essential to fully understand the ecology of diseases and anticipate the possible effects of global change on natural and agricultural systems. In a SW Atlantic salt marsh, we performed a field factorial experiment to evaluate the effect of herbivory, salinity, and nutrient availability, three main limiting factors for salt marsh plant growth, on the infection of the fungus *Claviceps purpurea* (ergot) upon the cordgrass *Spartina densiflora*. Results show that herbivory has no effect but both nutrients and salinity increase fungal infection. The combined effect of salinity and nutrients is not additive but interactive. Salinity stress increases infection at ambient nutrient levels but in combination with fertilizer it buffers the higher infection produced by increased nutrient availability. Since both, nitrogen availability and salinity are factors predicted to globally increase due to human impact on ecosystems, this interaction between environmental factors and ergot infection can have strong effects on natural and productive agricultural systems.

Keywords Plant–pathogen interactions · *Claviceps purpurea* · Ergot · Salt marshes · Nitrogen · Salinity

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Introduction

Environmental factors are widely recognized to affect plant–pathogen interactions (Colhoun et al. 1972; Schoeneweiss 1975). Abiotic stress, nutrient availability, and herbivory, for instance, can regulate the quantity and quality of food available for the pathogen, influence plant growth and development, or even affect plant resistance mechanisms, thus controlling (either positively or negatively) plant–pathogen interactions in multiple and complex ways (e.g. Singh 2002; Silliman et al. 2005; Walters and Bingham 2007). Natural systems are usually subject to a combination of environmental factors that can have separate and interactive effects on plant disease, but most of the works evaluating the effect of these factors on plant–pathogen interaction are usually focused in the isolation study of one environmental variable under agricultural or model systems (Coakley et al. 1999). In light of the continuous increase in agricultural nitrogen fertilization that runoff into natural systems, the increases of abiotic (e.g., thermal, hydric and salinity) stress and the human-induced changes in herbivory pressure, the evaluation of how multiple environmental factors influence plant–pathogen interactions under natural conditions becomes essential to fully understand the ecology of diseases and anticipate the possible effects of global change on natural and agricultural systems (Garrett et al. 2006).

Salt marshes are vegetated intertidal areas that, although very productive, are characterized by the presence of strong limiting factors for plant growth, including salinity, anoxia, nitrogen availability, and herbivory (Pennings and Bertness 2001; Jefferies et al. 2006). Those systems are also subject to periodic disease outbreaks because of high horizontal transmission rates favored in these dense, but relatively low-diversity, communities (Fisher et al. 2007; Daleo et al. 2009). This renders marshes good model systems to evaluate

the relative importance of nutrients, consumers, and abiotic stress on plant–pathogen interactions.

The ergot *Claviceps purpurea*, an ascomycetous toxic fungus, is a well-known pathogen of cereals and forage grasses. The fungus infects plant ovary and replace the tissue with a single sclerotium (known as ergot), making infected flowers unable to produce seeds (Fisher et al. 2005a). Three distinct groups or lineages are recognized within the species (Pazoutová et al. 2000). The largest group, G1, infect land grasses, G2 infect wetland grasses whereas G3 infect salt marsh grasses and is commonly associated to plants of the genus *Spartina* (Fisher et al. 2005b). Due to the wide host range and the economic damage it produces, the biology, genetics, and physiology of the fungus have been extensively studied (Tudzynsky and Shceffer 2004) but the ecology of its infectiousness in natural systems is still poorly understood.

The salt marshes occurring between southern Brazil (32° S) and northern Argentinean Patagonia (42° S) are dominated by the perennial grasses *Spartina densiflora* and *Spartina alterniflora* (Isacch et al. 2006) and by the intertidal burrowing crab *Neohelice granulata*, which inhabits the entire intertidal zone and can occur at densities higher than 60 crabs m^{-2} (Iribarne et al. 1997). Through grazing, crabs can exert strong control over marsh plant production by directly removing plant tissue (Alberti et al. 2007) as well as by facilitating fungal infection in crab-generated injuries (Daleo et al. 2009). In addition, abiotic stress (salinity) can influence the outcome of top-down (crab herbivory) and bottom-up (nutrient availability) control of plant production (Alberti et al. 2010), thereby potentially affecting resource availability for other species that use *Spartina* plants as food source. Thus, salt, nutrients, and herbivory can influence ergot–*Spartina* interactions. In this context, the objective of this work was to evaluate the separate and interactive effect of salinity, nutrient availability, and crab herbivory on the incidence of *C. purpurea* infection upon *Spartina* plants.

Methods

A fully factorial experiment was conducted in a *S. densiflora* marsh at Mar Chiquita coastal lagoon (Argentina, 37° 32′ S; 57° 19′ W). The factorial design ($2 \times 2 \times 2$) includes: herbivory manipulation (with and without crabs), salinity (with and without salt addition), and nutrients (with and without nutrient addition). The experiment started on March 2010 and each treatment combination was replicated six times (plots of 0.7×0.7 m separated by at least 1.5 m). Crab-exclusion plots were surrounded by a plastic mesh (10-mm opening) fence 0.6 m high and supported by iron stakes. Crab exclosures have been widely used in this system and the use of cage controls

revealed that there are no associated cage artifacts (e.g. Alberti et al. 2007; Daleo et al. 2007; Daleo and Iribarne 2009; Daleo et al. 2009; Alberti et al. 2010). Salt addition treatments received 20 g (40 g m^{-2}) of commercial pelletized salt spread superficially every 2 weeks. Nutrient addition treatments received 60 g (1,440 g m^{-2} $year^{-1}$) of a slow-release pelletized fertilizer (NPK, 29:5:5) monthly (see Alberti et al. 2010). Fertilizer was spread into six artificial holes (5 cm deep, 1 cm diameter) evenly distributed in each plot that were then filled with mud. In this system, crab herbivory decreases *S. densiflora* biomass nearly 20 % and nutrients addition increase it near 450 % (Alberti et al. 2010).

At the beginning of May 2011 (14 months after starting the experiment), the total number of spikes (unbranched inflorescences with flowers arranged along the axis) and the number of spikes with infection per plot was recorded (one control and one unfertilized exclosure were lost). Presence of infection was defined as the presence of at least one ergot (characteristic black sclerotium) per spike (Fisher et al. 2007). Proportion of infected spikes was then used as an estimation of infection incidence (Raybould et al. 1998). The null hypotheses of no effect of herbivory, salt, and nutrients on infection incidence were evaluated with a three-way ANOVA considering herbivory, salt, and nutrients as fixed factors. As plant-to-plant contact facilitates the spread of *C. purpurea*, spike density may contribute to infection incidence (Fisher et al. 2007), thus, spike density was included as co-variable. Post hoc comparisons were done with the Tukey's pairwise multiple comparison test. To evaluate the effectiveness of salt loading rates, sediment salinity was measured in each plot by collecting sediment samples (5 cm diameter, 8 cm deep), which were weighed, dried to constant weight, mixed with a known volume of distilled water, measured by refractometry after 48 h, and then corrected by the initial sample water volume, to reflect the original concentration of salt. Previous works in this system have proven the effectiveness of nutrient loading rates, which increased nitrates by more than an order of magnitude (1.37 ± 0.14 μM without nutrient addition vs. 85.24 ± 24.28 μM with nutrient addition, see Daleo et al. 2008).

Results

Salt addition increased pore-water salinity by near 20 % (0.015 g salt g $sediment^{-1}$ without salt addition vs. 0.019 g salt g $sediment^{-1}$ with salt addition). During the experiment, crab exclusion increased the number of live stems per plot near 50 % (i.e., from 32 ± 1.3 (mean ± SE) to 51 ± 1.6 stems per plot), nutrient addition increased it near 460 % (from 32 ± 1.3 to 147.5 ± 4.7), and salt addition decreased it near 15 % (from 32 ± 1.3 to 27.8 ± 1.5). Results show that there was no effect of

herbivory neither as a main factor ($F_{1,38}=0.54$, $P=0.5$) nor in combination with the other factors (herbivory × nutrients: $F_{1,38}=0.06$, $P=0.8$; herbivory × salt: $F_{1,38}=0.76$, $P=0.4$; herbivory × nutrients × salt: $F_{1,38}=0.01$, $P=0.9$, Fig. 1). Nutrients and salt, on the contrary, showed a significant interaction (nutrients × salt: $F_{1,38}=8.65$, $P<0.01$, Fig. 1); while salt addition increased infection at ambient nutrient levels, salt addition decreased infection levels at enriched nutrient levels (Fig. 1). Nutrient addition increased infection to a large extent when additional salt was not added, and much less so when additional salt was added (Fig. 1). Even though nutrients increased spike production more than 6-fold (from 8.4 to 52 spikes m^{-2} on average), spike density showed no effect when included as co-variate ($F_{1,37}=1.01$, $P>0.3$).

Discussion

Plants in natural systems are under the continuous influence of different biotic and abiotic stressors (Thaler and Bostock 2004). Plant response to one given stress can affect the probability of being affected by another stress (Rostás et al. 2003). Herbivores, for example, can influence pathogen infection by transporting disease between plants, inoculating disease propagules (Silliman and Newell 2003) and physically damaging plant protective barriers (Daleo et al. 2009) and even by activating plant-induced defenses (Hatcher et al. 2004). Although crabs can either directly decrease *Spartina* biomass by more than 20 % (Alberti et al. 2010), facilitate the infection of the fungus *Phaeosphaeria spartinicola* in damaged *Spartina* leaves (further reducing plant biomass; Daleo et al. 2009), and affect spike production (Canepuccia et al. 2008), our results show that there was no effect of crab exclusion on ergot incidence, suggesting that infection incidence is independent of plant biomass and that crabs do not inoculate the spores.
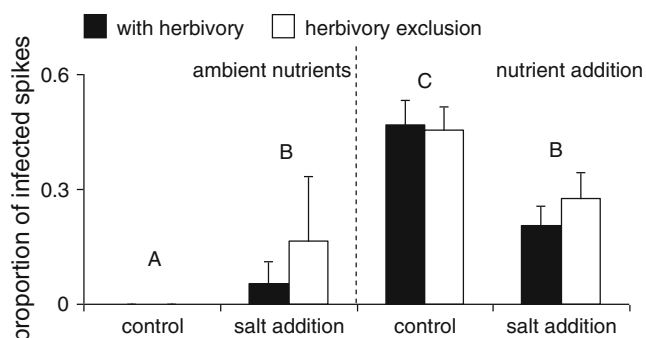

Fig. 1 The effect of crab herbivory, salinity, and nutrients on infection incidense of the pathogenic fungus *C. purpurea* on the saltmarsh plant *S. densiflora*. *Bars* are means and standard errors. *Letters* indicate significant differences ($P<0.05$) between treatments of the interaction between nutrients and salt (Tukey test)

The effect of nutrients on plant predisposition to pathogens is variable (Walters and Bingham 2007), nitrogen addition, for instance, is recognized to increase fungal disease severity in multiple ways, including increases in tissue N available as a resource for the pathogen, decreases in the production of defensive compounds, and increases in host density (see Mitchell et al. 2003 and references therein). Here we find that N fertilization can strongly increase *C. purpurea* infection on *S. densiflora*, although our experimental design does not allow us to discriminate the specific process involved. This result suggests important implications since nitrogen availability is one widespread component of global change (Vitousek et al. 1997). As *C. purpurea* infection can severely reduce seed production of infected plants (Fisher et al. 2005a, b) and produce alkaloids that decrease herbivory (Lev-Yadun and Halpern 2007), indirect effects of nutrient enrichment through increased *C. purpurea* infection can potentially affect plant community structure, as well as granivorous and herbivorous species (including livestock poisoning), and impact considerably natural and agricultural systems.

Salinity is also recognized to affect the vulnerability of plants to pathogenic fungi, mainly because the physiological response to dehydration stress involves a systemic increase of abscisic acid which is a key phytohormone in the plant response to phatogenic attacks and has been demonstrated to increase susceptibility (Xiong and Yang 2003; Atkinson and Urwin 2012). Results show that without nutrient addition, salt addition increased infection, suggesting that plants subject to salinity stress are prone to be infected by *C. purpurea*. At high nutrient levels, in contrast, salinity decreased infection. This may be because salinity negatively affects nitrogen uptake by higher plants (Linthurst and Seneca 1981; Esmaili et al. 2008). Thus, the positive effect of nutrients on infection levels is partially canceled when salinity increases. As soil salinity levels are also expected to rise due to global warming (Lynch and St. Clair 2004), especially on salt marshes (see Silliman et al. 2005), this interactive effect may ameliorate the possible effects of global change on ergot infection in natural communities.

Disease development has great between-year variability and is influenced by specific weather or geographic conditions; thus, experimental replication on other marshes or over different years should allow us to evaluate if the observed levels of infection are common. Our experimental design and results show that both nutrients and salt stress can affect *C. purpurea* infection on salt marshes and that the combined effect of the two factors is not additive but interactive given that salinity buffers the otherwise higher infection produced by increased nutrient availability. Understanding the outcome of host–pathogen interactions on the complex ecosystem context is essential to anticipate possible responses to global change (Dukes et al. 2009) and

our work highlights the importance of taking into account multiple environmental factors in order to predict human impacts on environmental stress–plant–pathogen interactions and their effects on ecosystems.

References

Alberti, J., M. Escapa, P. Daleo, O. Iribarne, B.R. Silliman, and M. Bertness. 2007. Local and geographic variation in grazing intensity by herbivorous crabs in SW Atlantic salt marshes. *Marine Ecology Progress Series* 349: 235–243.

Alberti, J., A. Mendez Casariego, P. Daleo, E. Fanjul, B.R. Silliman, M. Bertess, and O. Iribarne. 2010. Abiotic stress mediates top-down and bottom-up control in a Southwestern Atlantic saltmarsh. *Oecologia* 163: 181–191.

Atkinson, N.J., and P.E. Urwin. 2012. The interaction of plant biotic and abiotic stress: from genes to the field. *Journal of Experimental Botany*. doi:10.1093/jxb/ers100.

Canepuccia, A.D., M.S. Fanjul, M.E. Fanjul, F. Botto, and O.O. Iribarne. 2008. The intertidal burrowing crab Neohelice (=Chasmagnathus) granulata positively affects foraging of rodents in South Western Atlantic salt marshes. *Estuaries and Coasts* 31: 920–930.

Coakley, S.M., H. Scherm, and S. Chakraborty. 1999. Climate change and plant disease management. *Annual Review of Phytopathology* 37: 399–426.

Colhoun, J. 1972. Effect of environmental factors on plant diseasse. *Annual Review of Phytophatology* 11: 343–364.

Daleo, P., and O. Iribarne. 2009. Beyond competition: the stress-gradient hypothesis tested in plant–herbivore interactions. *Ecology* 90: 2368–2374.

Daleo, P., E. Fanjul, A. Mendez Casariego, B.R. Silliman, M.D. Bertness, and O. Iribarne. 2007. Ecosystem engineers activate mycorrhizal mutualism in saltmarshes. *Ecology Letters* 10: 902–908.

Daleo, P., J. Alberti, A. Canepuccia, M. Escapa, E. Fanjul, B.R. Silliman, M.D. Bertness, and O. Iribarne. 2008. Micorrizal fungi determines salt marsh plant zonation depending on nutrient supply. *Journal of Ecology* 96: 431–436.

Daleo, P., B. Silliman, J. Alberti, M. Escapa, A. Canepuccia, N. Peña, and O. Iribarne. 2009. Grazer facilitation of fungal infection and the control of plant growth in south-western Atlantic salt marshes. *Journal of Ecology* 97: 781–787.

Dukes, J.S., J. Pontius, D. Orwig, J.R. Garnas, V.L. Rodgers, N. Brazee, B. Cooke, K.A. Theoharides, E.E. Stange, R. Harrington, et al. 2009. Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America: What can we predict? *Canadian Journal of Forest Research* 39: 231–248.

Esmaili, E., S.A. Kapourchal, M.J. Malakouti, and M. Homaee. 2008. Interactive effect of salinity and two nitrogen fertilizers on growth and composition of sorghum. *Plant, Soil and Environment* 54: 537–546.

Fisher, A.J., T.R. Gordon, and J.M. DiTomaso. 2005a. Geographic distribution and diversity in Claviceps purpurea from salt marsh habitats and characterization of Pacific coast populations. *Mycological Research* 109: 439–446.

Fisher, A.J., J.M. DiTomaso, and T.R. Gordon. 2005b. Intraspecific groups of Claviceps purpurea associated with grass species in Willapa Bay, Washington, and the prospects for biological control of invasive Spartina alterniflora. *Biological Control* 34: 170–179.

Fisher, A.J., J.M. DiTomaso, T.R. Gordon, B.J. Aegerter, and D.R. Ayres. 2007. Salt marsh Claviceps purpurea in native and invaded Spartina marshes in northern California. *Plant Disease* 91: 380–386.

Garrett, K.A., S.P. Dendy, E.E. Frank, M.N. Rouse, and S.E. Travers. 2006. Climatic change effects on plant disease: genome to ecosystems. *Annual Review of Phytophatology* 44: 489–509.

Hatcher, P.E., J. Moore, J.E. Taylor, G.W. Tinney, and N.D. Paul. 2004. Phytohormones and plant-herbivore-pathogen interactions: integrating the molecular with the ecological. *Ecology* 85: 59–69.

Iribarne, O., A. Bortolus, and F. Botto. 1997. Between-habitat differences in burrow characteristics and trophic modes in the southwestern Atlantic burrowing crab Chasmagnathus granulata. *Marine Ecology Progress Series* 155: 137–145.

Isacch, J.P., C.S.B. Costa, L. Rodríguez-Gallego, D. Conde, M. Escapa, D.A. Gagliardini, and O.O. Iribarne. 2006. Distribution of saltmarsh plant communities associated with environmental factors along a latitudinal gradient in the south-west Atlantic coast. *Journal of Biogeography* 33: 888–900.

Jefferies, R.L., A.P. Jano, and K.F. Abraham. 2006. A biotic agent promotes large-scale catastrophic change in the coastal marshes of Hudson Bay. *Journal of Ecology* 94: 234–242.

Lev-Yadun, S., and M. Halpern. 2007. Ergot (Claviceps purpurea)—an aposematic fungus. *Symbiosis* 43: 105–108.

Linthurst, R.A., and E.D. Seneca. 1981. Aeration, nitrogen and salinity as determinants of Spartina alferniflora Loisel. growth response. *Estuaries* 4: 53–63.

Lynch, J.P., and S.B. St. Clair. 2004. Mineral stress: the missing link in understanding how global climate change will affect plants in real world soils. *Field Crops Research* 90: 101–115.

Mitchell, C.E., P.B. Reich, D. Tilman, and J.V. Groth. 2003. Effects of elevated CO2, nitrogen deposition, and decreased species diversity on foliar fungal plant disease. *Global Change Biology* 9: 438–451.

Pazoutová, S., J. Olsovská, M. Linka, R. Kolínská, and M. Flieger. 2000. Claviceps purpurea populations, chemoraces and habitat specialization. *Applied and Environmental Microbiology* 66: 5419–5425.

Pennings, S.C., and M.D. Bertness. 2001. Salt marsh communities. In *Marine community ecology*, ed. M.D. Bertness, S.D. Gaines, and M. Hay, 289–316. Sunderland: Sinauer.

Raybould, A.F., A.J. Gray, and R.T. Clarke. 1998. The long-term epidemic of Claviceps pupurea on Spartina anglica in Poole Harbour: pattern of infection, effects on seed production and the role of Fusarium heterosporum. *New Phytologist* 138: 497–505.

Rostás, M., M. Simon, and M. Hilker. 2003. Ecological cross-effects of induced plant responses towards herbivores and phytopathogenic fungi. *Basic and Applied Ecology* 4: 43–62.

Schoeneweiss, D.F. 1975. Predisposition, stress, and plant disease. *Annual Review of Phytopatology* 13: 193–211.

Silliman, B.R., and S.Y. Newell. 2003. Fungal farming in a snail. *Proceedings of the National Academy of Science of the United States America* 100: 15643–15648.

Silliman, B.R., J. van de Koppel, M.D. Bertness, L. Stanton, and I. Mendelsohn. 2005. Drought, snails, and large-scale die-off of southern U.S. salt marshes. *Science* 310: 1803–1806.

Singh, K. 2002. Transcription factors in plant defense and stress responses. *Current Opinion in Plant Biology* 5: 430–436.

Thaler, J.S., and R.M. Bostock. 2004. Interactions between abscisic-acid mediated responses and plant resistance to pathogens and insects. *Ecology* 85: 48–58.

Tudzynski, P., and J.A.N. Scheffer. 2004. *Claviceps purpurea*: molecular aspects of a unique pathogenic lifestyle. *Molecular Plant Pathololology* 5: 377–388.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7: 737–750.

Walters, D.R., and I.J. Bringham. 2007. Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. *Annals of Applied Biology* 151: 307–324.

Xiong, L., and Y. Yang. 2003. Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid–inducible mitogen-activated protein kinase. *The Plant Cell* 15: 745–759.