Field interactions between ozone and naturally occurring fungal disease in maize inbreds

Barrios-Perez, Ilse [1*]; Eastburn, Darin M [1]; Leakey, Andrew D.B [1]; Ainsworth, Elizabeth A. [1,2]; McIntyre, Lauren M. [3]; Brown, Patrick J [1]

(1) University of Illinois at Urbana-Champaign, IL (2) USDA ARS, Urbana-IL (3) University of Florida, Gainesville, FL

*brrsprz2@illinois.edu

www.maize03face.illinois.edu

@amaizeingO3

Introduction

Tropospheric ozone (O_3) is an air pollutant whose rising concentrations cost millions in crop losses worldwide annually. The physiological effects of elevated [O_3] on maize are beginning to be studied; however, there is evidence for a wide range of genotypic diversity in susceptibility, indicating the possibility of breeding for O_3 tolerance.

The change in [O_3] brings increased complexity to field stress interactions, which change across different years and sites leading to challenges in their study. Free Air Concentration Enrichment (FACE) facilities, which allow control of atmospheric gases in an open field setting, provide a useful tool for research.

Specifically, characterizing interactions between specific crop pathogens and elevated ozone can help understand O_3 responses and inform future pest management programs, by identifying the main level of interaction. Since O_3 and pathogen responses can interact on different levels:

⇒On the leaf surface level O_3 results in stomatal closure, which are the point of entry for certain pathogens.

⇒On a cellular level O_3 degrades into diverse Reactive Oxygen Species (ROS), mimicking the "Oxidative Burst" response to pathogen attack.

⇒On a whole plant level, diverse physiological responses to O_3 lead to accelerated plant senescence, affecting tissue availability for pathogen nutrition (e.g. biotrophs feed on live cells; necrotrophs, on dead cells; hemibiotrophs switch lifestyles)

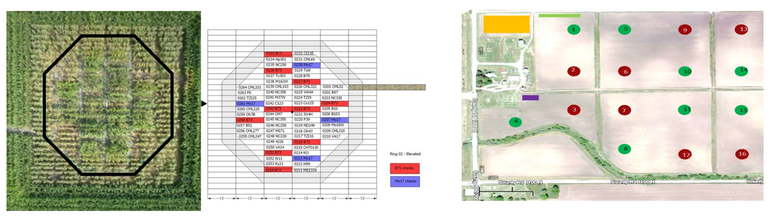
A disease survey was conducted within a germplasm O_3-tolerance study, to evaluate whether elevated ozone concentrations affected the incidence and severity of naturally occurring disease. In addition, if a genotype-independent ozone effect was present, what level of response was driving the interaction.

Experimental Design

The FACE system delivers ozone using octagonal pipe rings around the crop canopy and real-time measurement of natural wind direction and speed in order to meet a target gas concentration. Single-row 3.3m plots of maize inbreds were grown across 8 FACE rings at the SoyFACE facility in Savoy, IL during the summers of 2013-2015. Rings were paired in blocks of one ambient (~40 ppb) as control and one elevated [O_3] (~100 ppb) ring as treatment. Genotype layout was identical for both rings within a block. Number of genotypes was reduced (and replication increased) as a subset of tolerant/sensitive lines were chosen from the previous year's lines.

The O_3 fumigation took place for 8 h each day from shortly after emergence until physiological maturity. In 2014, the system maintained elevated [O_3]within 20% of the set-point for 80% of the time.

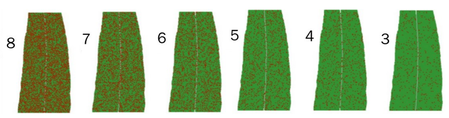
Year	Genotypes	N (paired rows)
2013	204	2
2014	51	4
2015	10	8/5

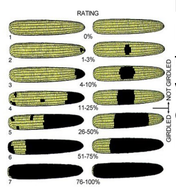


Aerial views of a single ring layout and the ring layout within the field

Traits

Foliar disease: Naturally occurring foliar disease was scored at two time points, after fifty (Round 1) and eighty percent (Round 2) of the lines had reached anthesis. At each time point two independent scorers evaluated the primary ear leaf of the first eight plants in each row for visual damage using a 9 point severity scale, where 0 = no lesions and 9 = dead. Multiple diseases could be evaluated in one plot, so each foliar disease was treated as a single trait.





Ear rot: Fungal ear rot was evaluated directly after a staggered hand harvest scheduled 8-10 weeks after anthesis. All ears from the first eight plants in each row were evaluated on a 6 point severity scale, where 0 = no rot present and 9 = complete ear covered in rot. Ear rot was evaluated as a single trait with additional notes regarding the specific types observed

Stalk rot: Stalk rot was evaluated as a presence/absence trait to be used as a proxy on plant stress. The same eight plants that were harvested. Plants were pushed to ~35° and proportion of lodged plants was recorded.

Statistical Analysis

Individual genotype responses: A normalized genotype response value was calculated using the scores from paired rows from each block:

(Ambient - Elevated)/(Ambient + Elevated)

This leads to negative values representing more disease incidence under Elevated [O_3] and positive values more disease incidence under Ambient [O_3]

Overall O_3 response: A generalized linear mixed model was fit to mean ring scores. The model for a single trait and year:

*Score = Maturity covariate + [O_3] + Genotype + Round + Block + [O_3]*Genotype + [O_3]*Block +E*

Fit in ASReml-R, with Blocks and their interactions as random effects. A negative binomial distribution and a first-order autoregressive (AR1) variance-covariance structure were specified, in order to account for non-normal and correlated residuals.

The multiple year model was fit to the foliar disease scores of the final subset of 10 genotypes, with the same specifications:

Score = Maturity covariate + Year + [O_3]+ Genotype+ Year[O_3] + [O_3]*Genotype + Year*Genotype + [O_3]*Year*Genotype + Block(Year) + Scorer + E*

Correlations: Phenotypic correlations were calculated from raw scores, and genotypic correlations from the genotypic estimates from the multiple year model.

Results: Per Trait/Year

Foliar Disease: Foliar diseases were evaluated all three seasons and the most commonly observed foliar diseases were Common Rust (*Puccinia sorghi;* biotroph), Northern Corn Leaf Blight (*Exserohilum turcicum,* hemibiotroph), Brown Spot (*Physoderma maydis,* biotroph) and Gray Leaf Spot (*Cercospora zeae-maydis*, necrotroph).

During 2013 only Common Rust showed a significant genotype-independent effect of elevated [O_3]. During 2014 Brown Spot showed a highly significant and strong overall [O_3] effect for lower severity under elevated [O_3]. Northern Leaf Blight showed a weaker and opposite response with higher severity under elevated [O_3].

During 2015 both Common Rust and Brown Spot showed responses similar to those of 2014. Northern leaf blight also showed a similar, although not significant at α=0.05, response to that of 2014.

Trait	2013	2014	2015
Foliar Disease: Common Rust	*	NS	**
Foliar Disease: Northern Leaf Blight	NS	•	NS
Foliar Disease: Brown Spot		***	***
Foliar Disease: Gray Leaf Spot	NS		NS
Foliar Disease: Goss's Wilt			**
Ear Rot		NS	NS
Stalk Rot	***		***

*p< 0.001 "***" 0.001 "**" 0.01 "*" 0.05 "•" 1 "NS"*

For significant traits: Blue cells indicate more disease incidence under Ambient [O_3] ; while yellow cells indicate more disease incidence under Elevated [O_3] . Blank cells indicate the trait was not measured during that field season, either due to absence (foliar disease) or technical difficulties (ear rot and *stalk rot)*

Goss's Bacterial Wilt (*Clavibacter michiganensis subsp. nebraskensis)* was present mostly in a single inbred, and was significantly more severe under elevated [O_3].

Ear Rot: Ear rot was recorded as a single trait, however rots present in the field included Diplodia (*Stenocarpella maydis)*, *Gibberella zeae* and *Fusarium verticillioides* ear rots. Ear rot was scored in 2014 and 2015. In both seasons there was not a significant genotype-independent effect of elevated [O_3]. Most of the variation was between genotypes.

Stalk Rot: Stalk rot was evaluated in 2013 and 2015. During both seasons there was a strong genotype-independent O3 effect, with higher incidence of stalk rot under Elevated [O_3]. This is consistent with the use of the trait as a proxy to measure plant stress, via the remobilization of stalk carbohydrates to the grain sink when photosynthesizing tissue is reduced, leaving the stalk susceptible to opportunistic rots.

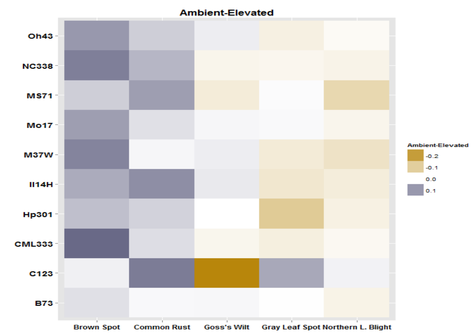
Results: 3 Year Foliar Disease

Disease scores for the final ten genotype subset from all three years were fitted using a multiyear model. Both Brown Spot and Common Rust had significant overall responses to [O_3]. Northern Leaf Blight showed a consistent trend, although not significant at the specified level. Common Rust shows a Year*[O_3] interaction. Non-significant Year*[O_3]*Genotype reflect consistent inbred responses between years.

Phenotypic and Genotypic correlations. Upper triangle: Genetic correlations. Lower triangle: Phenotypic correlations. In bold: Significant at p>0.05

NLB	-0.08	-0.11
-0.11	CRU	-0.14
-0.04	**-0.25**	BRS

Normalized score differences between treatments for each inbred/disease. Positive (blue) values indicate more disease incidence under Ambient [O_3] ; while negative (yellow-brown) values indicate more disease incidence under Elevated [O_3]

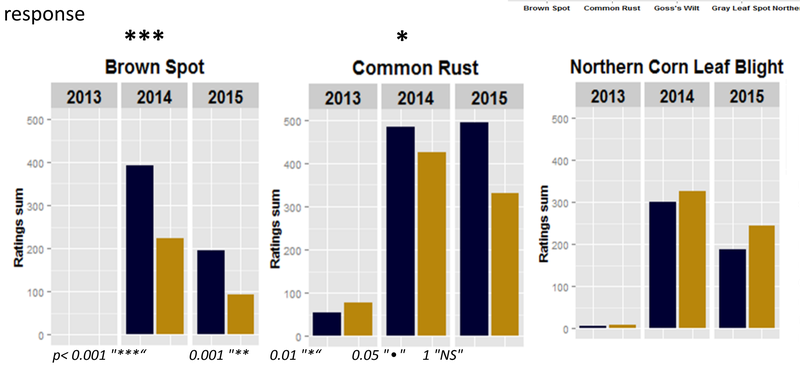


Disease correlations: Phenotypic and genotypic correlations between the three main diseases. Except for a mildly negative phenotypic correlation between Common Rust and Brown Spot, none of the correlations proved significant. Lack of correlation suggests pathogen-specific mechanisms, in spite of the common direction of the response between them.

Environmental Influence: 2014 and 2015 showed remarkably stronger effects due to environmental conditions conducive to fungal disease development

Weather patters for surveyed seasons. 2014 and 2015 are considered high-disease pressure years in comparison to 2013, given the above-average precipitation recorded, coupled with temperatures conducive to fungal pathogen development.

Year	Rain (in.)	Mean Temp (°F)
2013	13.54	69.6
2014	21.29	69.2
2015	19.45	70.5



*p< 0.001 "***" 0.001 "**" 0.01 "*" 0.05 "•" 1 "NS"*

Foliar disease incidence/ severity per year.

Total sum of ratings for each foliar disease that showed a significant responses to elevated O3 according to the multi-year model fitted on the values of the final 10 genotype set of inbreds.

Conclusions

⇒Interaction between O_3 and stresses appears to be specific to both a genotype's basal susceptibility to a particular pathogen, and to the oxidative stress itself. Genotype-independent signals of O_3 only appear significant in high disease-pressure environments

⇒When present, overall O_3 effect appears to correlate with a pathogen's liferstyle: biotrophic pathogens like Brown Spot and Common Rust show lower severity under high O_3, while the hemibiotrophic Northern Leaf Blight shows a weaker trend towards higher severity under elevated O_3. Stalk rot incidence, associated with loss of photosynthesizing tissue, also showed increased incidence under elevated O_3.

⇒However, given the difference between pathogens that responded similarly in the survey, actual mechanisms may be pathogen-specific

⇒Future work will consist of growth chamber studies to confirm and relate these responses to field observations. Further studies with the same set of inbreds and a variety of other oxidative stresses can help clarify the mechanisms behind responses in common.

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. (PGR-1238030). We thank the rest of the Maize O3 collaborators: Brad Dalsing, Gorka Erice, Christopher Montes, Alison Morse, Lorena Rios-Acosta, Crystal Sorgini, Tiago Tomaz, Craig Yendrek, Linda Young and Jessica Wedow; as well as the technical support at the SoyFACE facility.