Fusariosis de la espiga en trigo: interacción patógeno-huésped y estrategias para el manejo integrado

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Maestría en Ciencias Agrarias
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Dedicatoria

Dedico este trabajo a mí familia, que siempre ha estado ahí para apoyarme y que siempre ha creído en mí. Pero dedico este trabajo especialmente a mi tío, Rafael Enrique Bonilla Forero, que sé que, desde el cielo, así como lo estuvo en vida, está orgulloso de mí; a mis abuelos, María del Carmen Forero de Bonilla y Enrique Bonilla Zea, y a mis primas, Paola Alejandra Bonilla Díaz y Rafaela Bonilla Torres, que han sido fuertes en su ausencia.

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Resumen

La fusariosis de la espiga (FE), causada principalmente el complejo de especies de *Fusarium graminearum* (FGSC), representa una de las limitantes más importantes para la producción de trigo (*Triticum aestivum* L.). Esta enfermedad no sólo reduce el rendimiento de grano y la calidad física e industrial de este, sino que compromete además la inocuidad del grano debido a la producción de micotoxinas, en especial tricotecenos tipo B. La especie filogenética más frecuente en nuestro país es *F. graminearum sensu stricto* quimiotipo 15ADON, aunque se han registrado otras con capacidad potencial de producir NIV. El objetivo de esta tesis fue estudiar la interacción entre distintas especies del FGSC y genotipos de trigo, así como evaluar el efecto del uso de distintas estrategias para el manejo integrado en el control de FE, inocuidad en el grano y variables productivas. La primera parte de esta tesis evaluó la agresividad de nueve aislados representativos del FGSC, caracterizados por agresividad y quimiotipos 15ADON y NIV, sobre tres materiales de trigo de resistencia genética diferencial a FE, se demostró que la agresividad de los aislados varía de acuerdo con la resistencia del genotipo de trigo. También se analizó el efecto de la combinación de distintas estrategias de manejo en el control de FE; para ello se condujeron experimentos a campo, durante dos años consecutivos (2018 y 2019), combinando resistencia de cultivar, principios activos de los fungicidas y momento de aplicación. Se concluyó que, a bajos niveles de FE, la resistencia del genotipo de trigo es suficiente para controlar la FE y obtener bajos niveles de DON, mientras que la aplicación de fungicida es necesaria para alcanzar niveles aceptables de DON y calidad física aceptable del grano en años epidémicos. No fueron detectados residuos de ninguno de los principios activos analizados por encima del límite máximo de residuos (LMR).

PALABRAS CLAVE: *Fusarium*, trigo, Deoxinivalenol (DON), quimiotipos, resistencia

Summary

Fusarium Head Blight of wheat: Pathogen-host interaction and integrated management strategies

Fusarium Head Blight (FHB), mainly caused by the *Fusarium graminearum* species complex (FGSC), is a significant constraint to wheat (*Triticum aestivum* L.) production. This disease not only reduces grain yield and its physical and industrial quality but also jeopardizes grain safety due to the production of mycotoxins, especially type B trichothecenes. The most prevalent phylogenetic species in our country is *F. graminearum sensu stricto*, chemotype 15ADON, although others with the potential to produce NIV have been identified. The objective of this thesis was to study the interaction between different FGSC species and wheat genotypes, as well as to evaluate the effect of using various integrated management strategies on the control of FHB, grain safety and production variables. The first part of this thesis assessed the aggressiveness of nine representative isolates of the FGSC, characterized by aggressiveness and with 15ADON and NIV chemotypes, on three wheat materials with differential genetic resistance to FHB. The results demonstrated that isolate aggressiveness varies according to the resistance of the wheat genotype. The study also analyzed the effect of combining different management strategies on FHB control. Field experiments were conducted over two consecutive years (2018 and 2019), combining cultivar resistance, fungicide active principles and moment of application. It was concluded that, at low FHB levels, wheat genotype resistance is sufficient to control FHB and achieve low levels of DON. However, fungicide applications are necessary to reach acceptable levels of DON and maintain acceptable grain physical quality in epidemic years. No residues of any analyzed active principles were detected above the maximum residue limit (MRL).

KEYWORDS*:* *Fusarium*, wheat, Deoxynivalenol (DON), chemotypes, resistance

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1. Introducción

1.1. El cultivo de trigo

El trigo (*Triticum aestivum* L.) es el cultivo más importante en el mundo, según el área cosechada, con un promedio anual de 217,4 millones de hectáreas entre los años 2000 y 2020, y el cuarto cultivo en cuanto a producción, con un promedio de 673 millones de toneladas anuales para el mismo período (FAO, 2022).

Los principales países productores son China, India, Rusia, Estados Unidos y Francia, que en conjunto son responsables del 51,7 % (347,75 millones de toneladas) de la producción anual mundial. En el Cono Sur americano, en total se producen en promedio 23 millones de toneladas anuales y los cinco mayores productores son Argentina, Brasil, Chile, Paraguay y Uruguay, en orden decreciente de producción, representando un 98,2 % de la producción latinoamericana, según el promedio anual entre los años 2000 y 2020 (FAO, 2022).

Si bien la producción de trigo uruguaya representa apenas un 3,6 % de la producción latinoamericana anual (FAO, 2022), dentro del grupo de los cultivos cerealeros e industriales, hasta 2022, el trigo se ha mantenido en segunda posición en cuanto a área sembrada, por detrás de la soja, siendo así el cultivo de invierno más importante (DIEA y MGAP, 2023). El principal destino del grano de trigo es el abastecimiento de la demanda nacional, y, a pesar de que se observa una tendencia al descenso, el excedente se exporta, siendo Brasil el principal importador de trigo uruguayo (OPYPA y MGAP, 2019).

Estos datos son un claro reflejo de la importancia del trigo tanto para la alimentación humana como para la animal. Sin embargo, hay muchos factores que afectan su rendimiento y calidad. Las enfermedades representan uno de los factores limitantes para la producción en el país y son una de las principales causas de retiro de variedades

comerciales en producción (desuso o eliminación). Entre las enfermedades de mayor importancia se encuentra la fusariosis de la espiga (FE) o FHB (Fusarium Head Blight), según sus siglas en inglés, causada por especies de hongos del género *Fusarium*.

1.2. Fusariosis de la espiga

La FE en trigo es causada por diferentes especies del género *Fusarium*, entre las que se destacan las pertenecientes al complejo *Fusarium graminearum* (cuya forma sexual se conoce como *Gibberella zeae* Schw. & Petch). Es una de las enfermedades más destructivas, causante de importantes pérdidas económicas en el mundo en trigo y cebada, asociadas a la reducción de rendimiento, calidad e inocuidad del grano (D'Angelo et al., 2014; Paul et al., 2018; Pereyra, Castro, et al., 2014; Ward et al., 2008).

En una encuesta realizada por la revista *Molecular Plant Pathology* a 495 patólogos, miembros de la comunidad científica, sobre cuáles eran las enfermedades fúngicas vegetales más relevantes globalmente, *F. graminearum* Schwabe resultó en cuarta posición en importancia (Dean et al., 2012), lo que demuestra que este patógeno es una gran preocupación mundial y que es necesario seguir generando información y herramientas para combatirlo.

En Uruguay, la FE se reportó por primera vez en 1928 (Boerger, 1928). Sin embargo, la primera gran epidemia registrada fue en 1977, cuando, en conjunto con otras enfermedades, se estimó una reducción del rendimiento nacional de hasta el 50 % (Díaz y Kohli, 1997). Las estimaciones de pérdidas del rendimiento en trigo exclusivamente debidas a FE alcanzaron hasta un 31 % (Díaz, 1996).

En este país, la FE fue considerada una enfermedad secundaria hasta hace unas décadas. En un estudio realizado por Tavella et al. (1979), se observó que en un período

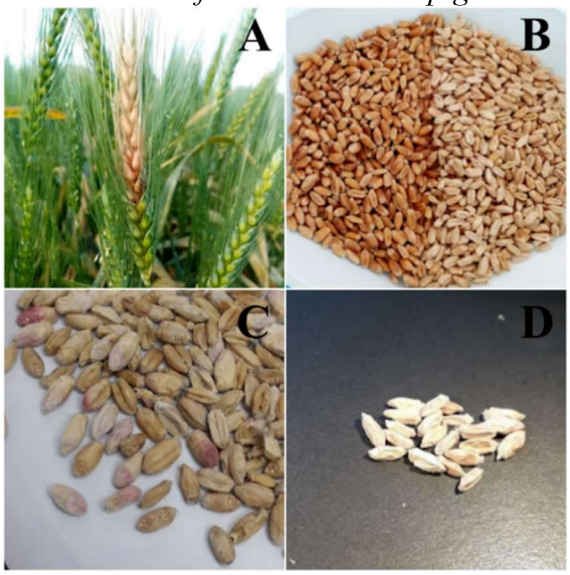
de 63 años (1915-1977) se daba un año favorable para la FE cada 16; así, esta enfermedad era esporádica. Sin embargo, entre 1977 y 1994, se dieron condiciones óptimas para la aparición de la enfermedad en tres zafras, lo que determinó una mayor frecuencia que varió las cifras a un año favorable de cada 11 (considerando el periodo comprendido entre 1914-1993) (Díaz, 1996). Ya durante la última década del siglo XX (hasta el 2001), la enfermedad fue importante durante cinco años, evidenciando un aumento en su frecuencia (Díaz et al., 2002). En la década de los 2000, en un año de cada dos, en promedio, se han registrado brotes de FE moderados o severos, que comprometieron el rendimiento del grano, así como la calidad, su comercialización y la de sus productos (Umpiérrez et al., 2011).

1.3. Sintomatología

El principal síntoma observable de la FE es una coloración blanquecina a pajiza de las espiguillas infectadas, producto de la necrosis de los tejidos y un secado prematuro (figura 1A). En condiciones muy favorables, la infección avanza desde el punto de infección hacia las espiguillas adyacentes. Sobre las glumas o bases de las espiguillas es posible visualizar una coloración salmón-anaranjada, correspondiente a masas de conidios (esporas asexuales). Sobre el final del ciclo, próximo a cosecha es posible visualizar peritecios de color negro-azulado (estructuras donde se producen las esporas sexuales o ascosporas) (Pereyra, Acosta, et al., 2014).

Además de los síntomas, es posible apreciar los efectos de la FE poscosecha, ya que esta enfermedad reduce la calidad física, los granos infectados son blanquecinos y menos desarrollados o chuzos (figura 1B, 1C y 1D). A pesar de que la FE compromete el rendimiento y la calidad física e industrial del grano, su mayor peligro reside en la acumulación de micotoxinas.

Figura 1.
Síntomas de la fusariosis de la espiga.



Nota. **A.** Espiga infectada. **B.** Granos sanos a izquierda y granos enfermos a derecha. **C.** Granos enfermos, algunos recubiertos con micelio salmón-rosa. **D.** Granos chuzos con *Fusarium* spp.

1.4. Importancia económica

La FE tiene un impacto directo en el número, tamaño y peso de los granos (Alconada y Kikot, 2013), lo que se traduce en menor rendimiento y peso hectolítrico (Brar et al., 2019; Salgado et al., 2014, 2015). Se han cuantificado reducciones de rendimiento de hasta un 50 %, las cuales se traducen en importantes pérdidas económicas (Agrios, 2005; D'Angelo et al., 2014; Paul et al., 2018; Pereyra, Castro, et al., 2014; Ward et al., 2008).

La FE puede disminuir también el vigor y germinación de la semilla (D'Angelo et al., 2014; Reynoso et al., 2013) y afectar la calidad industrial del grano mediante la reducción del contenido de proteína en este y alterando la proporción de gluteninas que influyen en la producción del gluten (Dexter et al., 1997). Además, produce exoenzimas, como las amilasas, que afectan la panificación (Pereyra, Castro, et al., 2014).

1.5. Ciclo biológico de la FE

Se requiere que tres factores interactúen entre sí para que se produzca una enfermedad en cultivos: un huésped susceptible, el agente patógeno y condiciones ambientales óptimas predisponentes (Agrios, 2005). En el caso de la FE, sus principales huéspedes son diferentes especies de gramíneas, entre las que se destacan trigo y cebada. El momento de mayor susceptibilidad va desde la floración (antesis), estadio Z61 según escala de Zadoks et al. (1974) hasta grano acuoso, Z85 (McMullen et al., 2012). Las condiciones ambientales juegan un rol fundamental en la FE; se consideran condiciones favorables para la infección: humedad relativa mayor a 80 %, temperaturas entre 10 °C y 30 °C y además espigas mojadas durante dos a tres días (Díaz et al., 2002; Panwar et al., 2017; Rossi et al., 2001).

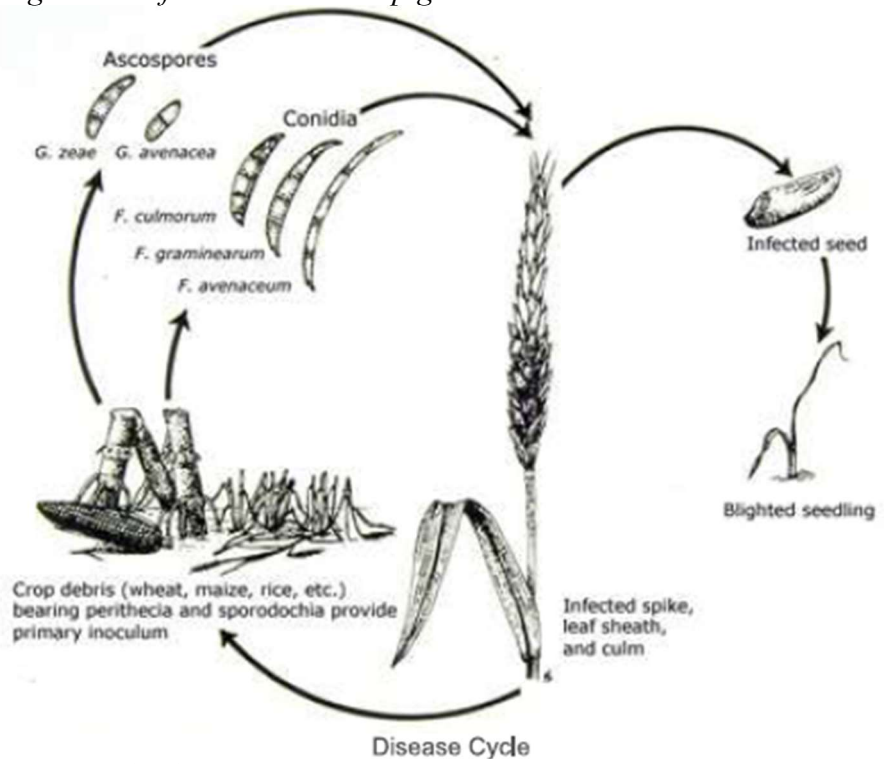
La FE es una enfermedad monocíclica, con una fase patogénica y otra saprofítica en los rastrojos infectados (Alconada y Kikot, 2013). En la etapa inicial, las ascosporas cumplen el papel de inóculo primario, estas alcanzan las anteras de las espigas durante la floración, y, si las condiciones son óptimas, se produce la infección inicial. Una vez colonizada una espiguilla, la FE puede avanzar por la espiga en forma tanto apical como basípeta (Alconada y Kikot, 2013; Pereyra, Castro, et al., 2014). Si dicha infección es lo suficientemente temprana (en antesis), acabará por impedir el desarrollo del grano. Sin embargo, también se pueden producir infecciones posteriores a la antesis y durante la fase de llenado de grano; como resultado, se generan granos desde chuzos a aparentemente sanos, pero contaminados por micotoxinas (Alconada y Kikot, 2013; Pereyra, Castro, et al., 2014; Schmale y Bergstrom, 2010).

Las anteras juegan un rol fundamental en la infección de la FE (Steiner et al., 2017; Strange y Smith, 1971; Tekle et al., 2012). Dos compuestos presentes en las anteras, colina y betaína, estimulan la germinación de esporas y el crecimiento del micelio (Strange y Smith, 1971). Las características de los tejidos de las anteras, su exposición y la mayor humedad allí retenida pueden además facilitar la entrada del hongo en la espiguilla y su crecimiento. Sin embargo, la antesis no es indefinida durante el ciclo:

Reis (1989) observó una duración de hasta cuatro días en una espiga, 12 en una planta y 30 en un cultivo, lo que refleja que hay una amplia ventana de infección para la FE. Se ha determinado una correlación directa entre el nivel de infección por FE y la presencia de anteras extendidas (Reis et al., 2016).

Tras la cosecha, *F. graminearum* podrá continuar el ciclo de dos formas (figura 2): en primer lugar, sobreviviendo de forma saprofítica en el rastrojo tanto de gramíneas como no gramíneas (trigo, cebada, sorgo, maíz, girasol, etc.); en segundo lugar, en situaciones en las que los granos contaminados llegaran a sembrarse, podrían verse comprometidas la germinación y el vigor de la semilla, y las plántulas podrían marchitarse (Schmale y Bergstrom, 2010). Es importante resaltar que se ha logrado aislar la forma sexual de *F. graminearum* (*G. zeae*) de rastrojo hasta tres años poscosecha (Pereyra, Castro, et al., 2014; Pereyra y Dill-Macky, 2008), así como que el inóculo en la semilla no tiene importancia epidemiológica para la FE.

Figura 2.
Ciclo biológico de la fusariosis de la espiga.

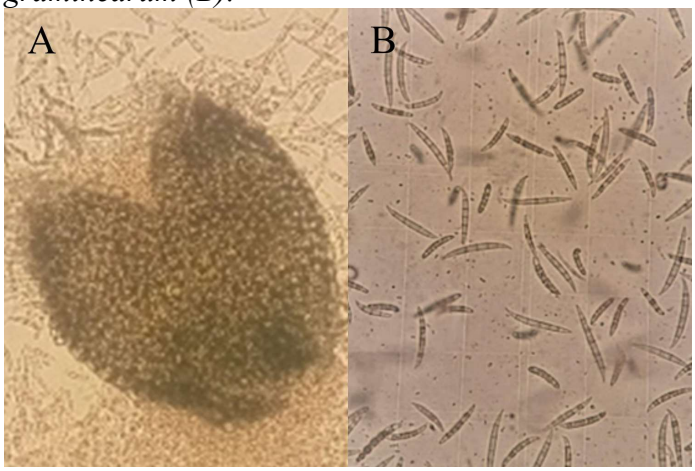


Nota. Schmale y Bergstrom (2010).

1.6. Complejo de especies de *Fusarium graminearum*

Fusarium graminearum es la forma en la que se conoce al anamorfo o forma asexual de *Gibberella zeae*. Esta especie pertenece al phylum ascomycota y, por lo tanto, su reproducción sexual se produce mediante la formación de ascosporas en los peritecios (figura 3A). Por otro lado, durante la reproducción asexual se forman macroconidios (figura 3B).

Figura 3.
Peritecios y ascosporas de Giberella zeae (A) y Macroconidios de Fusarium graminearum (B).



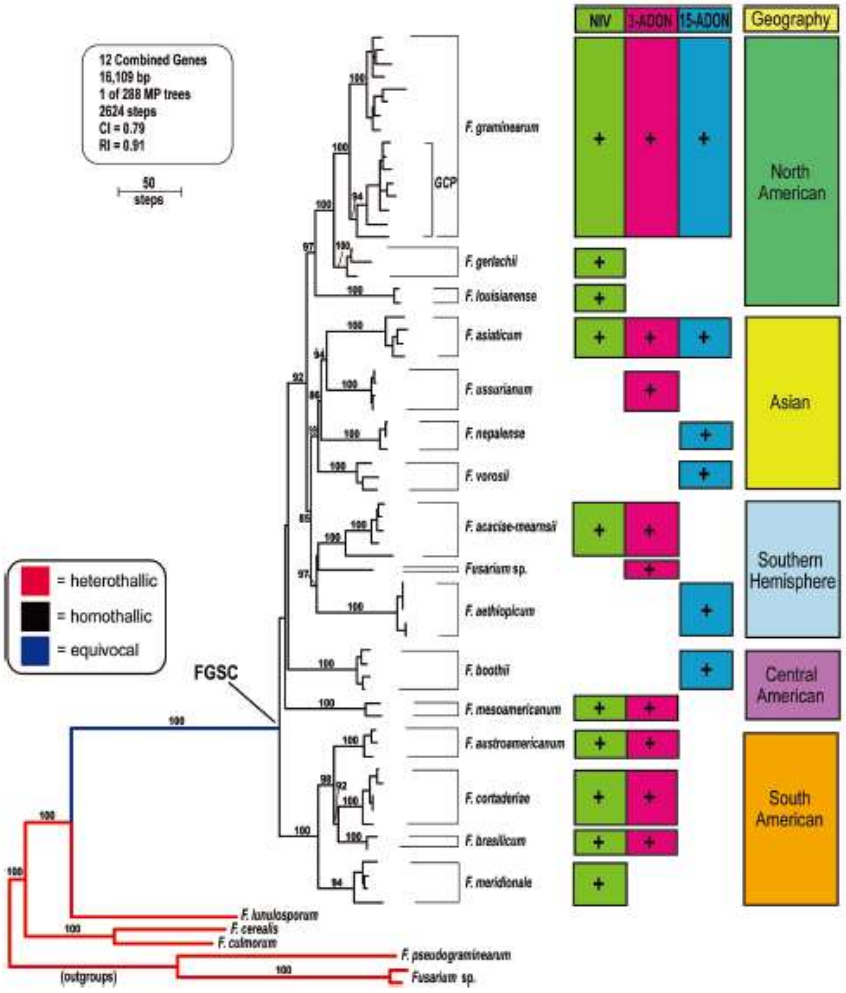
El complejo de especies (figura 4) de *F. graminearum* (FGSC, por sus siglas en inglés) está compuesto por 16 especies filogenéticas (Aoki et al., 2012; O'Donnell et al., 2008; Sarver et al., 2011). Tiene un amplio rango de huéspedes y se asocia con diversas patologías vegetales, entre las que se destaca la FE, pero también es el agente causal de la podredumbre de la corona de trigo (Stephens et al., 2008) y las podredumbres de semilla y plántula en soja, o de raíz, tallo y mazorca en maíz (Broders et al., 2007).

En una revisión bibliográfica llevada a cabo por Del Ponte et al. (2021) en la que se analizaron datos de todos los continentes e información de 16.274 cepas del FGSC, si bien se encontraron representantes de todas las especies del FGSC, se observó que apenas tres representan un 95,9 % de los aislados, siendo *F. graminearum sensu stricto*

la especie predominante con un 72, 2%, seguida por *F. asiaticum* con un 18,7 % y *F. meridionale* con un 5 %. Las demás especies se encontraron en proporciones entre el 1,7 % al 0,01 %.

Fusarium graminearum sensu stricto es la especie filogenética predominante asociada con la FE en trigo y cebada en Uruguay (Garmendia et al., 2018; Umpiérrez, Garmendia, Pereyra et al., 2013). En cuanto a agresividad y patogenicidad, aislados de *F. graminearum ss*. han mostrado ser más agresivos en trigo que otras especies estudiadas en Uruguay (Umpiérrez, Garmendia, Pereyra et al., 2013).

Figura 4.
Árbol filogenético del complejo de especies de Fusarium graminearum, producción de tricotecenos tipo B y distribución geográfica



Nota. Aoki et al. (2012)

1.7. Producción de micotoxinas

Varias especies del género *Fusarium* poseen la capacidad de producir diversas micotoxinas, metabolitos secundarios con efectos nocivos para la alimentación de personas y animales, sin ser tóxicos para el hongo (Wakulinski, 1989). En el caso del complejo de especies de *F. graminearum* se les conoce principalmente por ser productores de tricotecenos y zearalenona (O'Donnell et al., 2015; Scientific Committee on Food, 2002).

1.7.1. Tricotecenos

Los tricotecenos son un grupo de derivados sesquiterpenoides tricíclicos (terpenos de 15 carbonos) que contienen un anillo con un doble enlace entre los carbonos 9 y 10 y un grupo epoxi entre los carbonos 12 y 13, ambos enlaces esenciales para la toxicidad de estos compuestos (Asam et al., 2017; Desjardins y Proctor, 2007; Wakulinski, 1989; Wu et al., 2013). Sus mecanismos de toxicidad son muy diversos, pero el principal es la inhibición de la síntesis de proteínas ribosomales (Asam et al., 2017; Desjardins y Proctor, 2007).

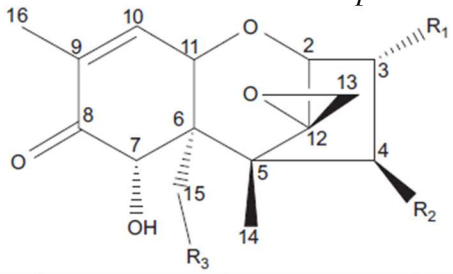
Los tricotecenos se clasifican en cuatro grupos, A, B, C y D. En el caso del complejo de especies *F. graminearum*, en grano de trigo, se las conoce principalmente por ser productoras de tricotecenos tipo B. La diferencia entre los tipos A y B radica en que el tipo B (figura 5) tiene un grupo cetosa en el carbono ocho que se remplaza por un grupo hidroxilo esterificado en el tipo A (figura 6) (Asam et al., 2017).

Los tricotecenos A y B se han detectado como contaminantes de cereales en diferentes localidades y en concentraciones potencialmente peligrosas (Asam et al., 2017). Desjardins y Proctor (2007) compilaron, a partir de varios estudios, valores de la dosis letal para el 50 % de la población (DL50) de los principales tricotecenos en ratones.

Las DL50 fueron 9,2; 10,5; 15,5; 34 y 46 mg/kg de masa corporal para HT2, T2, DAS, 3ADON/15ADON y DON, respectivamente.

Figura 5.

Estructura química de los tricotecenos tipo B.

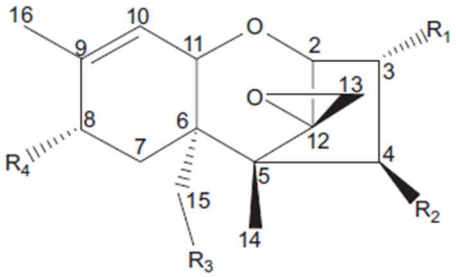


Compound	R_1	R_2	R_3
NIV	OH	OH	OH
FUSX	OH	OAc	OH
DON	OH	H	OH
3-ADON	OAc	H	OH
15-ADON	OH	H	OAc

Nota. NIV: nivalenol, FUSX: fusarenon X o 4-acetil-nivalenol, DON: deoxinivalenol, 3-ADON: 3-acetil-deoxinivalenol, 15-ADON: 15-acetil-deoxinivalenol. Asam et al. (2017)

Figura 6.

Estructura química de los tricotecenos tipo A.



Mycotoxin	R_1	R_2	R_3	R_4
HT2	OH	OH	OAc	$OCOCH_2CH(CH_3)_2$
T2	OH	OAc	OAc	$OCOCH_2CH(CH_3)_2$
MAS	OH	OH	OAc	H
DAS	OH	OAc	OAc	H

Nota. HT2: toxina HT2 T2: toxina T2, MAS: monoacetoxiscirpenol, DAS: diacetoxiscirpenol. Asam et al. (2017)

El deoxinivalenol (DON), también conocido como vomitoxina, debido a uno de sus síntomas más comunes en animales (Asam et al., 2017), es un tricoteceno tipo B y una de las principales preocupaciones asociadas a la FE. Tanto DON como sus formas acetiladas 3-acetil-deoxinivalenol (3ADON) y 15-acetil-deoxinivalenol (15ADON)

han sido detectados con frecuencia en muestras de grano de trigo en diferentes localidades alrededor del mundo (Weidenbörner, 2017). Sin embargo, únicamente DON está regulado por el *Codex Alimentarius* de la FAO y la OMS con un límite de 2000 µg/kg de grano de trigo (CAC, 1995). Esto se debe a que se ha observado que 3ADON se transforma en DON *in vivo* y que 15ADON tiene similar toxicidad que DON (JECFA, 2011).

En Uruguay, el Decreto n.º 533/001 (2001) establece como límite máximo de DON, para harina y derivados de trigo destinados a consumo humano, 1 mg/kg (1 ppm). Complementariamente, el Decreto n.º 470/002 (2002) prohíbe la importación o comercialización de harinas y alimentos elaborados a base de trigo que superen dicho nivel. Se debe resaltar que, para los productos destinados a alimentación animal, la Resolución S/N/001 Límites máximos de DON en alimentos para animales (2001) establece un límite máximo DON en alimentos para bovinos de carne, ovinos y aves de 5 ppm (5 mg/kg); bovinos de leche de 2 ppm (2 mg/kg), cerdos y equinos de 1 ppm (1 mg/kg) y para otros animales de 2 ppm (2 mg/kg) y un límite máximo de DON de 10 ppm (10 mg/kg) para las materias primas (granos y sus subproductos) destinados a la elaboración de alimentos para animales.

A pesar de que DON, 3ADON y 15ADON son los tricotecenos tipo B más frecuentes, el FGSC también está asociado, en trigo, a la producción de nivalenol (NIV) y de su forma acetilada 4-acetil-nivalenol, también conocida como fusarenona x. Si bien tienen menor incidencia y concentración que DON y sus derivados, la familia del nivalenol se encuentra en las muestras de trigo (Weidenbörner, 2017). En Uruguay, en un muestreo con 75 líneas de trigo, en apenas una fue detectada la presencia de NIV (Piñeiro, 1996). Contrariamente a lo que se puede pensar, ya que NIV es más toxico que DON, este no está regulado (JECFA, 2011; Van Der Lee et al., 2015).

Ciertas especies del género *Fusarium* también tienen la capacidad de producir tricotecenos tipo A, los más frecuentes son las toxinas T2 y HT-2, ambas detectadas en trigo y asociadas a la FE (Piñeiro, 1996; Weidenbörner, 2017). También recientemente,

Varga et al. (2015) detectaron que miembros del FGSC podían producir un nuevo tipo de tricoteceno tipo A, que nombraron NX-2 y a su derivado acetilado NX-3, ambos tóxicos.

Las cepas del FGSC se pueden clasificar por quimiotipo según su principal producción de micotoxinas; en concreto, tricotecenos tipo B, los quimiotipos más frecuentes son 15ADON, 3ADON y NIV (McMullen et al., 2012). Cepas con quimiotipos DON (15ADON y 3ADON) han mostrado ser más agresivas que cepas con quimiotipo NIV (Gale et al., 2010; Nicolli et al., 2018; Spolti et al., 2012) y pueden acumular hasta cuatro veces más toxina (Gale et al., 2010). En Uruguay, el quimiotipo más frecuente es 15ADON (Garmendia et al., 2018; Umpiérrez, Garmendia, Pereyra et al., 2013).

El FGSC siempre ha mostrado una marcada distribución geográfica (Aoki et al., 2012; Del Ponte et al., 2015; Van Der Lee et al., 2015), y la prevalencia de una u otra especie se rige por las condiciones ambientales durante la etapa de espigazón/floración de los cultivos hospedantes, e incluso durante la etapa poscosecha, así como la secuencia de especies vegetales en la rotación de cultivos de una región (Díaz y Pereyra, 2011). Sin embargo, esta distribución geográfica parece estar viéndose alterada; por ejemplo, en el hemisferio norte (EE. UU. y Canadá) se ha observado un cambio en la estructura poblacional, que ha favorecido el incremento de cepas con quimiotipo 3ADON (Puri y Zhong, 2010; Ward et al., 2008), mientras que en el sur de EE.UU. se ha encontrado una población en la que predomina *F. asiaticum* quimiotipo NIV (Gale et al., 2010).

1.7.2. Zearalenona

La zearalenona es una lactona del ácido 6-(10-hidroxi-6-oxo-trans-1-undecenil) β-resorcílico (Asam et al., 2017; Olsen, 1989). Es más conocida como micoestrógeno que como micotoxina, por su estructura similar a la del 17-b-estradiol (estrógeno). Consecuentemente, se ha asociado con diversos desórdenes reproductivos en ganado, especialmente en suinos (Olsen, 1989). No hay registro de una dosis letal asociada a

la ingesta de zearalenona ni en animales ni en personas (Asam et al., 2017; Desjardins y Proctor, 2007).

La zearalenona se considera térmicamente estable hasta los 150 °C, sin embargo, hay resultados contradictorios en cuanto a su degradación en el proceso de panificación. Su contenido parece reducirse durante la fermentación, pero, durante el horneado (~220 °C), algunos resultados apuntan a un incremento y otros a una reducción (Yu et al., 2022).

La zearalenona se ha encontrado como contaminante principalmente en maíz. Sin embargo, también se ha hallado en otros cereales como el trigo. De acuerdo con la FAO (2004), la zearalenona está regulada en 16 países con límites de entre 50 y 1000 µg/kg en maíz y otros cereales. Un ejemplo son los límites establecidos por la Comisión Europea: 100 µg/kg para cereales no procesados diferentes del maíz y 350 µg/kg para maíz no procesado (Commission Regulation (EC) n.º 1881/2006, 2006). En Uruguay no hay un límite específico para trigo, pero el Decreto n.º 315/994, (1994), enmendado por el Decreto n.º 155/006 (2006), establece un límite de 200 µg/kg en granos de maíz y cebada.

Hay pocos antecedentes en cuanto a detección de zearalenona en Uruguay; sin embargo, se ha verificado su presencia en trigo, maíz, cebada, arroz y sus derivados. En concreto, casi la totalidad de las muestras de trigo analizadas por Piñeiro (1996) tenían alguna concentración de zearalenona, aunque inferior a 100000 µg/kg.

1.7.3. Otras micotoxinas

Diferentes especies del género *Fusarium* también están asociadas a la producción de otras micotoxinas más peligrosas, pero encontradas en menor frecuencia en trigo. Un ejemplo, son las fumonisinas, aminopolialcoholes de cadena larga, catalogadas por la Agencia Internacional para la Investigación del Cáncer (IARC) como posibles

carcinógenos humanos (categoría 2B). Las especies del FGSC carecen del clúster de genes encargado de la producción de estas toxinas (Desjardins y Proctor, 2007). Otras toxinas potencialmente peligrosas que se ha demostrado que son producidas por el FGSC son la butenolida y las fusarinas; sin embargo, aún es necesario establecer los mecanismos de toxicidad y biosíntesis de ambas sustancias (Desjardins y Proctor, 2007).

1.8. Manejo integrado

El manejo integrado implica el uso combinado de las medidas disponibles para el control de las enfermedades. Se basa en la utilización de técnicas culturales, variedades resistentes y un monitoreo adecuado, de forma que se elimina o reduce el inóculo inicial y su eficacia, y como resultado retrasan el avance de la enfermedad, todo esto con la finalidad de usar una menor cantidad de pesticidas (Agrios, 2005).

En el caso de la FE, ninguna medida de manejo provee protección total ni garantiza nivel cero de micotoxinas (Pereyra, Castro, et al., 2014). Si bien la resistencia genética de la variedad reduce la severidad de la FE y la concentración de DON, es necesario el manejo integrado para hacer frente a epidemias moderadas a severas (Blandino et al., 2012; McMullen et al., 2012; Paul et al., 2019).

1.8.1. Variedades resistentes

El uso de variedades resistentes es la forma más eficiente y económica de control de enfermedades (Agrios, 2005). Para FE no existe inmunidad y la resistencia a FE es aditiva y controlada por el efecto de múltiples genes (Figlan y Mwadzingeni, 2022). Algunos autores hablan de hasta siete tipos de resistencia, siendo los principales para este trabajo el tipo II, a la propagación en espiga o resistencia horizontal, el tipo I, a la infección inicial o resistencia vertical (Agrios, 2005; McMullen et al., 2012) y el tipo III o resistencia a la acumulación de micotoxinas (Figlan y Mwadzingeni, 2022).

Las principales fuentes de resistencia tienen origen asiático; se cree que, debido a la presión de la FE en China, durante la década de 1950, productores locales seleccionaron variedades resistentes que hasta hoy son valiosas fuentes de resistencia (Zhu et al., 2019). De acuerdo con la revisión bibliográfica de Saharan (2020), para desarrollar variedades resistentes a FE se han empleado fundamentalmente las variedades Sumai 3 y Ning 7840. Sumai 3 surge en los 70 y es una cruza entre los cultivares Funo y Taiwanxiaomai llevada a cabo por el Suzhou Institute of Agricultural Sciences, y se atribuye su resistencia fundamentalmente al QTL (Quantitative Trait Loci) *Fhb1* (Zhu et al., 2019); mientras que Ning 7840 es una de las fuentes de máxima resistencia reportadas hasta el momento (que son: Ning 7840 y Ning 894037, Wangshuibai, las variedades japonesas Nobeokabozu y Nyu Bai (Nyubai) o el cultivar coreano Chokwang) (Saharan, 2020).

En Uruguay, desde 1981 se ha ido integrando la resistencia a FE en el mejoramiento de líneas y desde 1986 se han incluido fuentes de resistencia chinas. Sumai 3 ha destacado por tener los mejores resultados (Díaz y Pereyra, 2011); sin embargo, el uso de cultivares comerciales moderadamente resistentes contra la FE se introdujo en el país luego de la epidemia del 2001-2002 (Pereyra, Castro, et al., 2014). En la última evaluación nacional de cultivares, conducida en 2022, de 27 variedades evaluadas la mayoría tuvieron un comportamiento con clasificación (5-9) o susceptibilidad intermedia alta a alta a FE, y apenas 7 (26 %) tienen una clasificación de 3-4 o susceptibilidad intermedia-baja a intermedia a FE (Castro et al., 2023). Esto indica una alta vulnerabilidad frente a FE en años favorables para la enfermedad.

1.8.2. Medidas culturales

La prevención de la FE con medidas culturales consiste básicamente en buscar reducir el inóculo inicial y evitar la cosecha de granos contaminados. Se ha observado que la rotación con cultivos no susceptibles es medianamente eficaz, debido al amplio rango de huéspedes del hongo y a la capacidad de dispersión de las ascosporas que este

produce (Pereyra, Castro, et al., 2014; Pereyra y Dill-Macky, 2008). El laboreo reducido incrementa la descomposición del rastrojo en comparación con el no laboreo (siembra directa) y, en consecuencia, reduce el inóculo en los residuos (McMullen et al., 2012; Pereyra y Dill-Macky, 2008).

Algunas prácticas culturales se pueden llevar a cabo si se ha detectado infección para reducir el número de granos contaminados, entre ellas cosechar separadamente las áreas identificadas con menor infección de las de mayor infección o aumentar el caudal de viento en la cosechadora, inclusive separar por gravedad los granos infectados (FDK: *Fusarium damaged kernels*) (McMullen et al., 2012).

1.8.3. Aplicación de fungicidas

La aplicación de fungicidas es una práctica preventiva; se recomienda en el momento de mayor susceptibilidad, a inicio de floración (Z61) y considerando en conjunto las otras estrategias, como sistemas de alerta o rotaciones. Sin embargo, aplicaciones posfloración también han demostrado ser efectivas en la reducción del nivel de infección y el contenido de DON (D'Angelo et al., 2014; Díaz y Pereyra, 2011; Paul et al., 2019).

Los principios activos recomendados para el control de la FE son inhibidores de la desmetilación (DMI) en la biosíntesis del ergosterol, en concreto triazoles o mezclas de triazoles, mientras que los inhibidores externos de quinona (QoI) o estrobirulinas no son recomendados, ya que se han relacionado con mayor contenido de DON (McMullen et al., 2012). Su aplicación se recomienda en dosis adecuadas con picos de doble abanico tipo TwinJet60® que aseguran el mojado de las espigas (Pereyra, Castro, et al., 2014).

Actualmente se utilizan estos DMI tanto solos como en mezclas. En Uruguay metconazol + epoxiconazol y protioconazol + tebuconazol han demostrado alta eficiencia de control ante la FE, mientras que con tebuconazol y tebuconazol +

carbendazim se ha logrado eficiencia intermedia-alta (Pereyra y González, 2023). Si bien parece haber una tendencia a la reducción en la importación de fungicidas en el país (DIEA y MGAP, 2023), durante mucho tiempo el uso de fungicidas ha sido una práctica abusiva. Por ejemplo, para el tratamiento de la FE y otras enfermedades en trigo el uso de tebuconazol, tanto solo como en mezclas con compuestos como el azoxistrobin [eficaz en el tratamiento de enfermedades de hoja como la mancha amarilla, la septoriosis o la roya de hoja (Pereyra y González, 2023)], ha sido indiscriminado. Como consecuencia, se ha detectado que cepas del FGSC causantes de la FE en Uruguay han disminuido la sensibilidad a tebuconazol (Brancatti et al., 2022) con respecto a lo observado anteriormente por Garmendia et al. (2018) y Umpiérrez-Failache et al. (2013).

Estudios dirigidos en EE. UU. demostraron que, en aplicaciones en antesis, la combinación de cultivares moderadamente resistentes y el uso de fungicidas que combinen protioconazol + tebuconazol (Prosaro®) controlan en más del 70 % la enfermedad, incrementan el rendimiento de grano y el peso hectolítrico, a la vez que reducen el contenido en DON, respecto a cultivares susceptibles (Paul et al., 2018, 2019).

En el panorama actual, en el que la población está cada vez más concientizada y preocupada con el medioambiente, se han generado más datos y normativas respecto al uso de plaguicidas, para prevenir sus efectos nocivos. Internacionalmente es el *Codex Alimentarius* de la FAO el que recomienda los límites de los principios activos en plaguicidas, como es el caso de los triazoles.

En Uruguay, algunos de los límites máximos de residuos (LMR) de plaguicidas no vienen marcados *per se* en una normativa nacional, sino que la Resolución n.º 75/018 DGSA Límites máximos de residuos de plaguicidas (LMR) para productos de origen vegetal (2018) establece que aquellos principios activos no legislados en el país respetarán las tolerancias impuestas por el *Codex Alimentarius* de la FAO o por la normativa de la Comunidad Europea.

Es importante resaltar que un estudio llevado a cabo en China demostró que las buenas prácticas agrarias, que incluyen el uso adecuado de plaguicidas (siguiendo las recomendaciones de los fabricantes), son eficientes en cuanto al nivel residuos de triazoles en grano y rastrojo de trigo (Zhang et al., 2015).

Cuantificar los residuos de plaguicidas es importante, ya que no siempre va a ser posible realizar una aplicación óptima, normalmente debido a las condiciones ambientales.

En Uruguay existen escasos antecedentes sobre la cuantificación de residuos de fungicidas presentes en grano de trigo luego de la cosecha y ninguno de ellos encontró residuos por encima del LMR (Baráibar, 2018; Francia et al., 2017; Palladino et al., 2018).

1.8.4. Sistemas de alerta

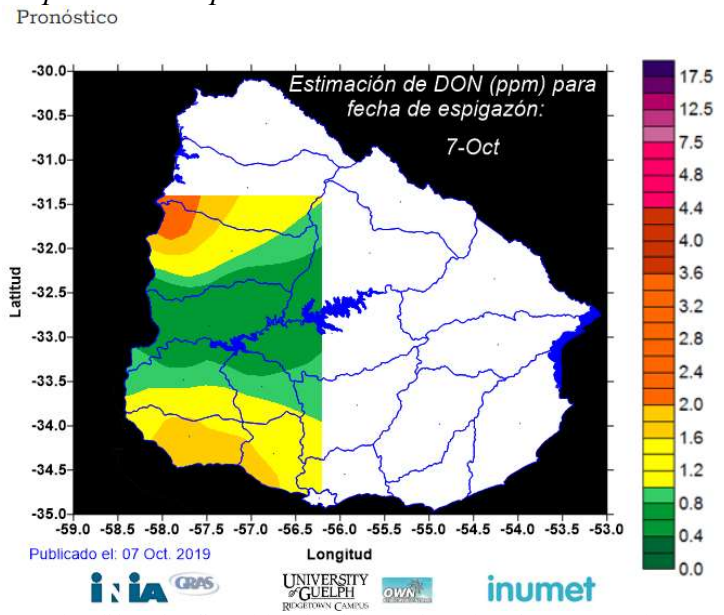
Las tecnologías de la información han tenido un desarrollo vertiginoso desde finales del siglo XX, por lo que se han incorporado también al manejo de patologías vegetales en la forma de sistemas de alerta. Antes de diseñar un sistema de predicción es necesario disponer de la mayor cantidad de información posible sobre la biología del patosistema (Agrios, 2005).

En tanto la FE posee una ventana relativamente estrecha de vulnerabilidad para la infección, dispersión del inóculo y acumulación de toxina DON, es posible predecir su riesgo de ocurrencia en función de variables climáticas (De Wolf et al., 2003). La eficiencia de los fungicidas depende en gran medida del momento y la calidad de la aplicación. Los sistemas de predicción de FE o contenido de DON en grano pueden constituir una herramienta útil para los productores y técnicos asesores del cultivo para apoyar la toma de decisiones en el control de FE con fungicidas o incluso para conocer

a priori sobre la contaminación de lotes con micotoxinas, especialmente DON, en la precosecha (De Wolf et al., 2000; Hooker et al., 2002; Moschini y Fortugno, 1996).

Existen varios modelos disponibles en el mundo para la predicción de FE o DON en grano (De Wolf et al., 2000; Hooker et al., 2002; Moschini y Fortugno, 1996). El modelo DONcast, fue diseñado para estimar la acumulación de deoxinivalenol (DON) para trigo en Canadá (Hooker et al., 2002) y fue adaptado para las condiciones de Uruguay por INIA en el marco del proyecto financiado por FAO *Apoyo en la prevención y control de* Fusarium *y micotoxinas en granos* (TCP/URU/2801). Este modelo considera humedad relativa, precipitaciones y temperaturas mínimas, medias y máximas en tres períodos críticos en torno a espigazón, floración y primeras etapas de llenado de grano (Díaz y Pereyra, 2011). Este sistema de alerta está disponible en la web http://www.inia.uy/gras/Alertas-y-herramientas/Pron%C3%B3stico-DON-para-trigo- y nos permite ver un mapa de la estimación de la cantidad de DON acumulada en grano de trigo introduciendo la fecha en la que tenemos un 75 % de espigazón (figura 8). Esta herramienta, en conjunto con otras prácticas y un criterio adecuado, permite evaluar si se debe o no realizar una aplicación de fungicida.

Figura 8.
Mapa DONcast para el 07 de octubre de 2019.



Nota. Disponible en: http://www.inia.uy/gras/Alertas-y-herramientas/Pron%C3%B3stico-DON-para-trigo-

1.9. Hipótesis

Las hipótesis de esta tesis fueron las siguientes:

- Existen diferencias en la agresividad en planta entre cepas del FGSC de quimiotipos 15ADON y NIV.
- La combinación de la resistencia genética del cultivar con una estrategia de manejo óptimo de fungicidas, en términos de momento y tipo de producto, influye significativamente en múltiples aspectos pues minimizan la severidad de la FE en campo y en grano cosechado.

1.10. Objetivos

1.10.1. Objetivo general

Estudiar la interacción entre distintas especies locales del complejo de especies de *Fusarium graminearum* y genotipos de trigo con niveles de resistencia contrastantes hacia la fusariosis de la espiga (FE), así como analizar el efecto de la combinación de distintas estrategias de manejo en el control de FE, respecto a inocuidad en el grano y variables productivas.

1.10.2. Objetivos específicos

Estudiar la interacción patógeno-hospedero, entre cepas representativas en Uruguay del complejo de especies *F. graminearum* (FGSC), de quimiotipo potencial conocido (15ADON y NIV), con genotipos de trigo con base de resistencia genética contrastante en cuanto a agresividad en planta.

Estudiar el efecto de distintas estrategias de aplicación de fungicidas, se estableció la eficiencia de manejo a través de la combinación de resistencia genética del cultivar y

el manejo del fungicida (momento y producto) sobre la FE, contenido de DON, variables productivas y residuos de fungicidas.

2. Aggressiveness of *Fusarium graminearum* isolates with 15ADON and NIV chemotypes from wheat in Uruguay

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2.1. Summary

Fusarium head blight (FHB) of wheat is mostly caused by the *Fusarium graminearum* species complex (FGSC) in the Southern Cone of South America. Wheat grains contaminated by FGSC may contain trichothecenes-B and their derivatives and represent a risk for people and animals. This work studied nine representative Uruguayan isolates of the FGSC, previously identified as potential producers of 15-acetyl deoxynivalenol (15ADON) and nivalenol (NIV), interacting with three wheat genotypes with different levels of resistance to FHB under greenhouse and growth chamber conditions. We conducted two identical experiments, in 2019 and 2020. Disease severity (%) was determined at 7, 14 and 21 days post-inoculation and the area under the disease progress curve (AUDPC) was quantified. We found that isolates *F. graminearum sensu stricto* chemotype 15ADON were more aggressive than the potentially NIV producing isolates. Additionally, we observed that isolates ranked differently according to the wheat genotype resistance resulting in a significative interaction ($p < 0.05$). Our results are consistent with previous findings and provide valuable data on the virulence of local FGSC populations and host resistance. Further studies are needed to include data on toxin accumulation.

KEYWORDS: Fusarium, wheat, interaction, resistance, chemotypes, Uruguay

2.2. Resumen

La fusariosis de la espiga (FE) en trigo es causada principalmente por el complejo de especies de *Fusarium graminearum* (FGSC) en el Cono Sur de América del Sur. Por otra parte, el trigo infectado con FGSC puede contener micotoxinas, como los tricotecenos tipo B, así como sus derivados acetilados y aglicosados, los cuales representan un riesgo para personas y animales. Este trabajo estudió nueve aislados de FGSC representativos de Uruguay de quimiotipos conocidos, 15-acetil-deoxinivalenol (15ADON) y nivalenol (NIV), interactuando con tres genotipos de trigo con comportamiento contrastante frente a FE en condiciones de invernáculo y cámara de crecimiento. Se llevaron a cabo dos experimentos idénticos, uno 2019 y 2020. La severidad de FE (%) fue evaluada a los 7, 14 y 21 días posinoculación y se cuantificó el área debajo de la curva de progreso de la enfermedad (AUDPC). Encontramos que los aislados *F. graminearum sensu stricto* con quimiotipo 15ADON fueron más agresivos que los potenciales productores de NIV. Además, observamos que los aislados variaban su comportamiento de acuerdo con la resistencia del genotipo de trigo, lo que resultó en una interacción significativa ($p < 0,05$). Nuestros resultados son consistentes con estudios anteriores y aportan información valiosa sobre la agresividad de la población local del FGSC y la resistencia del huésped. Son necesarios más estudiosque incluyan información sobre la acumulación de toxinas.

PALABRAS CLAVE: *Fusarium*, trigo, interacción, resistencia, quimiotipos, Uruguay

2.3. Introduction

Fusarium head blight (FHB) is a plant disease that affects wheat, barley, maize and other cereals. It is mainly caused by different species of the *Fusarium* genus, being some of the members of the *Fusarium graminearum* species complex (FGSC), the most common causal agents in Uruguay (Garmendia et al., 2018; Umpiérrez, Garmendia, Pereyra, et al., 2013), Argentina (Nogueira et al., 2018; Reynoso et al.,

2013), southern Brazil (Castañares et al., 2016; Del Ponte et al., 2015), Paraguay (Alvarenga et al., 2022) and other regions such as North America (McMullen et al., 2012; Ward et al., 2008) or some regions of Europe (Senatore et al., 2021).

The FGSC encompasses at least 16 different phylogenetic species (Aoki et al., 2012). Based on strain data collected from various continents and hosts, *Fusarium graminearum* Schawbe *(Gibberella zeae* Schw. & Petch*) sensu stricto* seems to be the dominant species worldwide, followed *by F. asiaticum*. Both phylogenetical species represent up to 80% of the analyzed strains (Del Ponte et al., 2021).

Fusarium species produce different mycotoxins, which are secondary metabolites, usually with harmful effects for people and animals. The FGSC produces type B trichothecenes, such as deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives (15ADON, 3ADON and 4ANIV, also known as fusarenon X). Isolates of the FGSC are also capable of producing type A trichothecenes as recently discovered metabolites NX-2 and its acetylated derivative NX-3 (Varga et al., 2015), and zearalenone (Scientific Committee on Food, 2002).

DON, also known as vomitoxin*,* is regulated by the FAO/WHO *Codex Alimentarius* (CAC, 2019) with a limit of 2000 µg/kg for wheat grain. 3ADON is converted to DON *in vivo* and 15ADON has similar toxicity as DON (JECFA, 2011). Even though NIV is more toxic than DON, it is not regulated (JECFA, 2011; van der Lee et al., 2015).

Isolates of the FGSC are differentiated by chemotypes, as DON, NIV, 15ADON or 3ADON, according to their primary mycotoxin production (McMullen et al., 2012). The most common strains (>85%) causing FHB in wheat and barley in Uruguay are *F. graminearum sensu stricto* and the predominant chemotype 15ADON, followed in a small proportion by the NIV chemotype and the hardly ever found 3ADON chemotypes (Garmendia et al., 2018; Umpiérrez, Garmendia, Pereyra, et al., 2013). *Fusarium graminearum ss* isolates have shown to be more aggressive on wheat than other species of the FGSC (Umpiérrez, Garmendia, Pereyra, et al., 2013).

Some studies on aggressiveness and pathogenicity of different strains and chemotypes of the FGSC found that DON (15ADON or 3ADON) chemotypes are generally significantly more aggressive than NIV (Gale et al., 2010; Nicolli et al., 2018; Spolti et al., 2012) and may accumulate up to four times more toxin (Gale et al., 2010). Record varied on the different resistant levels of the wheat genotypes and significantly cultivar × species interaction was detected (Spolti et al., 2012).

The FGSC shows a strong geographic structure (Aoki et al., 2012; Del Ponte et al., 2015; van der Lee et al., 2015) and may be altered by climate change. In Canada and northern US there has been a shift in the population structure of the FGSC with a significant increase towards 3ADON chemotypes in detriment of 15ADON isolates, along with greater levels of toxin accumulation but same disease severities (Puri & Zhong, 2010; Ward et al., 2008). In southern US, *F. asiaticum* NIV chemotypes prevailed (Gale et al., 2010). More studies must be conducted to describe the different *Fusarium* pathogen species causing FHB regionally, and their aggressiveness.

The aim of this paper was to study the host-pathogen interaction between nine Uruguayan representative strains of the FGSC, belonging to the 15ADON and NIV chemotypes and three wheat genotypes of contrasting FHB resistance.

2.4. Materials and methods

2.4.1. *Fusarium graminearum species* complex and inoculum production

Nine single-conidial isolates of the FGSC (table 1) were selected to assess the FHB development in wheat plants under greenhouse conditions. Fungal isolates were obtained from INIA and Facultad de Química (Universidad de la República) collections and were selected based on chemotype (15ADON or NIV), origin (year, location) and, if available, aggressiveness from previous studies.

Isolates from collections (conserved at 4 °C in silica gel or paper) were firstly transferred to petri dishes with potato dextrose agar (PDA- OXOID CM0139) and incubated in a growth chamber (Thermo Scientific Precision Model 818 Incubator at the National Agriculture Research Institute [INIA], La Estanzuela Station [latitude 34°20'17.1"S, longitude 57°41'26.7"W]), at 25 °C and 12-hour light and dark cycles for five to 10 days.

Once the viability of the isolates was checked, conidia were harvested by adding about 1 ml of deionized sterile water to each of four to 10 PDA cultures and gently scraping the mycelia with a sterile glass spatula. Suspensions were then filtered through two layers of cheesecloth and plated onto soybean medium (Pereyra & Dill-Macky, 2008). The plates were placed under light for ten days at 22-25 °C to promote abundant conidial production. The scraping process was repeated to make a macroconidia suspension and the concentration was adjusted to 1×10^5 con/ml. The inoculum was frozen at -20 °C in 1 ml Eppendorf vials and its viability was checked at each inoculation.

2.4.2. Experimental design and aggressiveness tests

Three different wheat genotypes with contrasting resistance to FHB, according to the national cultivar characterization (Castro et al., 2014), were selected: INIA Don Alberto (LE2331), highly susceptible, INIA Madrugador (LE2332), moderately susceptible to moderately susceptible-moderately resistant, and Sumai 3, source of resistance for FHB.

The experimental layout was a randomized complete design with 10 repetitions (pot) per combination (wheat genotype × isolate) and a mock-inoculated control check (deionized sterile water). The experiment was conducted twice (2019 and 2020) at the National Agriculture Research Institute (INIA), La Estanzuela Exp. Station (latitude 34°20'17.1"S, longitude 57°41'26.7"W) under greenhouse conditions (15-30 °C, natural light) from April-May until September-October, after infection and through the

FHB evaluation (21 days), plants were transferred to a growth chamber with optimal conditions for FHB.

Five seeds were sown on one-liter pots containing a 2:1:0.5 mixture of soil: commercial substrate (Vitaterra, Montevideo, Uruguay): expanded vermiculite (Agrinobre, Rio Grande do Sul, Brazil). When the plants reached approximately three leaves stage (Zadok's Growth Stage-ZGS13), they were thinned to two plants per pot. We applied urea fertilization (2-3 grams/pot) every two to three weeks from that moment until heading (ZGS55) (Zadoks et al., 1974). Plants were monitored daily, and pots with one or both plants at the beginning to mid-anthesis stage (ZGS61-ZGS65; Zadoks et al., 1974) were selected and grouped. Inoculations were made every second day according to the grouping. The treatment (isolate or mock-inoculated control check) was randomized among the available plants until a total of 10 plants were inoculated with the same isolate. The inoculation was performed by dispensing a 10 µl drop (1000 con/drop) of inoculum at both central spikelets with a precision micropipette. After 45 minutes at room temperature, the plants were transferred to a moisture chamber with 100% relative humidity (RH) for 72 hours. From then on and until the end of the assessment, the plants were kept in a growth chamber with a 25 °C temperature, 80% RH, and 12-hour photoperiod.

FHB development was assessed as severity (%) at 7, 14 and 21 days post infection (dpi). Severity was obtained by counting the total and symptomatic spikelets. Finally, the area under disease progression curves (AUDPC) was calculated according to (Madden et al., 2007) as:

$$AUDPC_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

$$y_i: severity\ (\%)\ at\ the\ 7, 14\ or\ 21\ dpi\ assessment$$

$$t_i: Day\ of\ assessment; \{0, 7, 14\ or\ 21\}$$

2.4.3. Statistical analysis

Statistical analyses were conducted using the 2020 version of INFOSTAT® (Di Rienzo et al., 2008); this software runs on the user installed version (4.02) of R (R core team, 2021).

For the general analysis, which included 2019 and 2020 experiments, and to minimize overdispertion and to ensure equal numbers of observations, we used the total number of spikelets and the number of diseased spikelets per each wheat genotype × isolate combination to assess FHB severity. We used a generalized linear mixed model with binomial distribution and logit link function. INFOSTAT® uses the *glmer* function of the lme4 package of R for generalized linear mixed models, the variance estimator was the restricted maximum likelihood (REML), the fixed effects were the wheat genotype, the isolate and their interaction, and the random effects were the year and the covariable days of flowering. When there were significant differences among factors, we ran pairwise comparisons.

AUDPC was analyzed with a linear mixed model after checking normality and homoscedasticity assumptions. INFOSTAT® uses the *lme* function of the nlme package of R for linear mixed models. The variance estimator and fixed effects were the same as for the severity analysis, and a single random effect, the year. When there were significant differences, we ran pairwise comparisons.

Additionally, AUDPC was analyzed independently for each experiment; to fulfill normality and homoscedasticity assumptions it was transformed to the square root of AUDPC, and each value was included (FHB AUDPC of every pot). The model was a general linear mixed model, the variance estimator was the restricted maximum likelihood (REML), the fixed effects were the wheat genotype, the isolate and their interaction, and the random effect was the covariable days of flowering. When there were significant differences among factors, we ran pairwise comparisons.

2.5. Results

2.5.1. Isolate aggressiveness

All isolates induced FHB symptoms on all wheat genotypes tested. Mock-inoculated spikes were asymptomatic. In the 2019 experiment, the maximum values of FHB severity were higher than those obtained in the 2020 experiment (table 2), yet statistical analyses of AUDPC in both experiments showed similar results. There were significant differences among wheat genotypes, isolates and their interaction ($p < 0.05$) in both experiments individually (data not shown). Individual AUDPC analysis results were consistent with the results of the general analysis (figure 1).

There were significant differences among wheat genotypes and isolates in severity at 7, 14 and 21 dpi and in AUDPC values (table 3). Particularly, there were significant differences among isolate x wheat genotype for severity at 14 and 21 dpi. In terms of FHB severity and AUDPCs values, wheat genotypes ranked as per their characterization to FHB susceptibility (Castro et al., 2014); INIA Don Alberto had significantly higher levels AUDPC and of severity than Sumai 3 at 7, 14 and 21 dpi, whilst INIA Madrugador had significant differences with both cultivars in AUDPC and severity at 14 dpi, but did not have significant differences on severity at 7 dpi with Sumai 3, or with INIA Don Alberto at 21 dpi (data not shown).

Isolates of the *F. graminearum sensu stricto* chemotype 15ADON were significantly more aggressive than isolates belonging to the NIV chemotype ($p < 0.05$) based on FHB severity at 7, 14 and 21 dpi and AUDPC at the general analysis. This was consistent with the results obtained independently for each experiment (2019 and 2020).

Isolate Fg-09-006 was consistently the most aggressive one (figures 1 and 2): each year according to root square analysis of AUDPC and at the general analysis at 7, 14

and 21 dpi severity and AUDPC values. It reached severity values from 17% (Sumai 3) to 79% (INIA Don Alberto) at 21 dpi. In most cases, the other isolates of *F. graminearum sensu stricto* did not report significant differences with Fg-09-006.

On the other hand, *F. asiaticum* isolate Fa-CL-4 was usually the one with lower levels of severity or AUDPC, and, in any case, did not have significant differences with *F. cortaderiae* isolates Fc -09-007 and Fc09-008, and was significantly different from *F. graminearum* isolates 09-006, 09-005 and 02-019 in AUDPC and severity at 7, 14 and 21 dpi.

2.5.2. Wheat genotype-FGSC isolate interaction

There were significant differences among isolate × wheat genotype combinations for FHB severity at 14 and 21 dpi (table 2). Whilst the wheat genotypes maintained the expected FHB severity levels, *Fusarium* isolates, especially the *F. graminearum sensu stricto*, varied in their aggressiveness depending on the wheat genotype (figures 3 and 4). This fact might have led to the statistically significant interaction.

2.6. Discussion

Both experiments (2019 and 2020) evidenced that all isolates were capable of inducing FHB on all wheat genotypes. Despite similar values of severity at seven days post-inoculation (dpi) observed in the 2019 and 2020 experiments (data not shown), higher severity values at 14 and 21 dpi were obtained in 2019. This could be due to additional stress factors in the 2019 experiment, which may have caused additional stress on the plants and contributed to higher susceptibility to FHB.

Our results are consistent with previous findings, at local and international level, as *F. graminearum ss* 15ADON isolates were more aggressive than NIV strains (Gale et al., 2010; Garmendia et al., 2018; Nicolli et al., 2018; Spolti et al., 2012; Umpiérrez, Garmendia, Pereyra, et al., 2013). Two out of four *F. graminerarum ss* isolates (09-

005 and 09-006) were constantly on the top of the aggressiveness tests; 09-006 was already tested in 2012 and was also one of the most aggressive isolates (Umpiérrez, Garmendia, Pereyra, et al., 2013). On the contrary, *F. asiaticum* CL4 was consistently the one that produced the lowest severity levels in plants. Finally, *F. asiaticum* isolate 12-077, originally obtained from barley grains and with a markedly high aggressive on barley in previous studies (Umpiérrez, Garmendia, Pereyra, et al., 2013), induced low FHB severity levels in this study.

We found a significant wheat genotype × isolate interaction at 14 and 21 dpi; such type of interaction has been described before, although it is rarely found. Spolti et al. (2012) reported that *F. graminearum ss* isolates were significantly more aggressive than *F. meridionale* isolates in moderately susceptible wheat genotype. However, they did not find differences in the susceptible material. From the same group, Nicolli et al. (2018) tested 15ADON, 3ADON and NIV, and observed the same results, reporting significant differences among cultivars in the moderately susceptible material, while there were not in the susceptible genotype; however, they could not test the interaction, as the experiments were conducted at different moments. Our interaction demonstrates that under different resistance levels of the wheat genotype the *Fusarium* isolates ranked otherwise; despite of *F. graminearum ss* always being on top, the isolate changed, and other isolates such as *F. brasilicum* under some circumstances tend to act as *F. graminearum ss*. Thus, resistance of the wheat genotype may play an important role on the advance of the FHB infection.

The moderately susceptible-moderately resistant material (INIA Madrugador) expressed similar severity levels as the moderately resistant genotype (Sumai 3) at 14 dpi, but was closer to the susceptible (INIA Don Alberto) at 21 dpi.

Despite not having a significant interaction at 7 dpi, we did observe a variation in the FHB infection on the different wheat genotypes, and the response agreed with their FHB characterization. INIA Don Alberto was always susceptible to FHB, whilst Sumai 3 had lower FHB severity levels, showing its resistance; on the other hand, INIA

Madrugador oscillated between the two performances, with similar FHB severity levels as the moderately resistant at 7 dpi and as the susceptible at 21 dpi.

Results from this study demonstrated that isolate aggressiveness changed accordingly to the wheat genotype resistance. Further studies to analyze host resistance role in the presence of different species at field conditions are needed. Also, more information on DON and NIV content for each combination of wheat genotype × isolate is needed.

2.7. Funding details

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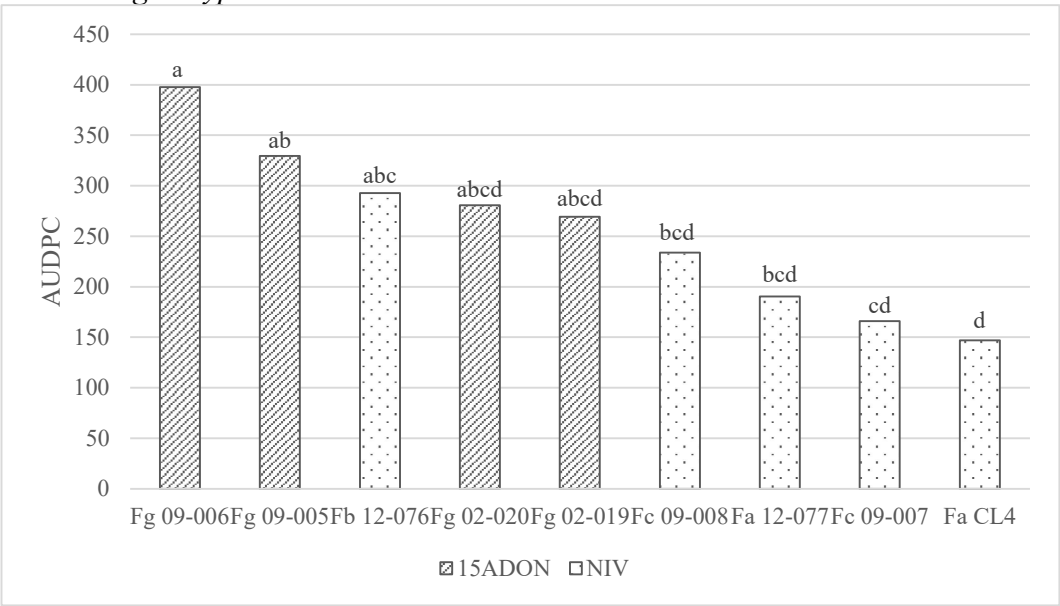
2.8. Disclosure statement

The authors report there are no competing interests to declare.

Figure 1.

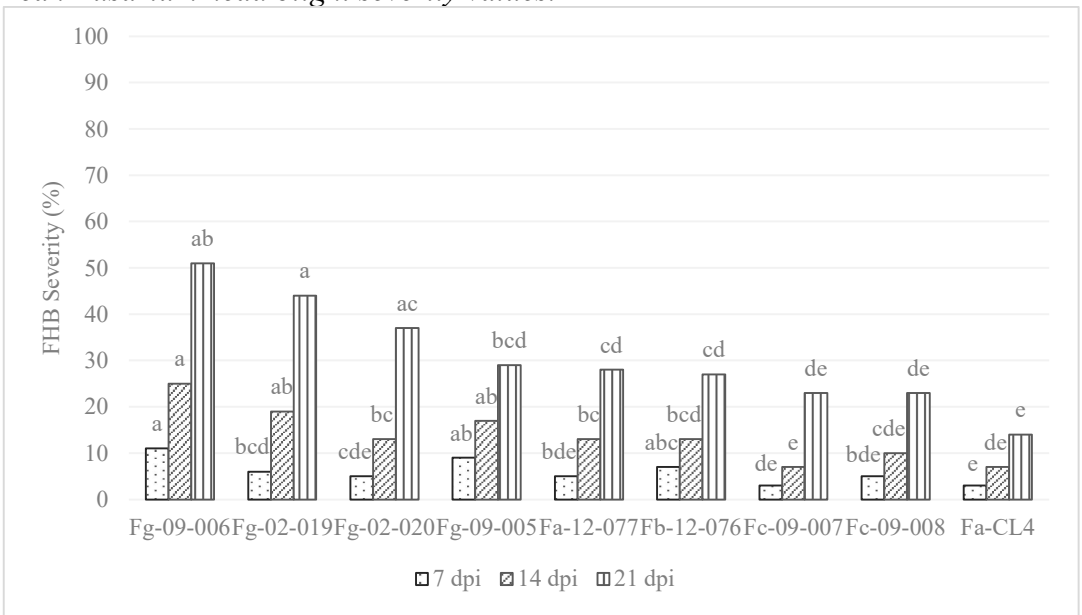
Mean values of the area under diseased progress curves (AUDPC) for each Fusarium genotype.



Note. AUDPC based on *Fusarium* head blight severity at 0, 7, 14 and 21 days post-inoculation (dpi) for three plant genotypes (INIA Don Alberto: susceptible to FHB, INIA Madrugador: moderately susceptible to moderately susceptible-moderately resistant, and Sumai 3, resistant to FHB), as per *Fusarium* genotype. Data from two experiments (2019 and 2020). Fg: *Fusarium graminearum*; Fb: *F. brasilicum*; Fc: *F. cortaderiae*; Fa: *F. asiaticum*. Potential chemotypes: 15ADON: 15-acetyldeoxynivalenol; NIV: nivalenol. Mean values with different letters are significantly different at p = 0.05 according to pairwise comparisons with F-value statistic from general linear mixed models within each dpi

Figure 2.

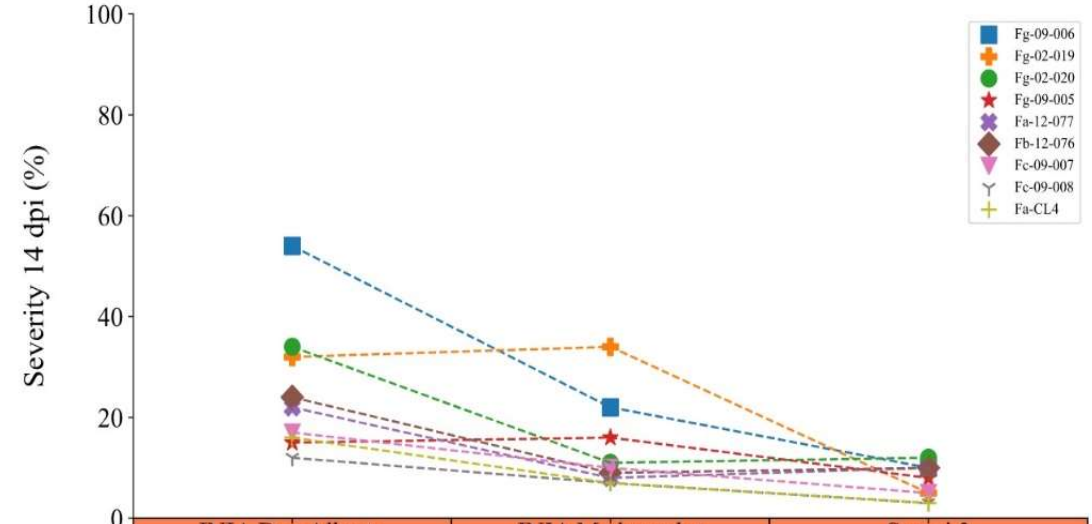
Mean Fusarium head blight severity values.



Note. Severity values assessed as percentual of symptomatic spikelets, at 7, 14 and 21 days post-inoculation (dpi) for three plant genotypes (INIA Don Alberto: susceptible to FHB, INIA Madrugador: moderately susceptible to moderately susceptible-moderately resistant, and Sumai 3, resistant to FHB), as per *Fusarium* genotype. Data from two experiments (2019 and 2020). Fg: *Fusarium graminearum*; Fb: *F. brasilicum*; Fc: *F. cortaderiae*; Fa: *F. asiaticum*. Potential chemotypes: 15ADON: 15-acetyldeoxynivalenol; NIV: nivalenol. Mean values with different letters are significantly different at p = 0.05 according to pairwise comparisons with maximum likelihood statistic χ^2 from generalized linear mixed models within each dpi.

Figure 3.

Mean values of Fusarium head blight severity at 14 days post-inoculation (dpi).

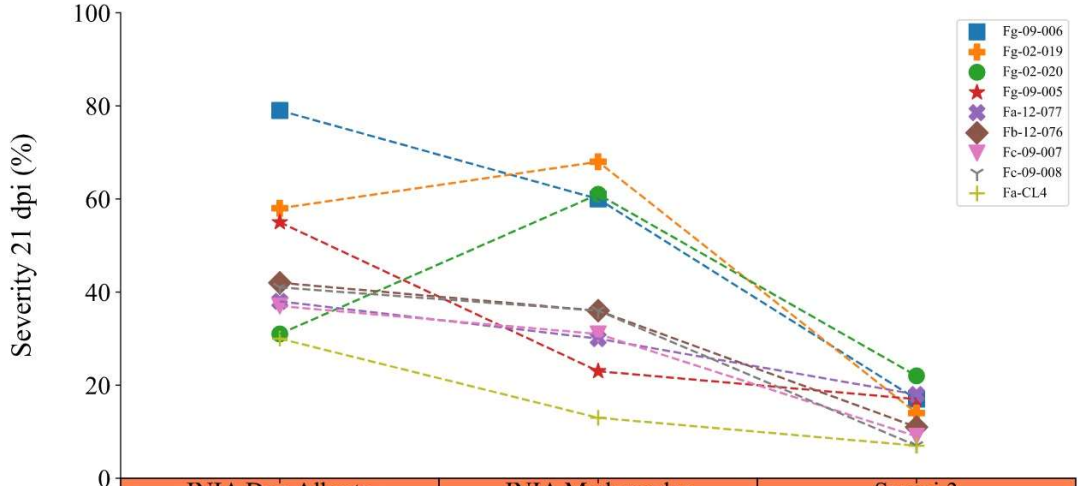


	INIA Don Alberto	INIA Madrugador	Sumai 3
Fg-09-006	a	bc	bd
Fg-02-019	ac	a	abc
Fg-02-020	ab	ce	a
Fg-09-005	aef	bd	abc
Fa-12-077	bce	ade	abc
Fb-12-076	cd	cde	cd
Fc-09-007	bdf	de	abc
Fc-09-008	bcf	de	ab
Fa-CL4	bc	de	ac

Note. Severity as percent of symptomatic spikelets, showing the interaction between wheat genotypes (INIA Don Alberto: susceptible to FHB, INIA Madrugador: moderately susceptible to moderately susceptible-moderately resistant, and Sumai 3, resistant to FHB) and FHB isolate. Data from two experiments (conducted in 2019 and 2020). Fg: *Fusarium graminearum*; Fb: *F. brasilicum*; Fc: *F. cortaderiae*; Fa: *F. asiaticum*. Potential chemotypes: 15ADON: 15-acetyldeoxynivalenol; NIV: nivalenol. Mean values with different letters are significantly different at $p = 0.05$ according to pairwise comparisons with maximum likelihood statistic χ^2 from generalized linear mixed models within each wheat genotype.

Figure 4.

Mean values of Fusarium head blight severity at 21 days post-inoculation (dpi).



	INIA Don Alberto	INIA Madrugador	Sumai 3
Fg-09-006	ab	cd	ab
Fg-02-019	bc	a	ab
Fg-02-020	ad	b	a
Fg-09-005	ac	ceg	b
Fa-12-077	ce	bdeg	ab
Fb-12-076	cd	acf	ab
Fc-09-007	cd	fgh	ab
Fc-09-008	ad	e	b
Fa-CL4	de	aeh	ab

Note. Severity as percent of symptomatic spikelets showing the interaction between wheat genotype (INIA Don Alberto: susceptible to FHB, INIA Madrugador: moderately susceptible to moderately susceptible-moderately resistant, and Sumai 3, resistant to FHB) and FHB isolate. Data from two experiments (conducted in 2019 and 2020). Fg: *Fusarium graminearum*; Fb: *F. brasilicum*; Fc: *F. cortaderiae*; Fa: *F. asiaticum*. Potential chemotypes: 15ADON: 15-acetyldeoxynivalenol; NIV: nivalenol. Mean values with different letters are significantly different at $p = 0.05$ according to pairwise comparisons with maximum likelihood statistic χ^2 from generalized linear mixed models within each wheat genotype.

Table 1.
Fusarium graminearum species complex (FGSC) selected isolates and chemotype.

Isolate code	Phylogenetical species	Chemotype	Plant species[1]	Year of collection	Location[2]	Reference
02-019	*F. graminearum*	15ADON	Wheat	2002	Rio Negro	(Cabrera, 2009) (Umpiérrez, Garmendia, Pereyra, et al., 2013)
02-020	*F. graminearum*	15ADON	Wheat	2002	Soriano	(Cabrera, 2009) (Umpiérrez, Garmendia, Pereyra, et al., 2013)
09-005	*F. graminearum*	15ADON	Wheat	2009	Rio Negro	(Umpiérrez, 2013) (Umpiérrez, Garmendia, Cabrera, et al., 2013)
09-006	*F. graminearum*	15ADON	Wheat	2009	Paysandú	(Umpiérrez, 2013) (Umpiérrez, Garmendia, Cabrera, et al., 2013)
09-007	*F. cortaderiae*	NIV	Wheat	2009	Cerro Largo	(Umpiérrez, 2013) (Umpiérrez, Garmendia, Cabrera, et al., 2013)
09-008	*F. cortaderiae*	NIV	Wheat	2009	Paysandú	(Umpiérrez, 2013) (Umpiérrez, Garmendia, Cabrera, et al., 2013)
CL4	*F. asiaticum*	NIV	Wheat	2011	Cerro Largo	(Umpiérrez, 2013)
12-076	*F. brasilicum*	NIV	Wheat	2012	Cerro Largo	(Umpiérrez, 2013)
12-077	*F. asiaticum*	NIV	Barley	2012	Soriano	(Garmendia et al., 2018)

Note. [1]Plant species from where the isolate was obtained.

[2]Region or Uruguay (Departamento) from which the isolate was collected.

Table 2.
Range of average values of *Fusarium* head blight severity values and area under disease progression curves (AUDPC).

		2019		2020	
	Factor	**Minimum**	**Maximum**	**Minimum**	**Maximum**
Severity (%) 7 dpi	I	3.55	15.31	2.30	10.37
	G	5.69	12.44	2.75	6.21
	I×G	0.64	20.94	0.00	12.80
Severity (%) 14 dpi	I	7.54	34.91	5.09	20.68
	G	12.67	38.89	5.37	16.59
	I×G	2.19	67.11	0.00	44.04
Severity (%) 21 dpi	I	28.47	61.39	6.54	39.50
	G	22.25	62.76	9.87	33.72
	I×G	12.19	88.07	0.45	67.55
AUDPC	I	185.11	566.36	77.07	342.82
	G	242.39	578.90	91.35	277.62
	I×G	90.29	924.60	1.56	617.83

Note. Severity assessed as percentual of symptomatic spikelets, at 7, 14 and 21 days post infection (dpi) and area under disease progression curves (AUDPC) based on severities at 0, 7, 14 and 21 dpi for each factor: isolate (I), wheat genotype(G) and their interaction (IxG).

Table 3.
Hypothesis test of fixed effects on Fusarium head blight severity values.

Dependent variable	**Factor**	**χ^2 or F-value**	**df**	**p-value**
Severity[1] 7 dpi	Isolate (I)	23.70	8	0.0026
	Wheat genotype (G)	12.77	2	0.0017
	I×G	21.47	16	0.1613
Severity[1] 14 dpi	I	43.56	8	<0.0001
	G	54.92	2	<0.0001
	I×G	32.23	16	0.0093
Severity[1] 21 dpi	I	185.25	8	<0.0001
	G	66.43	2	<0.0001
	I×G	40.14	16	0.0007
AUDPC[2]	I	2.71	8	0.0258
	G	10.95	2	0.0004
	I×G	0.70	16	0.7653

Note. Severity assessed as percentual of symptomatic spikelets. at 7, 14 and 21 days post infection (dpi) and on area under disease progression curves (AUDPC) based on severities at 0, 7, 14 and 21 dpi for three plant materials (INIA Don Alberto: susceptible to FHB, INIA Madrugador: moderately susceptible to moderately susceptible-moderately resistant, and Sumai 3, source of resistance for FHB) per genotype. Data from two experiments (conducted on 2019 and 2020).[1] p-values below 0.05 indicate significant differences among factors according to maximum likelihood statistic, χ^2 from generalized linear mixed models or [2] according to F-value statistic from general linear mixed model

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3. Strategies for integrated management of *Fusarium* Head Blight of wheat in Uruguay

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3.1. Abstract

Fusarium head blight (FHB) has been one of the main constraints for wheat production in Uruguay in the last 20 years. Field experiments were conducted in southwestern Uruguay in 2018 and 2019 to evaluate different strategies of integrated management of FHB and their effects on production and safety variables. We studied the combination of two levels of cultivar resistance to FHB (susceptible-S and moderately resistant-MR), two recommended fungicides (prothiconazole + tebuconazole Prosaro® and metconazole + epoxiconazole Swing® Plus) and three application times (ZadoksGS61-beginning of anthesis, ZGS65-50% anthesis and ZGS71-kernel watery ripe). FHB incidence and severity were assessed at the plot level at ZGS75-medium milk and ZGS83-early dough stages. Deoxynivalenol (DON) and fungicide residues were determined in harvested grain. We found a significant reduction of FHB due to cultivar resistance and a positive effect of integrating cultivar resistance and fungicide applied at ZGS61 or ZGS65 reaching the lowest DON contents, acceptable for grain commercialization. None of the grain samples had fungicide residues above the maximum residue limit (MRL) as per European Union (EU). Results from this

research emphasize the importance of integrated management of FHB and provide information on the effect of late applications on FHB control, DON content, production variables and fungicide residues in grains, with only a few precedents in the country.

KEYWORDS: *Fusarium* head blight, wheat, integrated management, Uruguay

3.2. Resumen

La fusariosis de la espiga (FE) ha sido una de las mayores limitantes de la producción de trigo en Uruguay en los últimos 20 años. Se llevaron a cabo experimentos de campo en el sudoeste de Uruguay en 2018 y 2019 para evaluar diferentes estrategias de manejo integrado de FE y su efecto sobre variables productivas e inocuidad. Se estudió la combinación de dos niveles contrastantes de resistencia a FE (susceptible y moderadamente resistente), dos fungicidas recomendados (protioconazol + tebuconazol Prosaro® y metconazol + epoxiconazol Swing® Plus), así como tres momentos de aplicación (inicio de antesis ZadoksGS61, 50 % de antesis ZGS65 y ¾ grano acuoso ZGS71). Se evaluaron incidencia y severidad de FE en la parcela en grano lechoso (ZGS75) y grano lechoso-pastoso (ZGS81). También se determinó el contenido de deoxinivalenol (DON) y residuos de fungicidas. Se observó una reducción significativa de FE, debido a la resistencia del cultivar, así como un efecto positivo de integrar resistencia y aplicación de fungicidas en ZGS61 o ZGS65, reportando niveles aceptables de DON. No se encontraron residuos de fungicidas por encima del límite máximo de residuos (LMR) de la Unión Europea (UE). Los resultados de este trabajo resaltan la importancia del manejo integrado de la FE y proveen valiosa información sobre el efecto de aplicaciones tardías, contenido de DON, variables productivas, así como sobre residuos de fungicidas de los cuales hay apenas algunos precedentes en el país.

PALABRAS CLAVE: fusariosis de la espiga, trigo, manejo integrado, Uruguay

3.3. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in Uruguay. Over the past eight growing seasons for cereals and oil crops, wheat has consistently ranked second in terms of sown area, just behind soybean (DIEA, 2020). One of the main diseases that affects wheat is fusarium head blight (FHB), caused by different *Fusarium* species. In the Southern Cone of South America, results from Brazil, Argentina, Uruguay and Paraguay show that FHB is mainly incited by members of the species complex of *Fusarium graminearum*, both in wheat and barley, with *Fusarium graminearum sensu stricto* Schwabe (*Gibberella zeae* Schw. & Petch) being the dominant species (Alvarenga et al., 2022; Castañares et al., 2016; Garmendia et al., 2018; Nogueira et al., 2018; Umpiérrez, Garmendia, Pereyra, et al., 2013).

The *Fusarium graminearum* species complex (FGSC) is known to produce mycotoxins, primarily type B trichothecenes, including nivalenol (NIV) and deoxynivalenol (DON), along with its acetylated compounds, 3ADON and 15ADON. DON (or *vomitoxin*) is toxic to different mammalian species such as mice, rats and pigs, causing emesis and reduction of body weight or reproductive complications, among other effects (CAC, 2019; JECFA, 2011). DON is one of the most regulated mycotoxins in wheat and barley grains and byproducts, despite it is not the only mycotoxin produced by the FGSC. A maximum limit of 2000 µg/kg for wheat grain, of 1000 µg/kg for flour, semolina and flakes and 200 µg/kg for cereal-based foods for infants and young children has been established by the *Codex Alimentarius* Commission (CAC, 2019). In Uruguay, DON is regulated by a national resolution establishing a maximum limit of 1000 µg/kg for wheat flour and wheat derivatives for human consumption and it is forbidden to import and trade flours over that limit (Decreto n.º 533/001, 2001; Decreto n.º 470/002, 2002).

Grain yield and quality greatly depend on the wheat genotype and environmental conditions (such as relative humidity above 80% and temperatures ranging from 10 °C to 25 °C) and the susceptibility of the host plant. The most susceptible phenological stage to FHB in wheat is anthesis, Zadoks growth stage (ZGS61) (Zadoks et al., 1974). However, infection may also occur up from ZGS61 to the soft dough kernel stage (ZGS85) (McMullen et al., 2012); late infected grains exhibit reduced numbers, smaller sizes and lower weights compared to healthy grains (Alconada & Kikot, 2013). Therefore, FHB causes important economic losses all over the world, not only due to the risk of toxin accumulation but also as a result of the reduction of grain yield, test weight and physical and industrial grain quality, as FHB index increases (Brar et al., 2019; D'Angelo et al., 2014; Paul et al., 2018; Pereyra, Castro, et al., 2014; Reynoso et al., 2013; Salgado et al., 2014, 2015; Ward et al., 2008). Integrated management, including the use of fungicides, has led to significantly higher grain yields and test weight in comparison to susceptible checks (Paul et al., 2019).

The susceptibility of the host plant depends on the resistance level of the wheat genotype. Although it has been proved that cultivar resistance alone is efficient in reducing FHB severity and DON accumulation, integrated management is necessary to overcome moderate to severe FHB epidemics, since under favorable years for FHB cultivar resistance can be insufficient to reach acceptable DON levels (Blandino et al., 2012; McMullen et al., 2012; Paul et al., 2019). Hence, chemical control is preventive and complementary to other management tools as there is not a single strategy that can avoid FHB infections (McMullen et al., 2012; Paul et al., 2018; Pereyra et al., 2014; Salgado et al., 2014). Fungicide applications leading to the best control efficiencies match the moment of maximum susceptibility at ZGS61 (Díaz & Pereyra, 2011). Furthermore, fungicide applications at post-anthesis (five to seven days after anthesis) have been equally effective in reducing both FHB index and DON levels when compared to optimal applications made during early anthesis (D'Angelo et al., 2014; Paul et al., 2019).

In Uruguay, all recommended fungicides for managing FHB and reducing DON are demethylation inhibitors (DMI). Among these, two highly efficient mixes of triazoles stand out: metconazole + epoxiconazole and prothioconazole + tebuconazole (Pereyra & González, 2022). Fungicides that contain quinone outside inhibitors (QoI), also known as strobilurins, are not recommended as they are associated with higher levels of DON (McMullen et al., 2012).

Despite the given efficacy of fungicides against FHB applied at the optimal stage of wheat growth, external factors such as weather conditions do not always allow these preventive applications to take place when needed, but rather become later applications, awakening concerns about fungicide residues. Most triazoles have an established maximum residue limit (MRL) given by the *Codex Alimentarius* or the European Commission Regulation which are used in Uruguay as the locally admitted maximum content when there is not a national regulation for a given compound (Resolución n.º 75/018 DGSA Límites Máximos de Residuos de Plaguicidas (LMR) para Productos de Origen Vegetal, 2018). An appropriate use of fungicides, according to manufacturer instructions, would not only be effective in the control of FHB but also would maintain fungicide residues below MRL (Zhang et al., 2015).

In Uruguay, there are just a few precedents on fungicide residues quantification on wheat and none of these studies has detected fungicide residues over the MRL (Baráibar, 2018; Francia et al., 2017; Palladino et al., 2018).

The main objective of this study was to optimize FHB integrated control practices for Uruguayan cropping systems, whilst minimizing deoxynivalenol (DON) and fungicides residues in wheat grain, through different integrated management strategies that combine host resistance and fungicide treatments (considering active ingredients and moment of application). In order to do that, we evaluated the effect of the implemented management of FHB, based on AUDPC (area under diseased progression curve) obtained as incidence and severity, that became FHB index, and post-harvest

on *Fusarium* damaged kernels (FDK), DON content, grain yield (kg/ha), test weight (TW) and thousand kernel weight (TGW).

3.4. Materials and methods

3.4.1. Experimental field conditions and design

We conducted four field experiments in 2018 and 2019 at La Estanzuela (LE) Experimental Station, National Agricultural Research Institute (INIA), located in the Department of Colonia, southwestern Uruguay (34°20' S, 57°41'W). Trials were sown on June 29th (COL_18_1) and August 7th (COL_18_2) in 2018, and May 31st (COL_19_1) and July 5th (COL_19_2) in 2019.

The experimental layout was an augmented factorial design with four completely randomized blocks with 14 treatments 12 + 2 (2 × 2 × 3 + 2). Factors included: two wheat cultivars (CULT) with contrasting FHB resistance, INIA Don Alberto (IDA), susceptible to FHB (Castro et al., 2014), and Génesis 6.87 (LE2387), moderately resistant to FHB (Castro et al., 2022); two of the most common commercial fungicides to control FHB in Uruguay (FUNG): Prosaro® (Bayer CropScience; Uruguay): prothioconazole 125 g/l + tebuconazole 125 /g/l and Swing® Plus (BASF; Uruguay): metconazole 27.5 g/l + epoxiconazole 37.5 g/l and three fungicide application moments (MOM): early-anthesis (ZGS61), mid anthesis (ZGS65) and at watery ripe grain (ZGS71) stages (Zadoks et al., 1974). Additionally, we included a non-treated control, for each cultivar.

All 14 treatments were completely randomized within each block and experiment. Each plot consisted of six furrows of five meters long, spaced at a distance of 0.16 meters, which were sown using a self-propelled conventional plot seeder (Plotman, Wintersteiger® Austria).

Artificial inoculation was performed at two phenological stages, flag leaf fully emerged (ZGS39) and half of the spike visible (ZGS55; Zadoks et al., 1974) with grain spawn. At each moment, 40 g/m^2 of *F. graminearum* infected maize grains were spread on the soil of each plot. After the first inoculation, and until the last disease assessment, plots were irrigated with rotative XCEL-WOOBBLER™ #14 noozle-blue sprinklers (Senninger®) for ten minutes each time, twice to four times per day, about 7 mm per day. The aim of these irrigations was to wet and dry infected maize kernels, so that *F. graminearum* spores were released and to keep spikes humid enough to enhance FHB infection and subsequent colonization.

3.4.2. Colonized grain inoculum

Artificial inoculum consisted of maize grains inoculated with a mixture of 12-13 representative Uruguayan isolates of *F. graminearum sensu stricto*, chemotype 15ADON, which is the dominant species within the FGSC prevalent in the Uruguayan wheat growing region. Isolates were obtained from wheat grains from different cultivars, locations and years, and hold proven pathogenicity to wheat (Umpiérrez-Failache et al., 2013, Vero et al., personal communication). Inoculum was prepared and applied according to the modifications of Campbell & Lipps (1998) and Dill-Macky (2003) procedures.

Briefly, we soaked maize grains in tap water for 24 hours, then water was fully drained, grains were put into stainless steel trays covered with a double layer of aluminium foil and sterilized in an autoclave for 30 min at 120 °C. Each isolate of *F. graminearum* was grown on potato dextrose agar (PDA-OXOID CM0139) and incubated in a growth chamber (Model 818 Incubator Thermo Scientific Precision) at 25 °C and 12 h light and dark cycle for five to 10 days, then transferred into sterile grains in each tray to produce abundant mycelia. Colonized kernels were stirred every seven days to break mycelial lumps. After 14 days, grains were uniformly distributed on a surface disinfected plastic layer and dried at room temperature with sufficient natural light to

promote perithecia development. Inoculum was then stocked under room temperature and dry conditions until use.

3.4.3. Applications of fungicides

Fungicides were applied at ZGS61 early anthesis, ZGS65 mid-anthesis and ZGS71 grain water ripe stages (Zadoks et al., 1974), using a CO_2 backpack (ScubaType W203S, BellSpray Inc, Opelousas) connected to a bar with four Twin Jet 60° noozles (TeeJet® technologies, Wheaton) 25 cm apart from each other.

3.4.4. FHB assessment

We visually assessed FHB incidence and severity at the plot level based on a double-digit scale (0-10/0-10), where the first number corresponds to the incidence, (percentage of infected spikes in a plot) and the second digit to severity (the percentage of symptomatic spikelets within the diseased spikes in a plot).

Additionally, we determined diseased and total spikelets in 20 tagged spikes per plot. Incidence was the percentage of spikes with FHB symptoms and severity the total of diseased spikelets in 20 spikes per plot. Both assessments were recorded simultaneously at medium milky grain (ZGS75) and early dough grain stages (ZGS83; Zadoks et al., 1974).

For both methodologies, we obtained the FHB index as the product of incidence and severity. We calculated the area under the disease progress curves (AUDPC) based on the FHB index in the two assessment moments and origin at early-anthesis (ZGS61), where we assumed that there was no infection (FHB index = 0) according to Madden et al. (2007).

$$AUDPC_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

3.4.5. Post-harvest determinations

We determined grain yield (kg/ha), test weight, TW (kg/hl) and thousand grain weight, TGW (g) in all plots.

The percentage of *Fusarium* damaged kernels (FDK) was determined by visual evaluation following the method of the Canadian Grain Commission (Official Grain Grading Guide, 2019), in representative samples of each plot (FDK). The same methodology was applied to the grains obtained from the 20 tagged spikes (SpkFDK).

We also quantified deoxynivalenol (DON, ppm) with AgraQuant® Deoxynivalenol direct competitive enzyme-linked immunosorbent assay (ELISA) kits in all plots of two blocks per trial at the three following trials: COL_18_1, COL_19_1 and COL_19_2.

Finally, fungicide residues were quantified in collaboration with Dr. L. Pareja group at CENUR (Udelar), by liquid chromatography mass-spectrometry (HPLC-MS/MS) in 16 samples per trial, corresponding to the late fungicide applications treatments (ZGS71) and five extra samples at COL_19_1 corresponding to the mid-anthesis fungicide application treatments (ZGS65). These treatments were selected on the basis of a greater probability of detecting fungicides residues.

The limits of quantification (LOQ) were 100µg/kg, 10µg/kg, 10µg/kg, 10µg/kg for prothioconazole, tebuconazole, epoxiconazole and metconazole, respectively.

3.4.6. Statistical analysis

Statistical analyses were conducted using InfoStat® (Di Rienzo et al., 2008), which runs on the user installed version (4.02) of R (R core team, 2021).

AUDPC and SpkAUDPC were transformed to square root to fit normality assumptions. The general linear model procedure was used to analyze √AUDPC, √SpkAUDPC, grain yield, TW, TGW and DON at the individual environments. INFOSTAT® uses the *gls* function of the *nlme* package of R, the variance estimator was the restricted maximum likelihood (REML) and when significant differences were found among treatments, we ran contrasts to compare the different factors (CULT, FUNG, MOM and their interactions). When contrasts were significant, we ran pairwise comparisons. The model was given by:

$$Y_{ij} = \beta_0 + \beta_1 X_1 + \varepsilon_{ij}$$

$$y: response\ variable\ (\sqrt{AUDPC}, \sqrt{SpkAUDPC}, DON, Grain\ yield, TW\ or\ TGW)$$

$$\beta_0: Intercept$$

$$\beta_i: Regression\ coefficient\ of\ the\ factor\ effect\ (slope)$$

$$X_1: Treatments; \{1-14\}$$

$$\varepsilon_{ij}: independent\ identically\ distributed\ normal\ error$$

For FDK and SpkFDK, the total weight of diseased grains and the total weight of the two samples was used in a generalized linear model with binomial distribution and logit link function. We used the *glm* function of the stats package of R in INFOSTAT®, the variance estimator was the maximum likelihood (ML) and when significant differences were found among treatments, we ran contrasts to compare the different factors (CULT, FUNG, MOM and their interactions). When contrasts were significant, we ran pairwise comparisons. The model was given by:

$$Ln\left(\frac{p_{ij}}{1-p_{ij}}\right) = \mu + \tau_i + \varepsilon$$

$$p_{ij}: Infection\ probability = \frac{grains\ with\ symptoms\ (g)}{total\ weight\ of\ the\ sample\ (g)}$$

$$\mu: Intercept$$

$$\tau_i: Effect\ of\ the\ treatments; \{1-14\}$$

$$\varepsilon: error$$

$$Ln\left(\frac{p_{ij}}{1-p_{ij}}\right) is\ the\ logit\ transformation\ of\ the\ FDK\ probaility$$

We selected the three most contrasting environments (COL_18_1, COL_19_1 and COL_19_2) to analyze all combined data. Linear and generalized mixed models were used agreeing with the selected model for the response variable and the individual environments, the random effect was the essay. INFOSTAT® used the lme function of the *nlme* package of R for linear mixed models and the glmer function of the lme4 package of R for generalized linear mixed models. The variance estimators were the same as for the individual analysis of the environments and when significant differences were found among treatments, we followed the same procedure as previously described.

Finally, the Spearman's correlation coefficients were obtained between FHB associated variables: AUDPC, SpkAUDPC, FDK, SpkFDK and DON.

3.5. Results

3.5.1. FHB and environmental conditions

All four environments had diverse weather conditions in the ten days following anthesis: COL_18_1 (day of flowering October 15th) had average daily temperatures from 11.6 °C to 21.1 °C, relative average humidity over 85% and five rain events (total of 9.3 mm). COL_18_2 (day of flowering November 5th) had warmer daily average temperatures from 17.4 °C to 26.1 °C, relative average humidity over 79% and five rain events (total of 57.9 mm). COL_19_1 (day of flowering October 7th) had average temperatures from 10 °C to 21.6 °C, relative average humidity over 56% and seven rain events (total of 104.7 mm). COL_19_2 (day of flowering October 22nd) had similar average temperatures to the other essay of the same year that ranged from 14.7 °C to 22.3 °C, relative average humidity over 54% and three rain events (total of 16

mm). According to the meteorological station of the national meteorological service (Instituto Uruguayo de Meteorología-INUMET), located at Colonia, average monthly values for October and November (1991-2020) were 16.9 °C and 19.7 °C for temperature, and 113 mm and 104 mm for accumulated precipitation, respectively (INUMET, 2023).

The amount of total rainfall from heading to harvest in 2018 (97.5 mm) was less than half than the amount registered for the same period in 2019 (222.4 mm). Yet, similar number of precipitation events occurred in this phenological period both in 2018 and 2019 (20 vs. 22, respectively). The successful achievement of FHB infection was attributed to the synergistic effect of temperature, natural rainfall and the artificial irrigation regime implemented in this work.

FHB was present in all experiments (environments). We included non-treated checks of each cultivar per repetition in every experiment, as references of the levels of FHB infection reached in each environment and reflected the differences among varieties (figure 1). There were significant differences (p-value < 0.05) between the non-treated cultivars in all environments for most variables. The highest levels of FHB in terms of AUDPC, FDK and specially DON occurred in COL_19_1, followed by COL_19_2 and COL_18_1. On the other hand, COL_18_2 had the lowest FHB infection, and for this reason it was excluded from the combined data analysis.

3.5.2. Effects of different management factors on FHB and DON

There were significant differences among treatments (p-value < 0.05) for AUDPC and SpkAUDPC within environments, except in COL_18_2. FDK had significant differences between treatments at COL_18_2, COL_19_1 and the combined data with p-values of 0.0157, <0.0001 and <0.0001, respectively, and SpkFDK only had significant differences in COL_19_1 (high levels of FHB) and in the combined data (table 1). The lower the FHB levels, the more challenging it becomes to detect

significant differences among treatments. In other words, significant differences were more evident in experiments when FHB levels were high.

There was a significant cultivar effect due to FHB cultivar resistance for all the variables (AUDPC SpkAUDPC, FDK, SpkFDK, yield, TW, TGW and DON, with p-values <0.0001 at the combined data and at least one of the individual experiments. Fungicide effect was only detected at high levels of FHB (in COL_19_1). Finally, even though there were not significant differences among fungicide application moments for FDK and SpkFDK at individual environments, there was a significant moment effect for FDK at the combined data. We also found significant differences among moments for AUDPC, SpkAUDPC and DON accumulation (table 1). There were not significant differences among early (ZGS61) and mid-anthesis (ZGS65) applications; however, late ones (ZGS71) showed significantly higher levels of FHB and DON.

There was a significant CULT × MOM interaction for AUDPC in COL_18_ and COL_19_2 and for SpkAUDPC in COL_18_1, COL_19_1 and COL_19_2 (table 1) and for the combined data, as well as for DON in both 2019 experiments (table 1). Early and mid-anthesis fungicide applications were effective in significantly reducing FHB and DON levels in INIA Don Alberto (susceptible cultivar), as compared to the application at the kernel water ripe stage. Although the ZGS61 and ZGS65 treatments in the moderately resistant cultivar Génesis 6.87 fungicide applications showed lower FHB levels than the ZGS71 application, the model did not detect significant differences among application moments.

Least square means obtained from the statistical models were transformed to the original scale (table 2). Even when maximum and mean FHB levels assessed on the 20 tagged spikes were smaller than those obtained by the visual scale, in terms of AUDPC, both methodologies showed similar results as we found significant differences only among wheat genotype within environments in favor of the moderately resistant variety. Moreover, the FDK values were similar in all

environments, and, usually, the two samples (tagged spikes and plot) also pointed the same results.

The resistant cultivar, Genesis 6.87, had significantly lower AUDPC, SpkAUDPC, FDK, SpkFDK and DON content as compared to INIA Don Alberto when all data was combined (table 2, $p < 0.05$). On average, the use of cv. Genesis 6.87 led to a reduction of FHB over 50% for all variables at the combined data compared to cv. INIA Don Alberto, with reductions of 77.7% on AUDPC (range 66.82%-84.11%), 90,98% on SpkAUDPC (range 85.60%-96.93%), 72.73% on FDK (range 48.89%-67.35%), 84.62% on SpkFDK (7.89% COL_19_1), 71.06% on DON (range 69.23%-80.62%).

We only found significant differences between fungicides (table 2) for AUDPC at COL_19_1 (high levels of FHB) and for DON at COL_19_2 (intermediate levels of infection) in favor of metconazole + epoxiconazole treatments (Swing® Plus). However, since those infection levels were extremely high and we only detected differences at two analyses, it could be considered that there were no significant differences between the tested fungicides mixtures prothioconazole + tebuconazole (Prosaro®) and metconazole + epoxiconazole (Swing® Plus).

In experiments with significant effects of application moment (detected for AUDPC at COL_18_1, COL_19_1, COL_19_2 and the combined data, for SpkAUDPC at COL_18_1, COL_19_1, COL_19_2 and the combined data, for FDK at the combined data and for DON content at COL_19_1, COL_19_2 and the combined data (table 1), there were no significant differences between treatments where fungicides were applied at early (ZGS61) and mid-anthesis (ZGS65). Conversely, we observed a significant increase of FHB (as AUDPC, SpkAUDPC and DON content) in late fungicide applications (ZGS71). Thus, fungicide applications as late as watery ripe grain were not effective to prevent FHB infection and development (table 2). Although the moment effect was harder to detect for FDK (both assessment methodologies), there were significant differences within moments for DON, even though FDK and DON are highly correlated. We obtained high levels of DON in all the experiments

with intermediate to high FHB, and above the recommended limit of 2000 µg/kg of wheat grain (CAC, 1995).

3.5.3. Effects of different management factors on grain yield and physical quality variables

We found significant differences among the 14 treatments (the different combinations of the two cultivars, the two fungicides and the three application moments, plus the two checks without fungicide) for grain yield, TW and for TGW at all the environments (COL_18_1, COL_18_2, COL_19_1_ COL_19_2 and the combined data). The difference was mostly due to the higher potential yield of Génesis 6.87 in comparison to INIA Don Alberto (table 3), associated to the resistance to FHB. As a result, we detected a CULT effect ($p < 0.05$) for all the environments and variables. We did not find significant differences among FUNG and MOM of fungicide application at the different specific environments with two exceptions: where we detected a MOM effect for TW at COL_18_1 and for TGW at COL_18_2 (table 3). Yet, when we analyzed the combined data, there were significant differences among MOM treatments for grain yield, TW and TGW (table 3), significantly higher values for all variables at early and mid-anthesis applications in contrast with late applications (table 4).

There was a significant CULT × MOM effect only in one specific environment, COL_19_2 for TWG (table 3). However, there were significant differences at the combined data for grain yield, TW and TGW (table 3). The significant effect of CULT × MOM might be associated to the strong cultivar effect. On one side, we detected significant differences within moments for grain yield, TW and TGW in the susceptible variety INIA Don Alberto, while in the resistant cultivar Génesis 6.87 the model did not detect any difference resulting in a significant interaction.

We found significant differences between CULT for all variables associated to productivity (grain yield) and physical quality of the grain (TW and TGW). INIA Don

Alberto had significantly lower grain yield (-39.94%), TW (-3.423%) and TGW (-15.985%) than Génesis 6.87 (table 4).

3.5.4. Correlation among FDK, SpkFDK and DON

The Spearman's correlation coefficients (rho) pointed that the relationships between variables decreased along levels of FHB. Significant rho ($p < 0.05$) ranged from 0.35 to 0.96 considering all environments. At intermediate (COL_19_2) and high levels (COL_19_1) of disease and at the combined data rho values ranged from 0.72 to 0.96, whilst at low levels of FHB (COL_18_1 and COL_18_2) rho values were from 0.35 up to 0.73 (table 5). The above mentioned was also consistent with the two assessment methods, since the 20 spikes counting had high correlation to the visual assessment.

3.5.5. Fungicide residues

Epoxiconazole was detected in 18.9% of the analyzed samples. Values ranged from 10 µg/kg (limit of quantification-LOQ) to 24 µg/kg. Yet, in all cases, epoxiconazole levels were below the maximin residue limit (MRL) of 600 µg/kg given by the European Commission Regulation (EU) No 978/2011 (2011). Metconazole was detected in a single sample that came from a plot of Génesis 6.87 applied with Swing® Plus at ZGS71(14 µg/kg; LOQ: 10 µg/kg), and thus below the MRL of 150 µg/kg given by the European Commission Regulation (EU) 2016/1902 (2016) (table 6).

Prothioconazole and tebuconazole were not detected above the LOQs of 100 µg/kg and 10 µg/kg, respectively, in any of the samples where these fungicides had been applied, yet as late as kernel watery-ripe grain stage, ZGS71.

3.6. Discussion

This research got the challenge of achieving a FHB integrated disease management in wheat, testing host resistance, fungicide selection and moment of application in four experiments. The four trials had different environmental conditions and, consequently, very contrasting FHB levels. As the level of FHB increased, it was easier to observe significant differences for disease variables into each environment; therefore, COL_19_1 was the environment which weighted the more on the combined data analysis. Accordingly, we decided to remove COL_18_2 from the bulk analysis, due to a high yellow rust infection, poor crop density, less and smaller spikes, and consequently lower FHB infection.

We found that all treatments were effective in reducing FHB if compared to the non-treated susceptible cultivar, but there was a clear effect of the resistance of the wheat genotype on FHB (AUDPC), DON and yield and grain physical quality (TW and TGW), which we observed also when comparing the non-treated cultivars. These results were consistent with previous reports (Blandino et al., 2012; Brar et al., 2019; D'Angelo et al., 2014; Duan et al., 2019; Paul et al., 2018, 2019; Salgado et al., 2011, 2014).

Previously, Paul et al. (2018) tested two different mixes of triazoles prothioconazole + tebuconazole against metconazole applied at different moments and found that there were no significant differences on FHB index, grain yield and test weight. Yet, there were statistically significant differences on DON content when fungicides were applied at anthesis versus late applications. We also tested two mixes of triazoles prothioconazole + tebuconazole (Prosaro®) versus metconazole + epoxiconazole (Swing® Plus) and found similar results: there were no significant differences in quality associated variables and FDK. We only observed significant differences among fungicides with Swing® Plus reducing up to 23.2% AUDPC at extremely high levels of FHB (COL_19_1 and combined data) and reducing DON up to 39.5% at intermediate levels of FHB (COL_19_2). It seems that fungicides including

metconazole tend to control better FHB from intermediate to high levels of FHB, and this should be analyzed more deeply.

Brancatti et al. (2022) tested the four active principles used in this study for half maximal effective concentration EC50 (ppm) on all the isolates used for artificial inoculum and three samples from COL_2018_1. They found that greater concentrations of tebuconazole were required to inhibit *Fusarium* growth (up to 5.5 ppm, with a mean value of 2.8 ppm) whilst metconazole, epoxiconazole and prothioconazole had mean EC50 values of 0.08 ppm, 0.95 ppm and 0.37 ppm, respectively. The three field samples came from fungicide applied plots (two with Prosaro® and one with Swing® Plus), epoxiconazole and metconazole pointed the same that the isolates results; on the other hand, the two samples applied with Swing® Plus showed resistance to prothioconazole with a mean EC50 of 10.95 ppm and 0.31 ppm for tebuconazole, opposite to the observed on the isolates.

Fungicide applications at anthesis are effective against FHB (D'Angelo et al., 2014; Díaz & Pereyra, 2011; Paul et al., 2018), but most studies focused mainly on early applications (around heading), beginning of anthesis (ZGS61) or 50% anthesis (ZG65) (Zadoks et al., 1974). In this study we tested the two last mentioned stages and included a late application at kernel watery ripe (ZGS71). Our results indicated that applications at ZGS65 were as effective as ZG61. Contrarily, late applications (ZGS71) lead to significantly higher AUDPC values and DON concentrations comparable to the ones obtained in the non-treated checks. Hence, when conditions do not allow fungicides applications at flowering, doing so at early grain filling stages would not be recommended.

We analyzed the interactions among the three studied factors (cultivar resistance, fungicides and moments of fungicide applications) and found a significant moment by genotype interaction. It stands out that at higher levels of FHB it was harder to detect the interaction. The interaction indicated that there were not significant differences among moment of application of fungicides on the moderately-resistant cultivar

(Génesis 6.87), whilst the model detected significant differences on the susceptible cultivar (INIA Don Alberto) at low levels of FHB.

Our results support that only at lower levels of FHB, the genetic resistance of the cultivar or early fungicide applications on the susceptible material might be sufficient to control FHB and obtain low or no DON, even though, we observed that integrated management was required to reach acceptable levels of DON in 2019 and fulfill quality requirements. In both 2019 experiments, we obtained FHB indexes over 10%, which some authors have established as FHB epidemics (D. A. Shah et al., 2014; L. Shah et al., 2018).

Both cultivars have similar grain yield potentials, according to the documented record of grain yields obtained from the National Characterization of Cultivars (ENC) trials managed with fungicide. For Génesis 6.87, grain yields were as high as 9312 kg/ha in 2016 and of 8280 kg/ha in 2014, with and without fungicide, respectively (Castro, Pereyra, et al., 2022). For INIA Don Alberto they were of 9350 kg/ha in 2014 and 7987 kg/ha in 2011, with and without fungicide, respectively (Castro, Pereyra, et al., 2022). Significant differences on quality and yield of the grain were mostly given by the potential of each cultivar but this is closely related to the resistance to FHB, as the infection may directly affect grain filling.

Finally, we assessed correlations between variables and found that the visual scale (1-10/1-10) used to assess field FHB incidence and severity is a good estimate as there are high significant correlations with the 20 spikes assessment method. The visual evaluation speeds up the job at field and provides valid data. The correlations increased along with FHB levels and both AUDPC and FDK significantly correlated with DON.

The results of this study provide valuable data on the integrated management for FHB in Uruguay and contribute to the few precedents on fungicide residues on wheat (Baráibar, 2018; Francia et al., 2017; Palladino et al., 2018). Our results were consistent with those obtained by these authors in the sense that we did not detect any

of the analyzed active principles above the maximum residue limit (MRL). However, as Baráibar (2018), we detected epoxiconazole more frequently, which is worth to be analyzed more deeply.

3.7. Funding details

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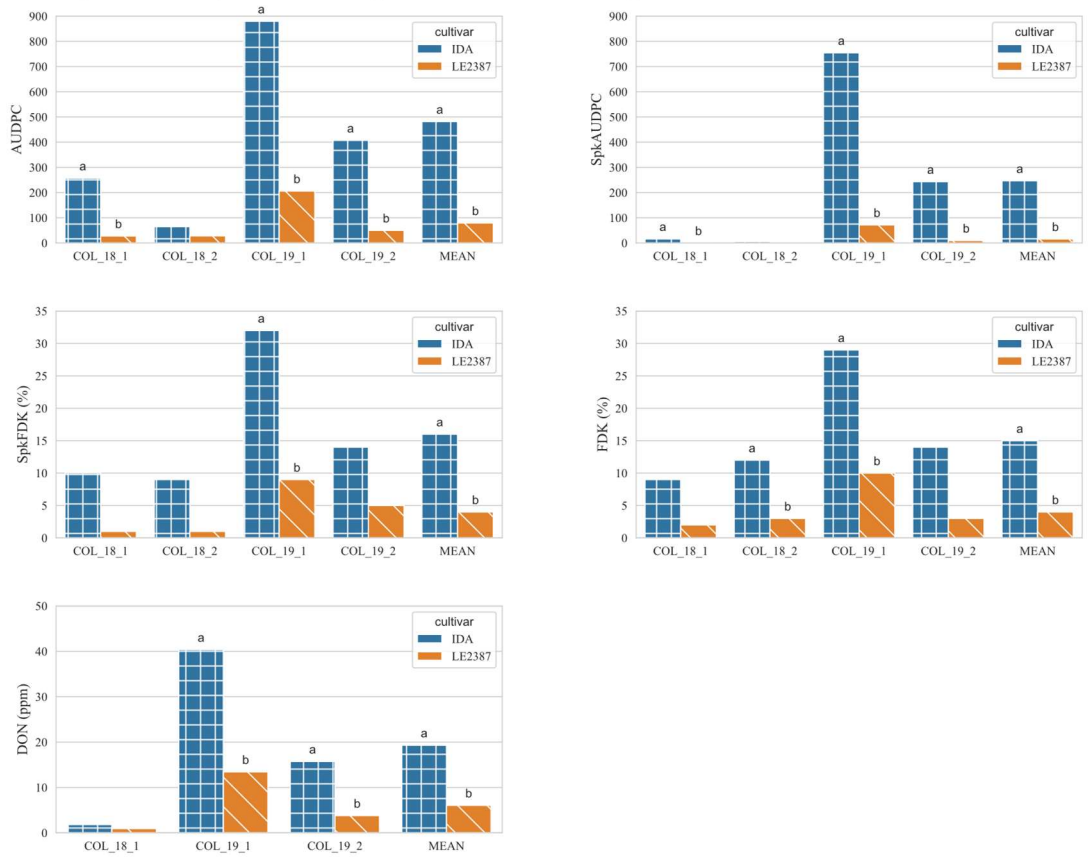
3.8. Disclosure statement

The authors report there are no competing interests to declare.

Figure 1.

Average values of AUDPC, SpKAUDPC, FDK, SpkFDK and DON content.



Note. Average values of area under diseased progress curves based on *Fusarium* head blight (FHB) index assessed on whole plots (AUDPC), in 20 spikelets per plot (SpkAUDPC), *Fusarium* damaged kernels from whole plots (FDK), from 20 spikelets per plot (SpkFDK)and deoxynivalenol (DON) content for two cultivars (susceptible and moderately resistant) checks without fungicide application, in four experiments (environments) and mean from the general mixed model of the three most contrasting environments[1]. Data transformed from least square means from the general linear models for square root of AUDPC, square root of the Spk (Spikelets) AUDPC, Deoxynivalenol (DON) .

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

INIA Don Alberto (IDA), susceptible to FHB (Castro et al., 2014)

Génesis 6.87 (LE23.87), moderately resistant to FHB (Castro, Germán, et al., 2022)

Linear general model (√AUDPC, √SpkAUDPC and DON)

Generalized linear models (FDK and SpkFDK)

For DON only two repetitions for experiment included, and no data was taken for COL_18_2.

Mean values with the same letter are not significantly different at p = 0.05, within given variable and environment, according to pairwise comparisons based on *F* test from linear general and mixed models[4] or according to maximum likelihood statistic, χ^2 from generalized linear and mixed models.

Table 1.

p-values of type III fixed effects (treatment) and of pairwise comparisons for the factors (cultivar, fungicide, moment, and their Interactions) for AUDPC, SpKAUDPC, FDK, SpkFDK and DON content.

Factor	Environment	√AUDPC[2]	√SpkAUDPC[2]	FDK[3]	SpkFDK[3]	DON[2-4]
Treatment[5]	**COL_18_1**	<0.0001	<0.0001	0.8979	>0.9999	0.7370
	COL_18_2	0.3108	0.0712	0.0157	0.9940	NA
	COL_19_1	<0.0001	<0.0001	<0.0001	0.0001	<0.0001
	COL_19_2	<0.0001	<0.0001	0.5479	>0.9999	<0.0001
	Mean[1]	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Cultivar (CULT)	**COL_18_1**	<0.0001	<0.0001	-	-	-
	COL_18_2	-	-	0.0001	-	NA
	COL_19_1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	COL_19_2	<0.0001	<0.0001	-	-	<0.0001
	Mean[1]	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Fungicide (FUNG)	**COL_18_1**	0.1569	0.6318	-	-	-
	COL_18_2	-	-	0.3456	-	NA
	COL_19_1	0.0269	0.1289	0.5100	0.8440	0.8816
	COL_19_2	0.5113	0.9200	-	-	0.0013
	Mean[1]	**0.0073**	**0.4686**	**0.1434**	**0.5989**	**0.5153**
Moment (MOM)	**COL_18_1**	<0.0001	0.0001	-	-	-
	COL_18_2	-	-	0.9067	-	NA
	COL_19_1	0.0019	<0.0001	0.4376	0.3425	0.0002
	COL_19_2	<0.0001	<0.0001	-	-	<0.0001
	Mean[1]	**<0.0001**	**<0.0001**	**0.0124**	**0.2242**	**0.0032**
CULT×FUNG	**COL_18_1**	0.2891	0.6304	-	-	-
	COL_18_2	-	-	0.8525	-	NA
	COL_19_1	0.3682	0.6814	0.9183	0.7626	0.2555
	COL_19_2	0.3470	0.0709	-	-	0.0578
	Mean[1]	**0.1058**	**0.2966**	**0.6394**	**0.8231**	**0.9808**
CULT×MOM	**COL_18_1**	0.0044	0.0011	-	-	-
	COL_18_2	-	-	0.7934	-	NA
	COL_19_1	0.1279	0.0054	0.8816	0.1425	0.0098
	COL_19_2	0.0055	0.0001	-	-	0.0001
	Mean[1]	**<0.0001**	**<0.0001**	**0.6874**	**0.0203**	**0.0615**
FUNG×MOM	**COL_18_1**	0.7375	0.7872	-	-	-
	COL_18_2	-	-	0.9864	-	NA
	COL_19_1	0.4517	0.6201	0.6429	0.8653	0.3288
	COL_19_2	0.4123	0.3085	-	-	0.1685
	Mean[1]	**0.2322**	**0.8864**	**0.8654**	**0.4223**	**0.8884**
CULT×MOM×FUNG	**COL_18_1**	0.5346	0.3198	-	-	-
	COL_18_2	-	-	0.8761	-	NA
	COL_19_1	0.9430	0.9307	0.8514	0.8863	0.1723
	COL_19_2	0.2872	0.1204	-	-	0.7377
	Mean[1]	**0.3937**	**0.8629**	**0.9686**	**0.7714**	**0.8126**

Note. Probabilities (p-values) of type III fixed effects (treatment) and p-values of pairwise comparisons for the factors (cultivar, fungicide, moment, and their Interactions) for square root of area under disease progression curves based on Fusarium head blight (FHB) index assessed on whole plots (AUDPC), from 20 spikelets per plot (SpkAUDPC), *Fusarium* damaged kernels from whole plots (FDK), from 20 spikelets per plot (SpkFDK), and deoxynivalenol (DON) content in four experiments (environments) and mean from the general mixed model of the three most contrasting environments1

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

[2] Linear general model (√AUDPC, √SpkAUDPC and DON)

[3] Generalized linear models (FDK and SpkFDK)

[4] Only two repetitions for experiment included, and no data was taken for COL_18_2. NA= Not available.

[5] Fourteen treatments analyzed (susceptible and resistant checks included). When no significant differences were found among treatments ($p > 0.05$), we did not run further contrasts.

Table 2.

Average values for the factors (cultivar, fungicide and fungicide application moment), for AUDPC, SpkAUDPC, FDK, SpkFDK DON content.

Environment	Factors	AUDPC[4]	SpkAUDPC[4]	FDK (%)[5]	SpkFDK (%)[5]	DON[4-6] (ppm)
	CULTIVAR					
COL_18_1	IDA[2]	109.14[a]	3.45[a]	3.17	2.67	0.99
	LE2387[3]	17.92[b]	0.11[b]	1.17	0.17	0.82
COL_18_2	IDA	67.49	1.93	7.50[a]	4.83	NA
	LE2387	43.65	0.55	3.83[b]	0.83	NA
COL_19_1	IDA	302.67[a]	235.40[a]	16.33[a]	13.17[a]	19.89[a]
	LE2387	100.42[b]	33.89[b]	5.33[b]	3.83[b]	6.12[b]
COL_19_2	IDA	203.39[a]	150.93[a]	7.50	7.00	9.32[a]
	LE2387	32.31[b]	7.38[b]	1.50	0.67	1.81[b]
Mean[1]	IDA	195.25[a]	95.16[a]	7.33[a]	6.50[a]	10.07[a]
	LE2387	43.54[b]	8.58[b]	2.00[b]	1.00[b]	2.91[b]
	FUNGICIDE					
COL_18_1	P+T[7]	68.62	1.84	2.17	1.50	0.88
	E+M[8]	58.44	1.72	2.17	1.33	0.92
COL_18_2	P+T	62.28	1.4	6.17	3.67	NA
	E+M	48.86	1.08	5.17	2.00	NA
COL_19_1	P+T	227.97[a]	145.69	11.17	8.17	12.93
	E+M	175.12[b]	123.61	10.50	8.83	13.08
COL_19_2	P+T	118.30	71.50	5.00	4.67	6.94[a]
	E+M	117.40	86.82	4.00	3.00	4.20[b]
Mean[1]	P+T	128.40[a]	52.02	5.00	4.00	6.90
	E+M	110.38[b]	51.73	4.33	3.50	6.08
	MOMENT[9]					
COL_18_1	ZGS61	28.46[b]	0.24[b]	1.25	0.75	0.73
	ZGS65	30.36[b]	0.43[b]	1.50	0.25	0.96
	ZGS71	131.76[a]	4.67[a]	3.75	3.25	1.02
COL_18_2	ZGS61	51.43	0.96	5.50	3.00	NA
	ZGS65	62.59	1.55	5.25	3.00	NA
	ZGS71	52.69	1.21	6.25	2.50	NA
COL_19_1	ZGS61	153.88[b]	75.76[b]	10.75	7.25	9.26[b]
	ZGS65	160.21[b]	77.18[b]	9.75	6.75	10.76[b]
	ZGS71	290.54[a]	251.00[a]	12	11.5	18.99[a]
COL_19_2	ZGS61	83.79[b]	46.68[b]	4.75	2.50	4.59[b]
	ZGS65	80.49[b]	36.00[b]	2.75	3.50	3.19[b]
	ZGS71	189.28[a]	154.80[a]	6.00	5.50	8.92[a]
Mean[1]	ZGS61	79.59[b]	27.63[b]	4.50[ab]	2.50	4.88[b]
	ZGS65	80.76[b]	25.77[b]	3.50[b]	3.00	4.95[b]
	ZGS71	197.83[a]	102.23[a]	6.00[a]	5.75	9.65[a]

Note. Average values for the factors, for area under disease progression curve index assessed on whole plots (AUDPC), in 20 spikelets per plot (SpkAUDPC), *Fusarium* damaged kernels (FDK) from whole plots, SpkFDK, and from 20 spikelets per plot (SpkFDK) and deoxynivalenol (DON) content in four experiments (environments) and mean from the general mixed model of the three most contrasting environments[1]. Mean values with the same letter are not significantly different at p = 0.05, within a given variable and environment, according to pairwise comparisons based on *F* test from linear general and mixed models[4] or according to maximum likelihood statistic, χ^2 from generalized linear and mixed models[5].

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

[2] INIA Don Alberto (IDA), susceptible to FHB (Castro et al., 2014)

[3] Génesis 6.87 (LE2387), moderately resistant to FHB (Castro, Germán, et al., 2022)

[4] Values transformed from the least square means of the linear general model (√AUDPC, √SpkAUDPC and DON)

[5] Values transformed from the least square means of the generalized linear models (FDK and SpkFDK)

[6] Only two repetitions for experiment included, and no data was taken for COL_18_2. NA= Not available.

[7] Prosaro®: (P+T) Prothioconazole 12,5% + Tebuconazole 12,5% (750 ml/ha)

[8] Swing® Plus : (E+M) Epoxiconazole 3,75 % + Metconazole 2,75 % (1500 ml/ha)

[9] Moment of application of fungicides, according to Zadoks growth scale (Zadoks et al., 1974). ZGS61 corresponds to early anthesis, ZGS65 to mid-anthesis and ZGS71 to kernel water ripe.

Table 3.

p-values of type III fixed effects (treatment) and of the pairwise comparisons for the factors (cultivar, fungicide, moment of fungicide application, and their interactions) for grain yield, test weight (TW) and thousand grains weight (TGW).

Factors	Environment	Grain yield[3]	TW[3]	TGW[3]
Treatment[2]	**COL_18_1**	<0.0001	<0.0001	<0.0001
	COL_18_2	0.0002	0.0242	0.0436
	COL_19_1	<0.0001	<0.0001	<0.0001
	COL_19_2	0.0107	0.0010	0.0216
	Mean[1]	**<0.0001**	**<0.0001**	**<0.0001**
Cultivar (CV)	**COL_18_1**	<0.0001	<0.0001	<0.0001
	COL_18_2	<0.0001	0.0001	0.0254
	COL_19_1	<0.0001	<0.0001	<0.0001
	COL_19_2	0.0005	0.0011	0.0611
	Mean[1]	**<0.0001**	**<0.0001**	**<0.0001**
Fungicide (FUNG)	**COL_18_1**	0.6694	0.4440	0.8901
	COL_18_2	0.4226	0.9490	0.2817
	COL_19_1	0.5086	0.6867	0.1431
	COL_19_2	0.2539	0.7146	0.5466
	Mean[1]	**0.1887**	**0.5164**	**0.7871**
Moment (MOM)	**COL_18_1**	0.0671	0.0012	0.2859
	COL_18_2	0.5831	0.9068	0.0023
	COL_19_1	0.3111	0.2235	0.1200
	COL_19_2	0.2748	0.2707	0.1117
	Mean[1]	**0.0140**	**0.0145**	**0.0187**
CV×FUNG	**COL_18_1**	0.1880	0.8001	0.9247
	COL_18_2	0.5243	0.4261	0.2412
	COL_19_1	0.2030	0.5178	0.9680
	COL_19_2	0.8142	0.8740	0.1021
	Mean[1]	**0.6883**	**0.7587**	**0.3781**
CV×MOM	**COL_18_1**	0.1464	0.0528	0.1870
	COL_18_2	0.8022	0.4665	0.8039
	COL_19_1	0.2706	0.1609	0.0922
	COL_19_2	0.1937	0.2768	0.0179
	Mean[1]	**0.0412**	**0.0244**	**0.0078**
FUNG×MOM	**COL_18_1**	0.4403	0.4339	0.1838
	COL_18_2	0.6562	0.7734	0.7083
	COL_19_1	0.5137	0.8830	0.6749
	COL_19_2	0.7887	0.6430	0.6466
	Mean[1]	**0.2698**	**0.9494**	**0.9462**
CV×MOM×FUNG	**COL_18_1**	0.6025	0.0228	0.9180
	COL_18_2	0.9917	0.1651	0.8264
	COL_19_1	0.8684	0.9948	0.7871
	COL_19_2	0.3121	0.2180	0.8210
	Mean[1]	**0.2899**	**0.1735**	**0.8139**

Note. Probabilities (p-values) of type III fixed effects (treatment) and p-values of the pairwise comparisons for the factors (cultivar, fungicide, moment of fungicide application, and their interactions) for grain yield, test weight (TW) and thousand grains weight (TGW) for whole plots in four experiments (environments) and mean from the general mixed model of the three most contrasting environments1

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2[2] Fourteen treatments analyzed (susceptible and resistant checks included). When no significant differences were found among treatments ($p > 0,05$), we did not run further contrasts.

[3] Linear general model (Grain yield, TW, TGW)

Table 4.

Average values for the factors (cultivar, fungicide, and fungicide application moment), for grain yield, test weight (TW) and thousand grains weight (TGW).

Environment	Factor	Grain yield[4] (kg/ha)	TW[4] (kg/hl)	TGW[4] (g)
	CULTIVAR			
COL_18_1	IDA[2]	4359.07[a]	82.27[b]	31.91[b]
	LE2387[3]	6051.61[b]	83.55[a]	39.02[a]
COL_18_2	IDA	2146.46[b]	78.49[a]	37.00[b]
	LE2387	2892.50[a]	77.10[b]	37.99[a]
COL_19_1	IDA	3961.84[b]	73.93[b]	30.00[b]
	LE2387	6125.25[a]	78.21[a]	36.36[a]
COL_19_2	IDA	5579.01[b]	76.49[b]	32.27
	LE2387	6578.70[a]	78.89[a]	33.86
Mean[1]	IDA	4633.31[b]	77.56[b]	31.39[b]
	LE2387	6252.41[a]	80.22[a]	36.41[a]
	FUNGICIDE			
COL_18_1	P+T[5]	5163.43	82.83	35.40
	E+ M[6]	5247.25	82.99	35.52
COL_18_2	P+T	2565.90	77.78	37.73
	E+M	2473.06	77.80	37.26
COL_19_1	P+T	4947.44	75.96	32.79
	E+M	5139.65	76.18	33.56
COL_19_2	P+T	5925.36	77.56	33.32
	E+M	6232.35	77.82	32.82
Mean[1]	P+T	5345.41	78.79	33.84
	E+M	5540.30	79.00	33.97
	MOMENT [7]			
COL_18_1	ZGS61	5471.63	83.26[a]	36.28
	ZGS65	5242.39	83.13[a]	35.52
	ZGS71	4902.01	82.34[b]	34.60
COL_18_2	ZGS61	2451.97	77.88	37.37[b]
	ZGS65	2597.58	77.71	36.59[b]
	ZGS71	2508.89	77.79	38.53[a]
COL_19_1	ZGS61	5305.91	76.25	33.53
	ZGS65	5068.78	76.54	33.60
	ZGS71	4755.94	75.41	32.41
COL_19_2	ZGS61	6206.96	77.96	33.66
	ZGS65	6255.75	78.18	33.72
	ZGS71	5773.86	76.92	31.82
Mean[1]	ZGS61	5661.50[a]	79.16[a]	34.49[a]
	ZGS65	5523.14[a]	79.29[a]	34.28[a]
	ZGS71	5143.94[b]	78.22[b]	32.94[b]

Note. Average values for the factors (cultivar, fungicide, and fungicide application moment), for grain yield, test weight (TW) and thousand grains weight (TGW). assessed on whole plots, in four experiments (environments) and mean from the general mixed model of the three most contrasting environments[1]

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

[2] INIA Don Alberto (IDA), susceptible to FHB (Castro et al., 2014)

[3] Génesis 6.87 (LE2387), moderately resistant to FHB (Castro, Germán, et al., 2022)

[4] Values transformed from the least square means of the linear general model (Grain yield, TW, TGW)

[5] Prosaro®: (P+T) Prothioconazole 12,5% + Tebuconazole 12,5% (750 ml/ha)

[6] Swing® Plus: (E+M) Epoxiconazole 3,75 % + Metconazole 2,75 % (1500 ml/ha)

[7] Moment of application of fungicides, according to Zadoks growth scale (Zadoks et al., 1974). ZGS61 corresponds to early anthesis, ZGS65 to mid-anthesis and ZGS71 to kernel water ripe.

Mean values with the same letter are not significantly different at p = 0.05, within a given variable and environment, according to pairwise comparisons based on *F* test from linear general and mixed models[4].

Table 5.
Spearman's correlation coefficients matrix for values of *Fusarium* damaged kernels (FDK), SpkFDK, area under diseased progress curves (AUDPC), SpkAUDPC and DON content

		FDK (%)	SpkFDK (%)	AUDPC	SpkAUDPC	DON[2] (ppm)
COL_18_1	**FDK (%)**	**1,00**	<0,0001	<0,0001	<0,0001	0,98
	SpkFDK (%)	0,67	**1,00**	<0,0001	<0,0001	0,22
	AUDPC	0,68	0,77	**1,00**	<0,0001	0,43
	SpkAUDPC	0,56	0,68	0,72	**1,00**	0,36
	DON[2] (ppm)	-0,01	0,24	0,16	0,18	**1,00**
COL_18_2	**FDK (%)**	**1,00**	<0,0001	0,10	<0,0001	NA
	SpkFDK (%)	0,72	**1,00**	0,05	<0,0001	NA
	AUDPC	0,22	0,27	**1,00**	0,01	NA
	SpkAUDPC	0,49	0,53	0,35	**1,00**	NA
	DON[2] (ppm)	NA	NA	NA	NA	NA
COL_19_1	**FDK (%)**	**1,00**	<0,0001	<0,0001	<0,0001	<0,0001
	SpkFDK (%)	0,90	**1,00**	<0,0001	<0,0001	<0,0001
	AUDPC	0,80	0,80	**1,00**	<0,0001	<0,0001
	SpkAUDPC	0,86	0,92	0,83	**1,00**	<0,0001
	DON[2] (ppm)	0,96	0,92	0,83	0,93	**1,00**
COL_19_2	**FDK (%)**	**1,00**	<0,0001	<0,0001	<0,0001	<0,0001
	SpkFDK (%)	0,79	**1,00**	<0,0001	<0,0001	<0,0001
	AUDPC	0,88	0,75	**1,00**	<0,0001	<0,0001
	SpkAUDPC	0,90	0,77	0,91	**1,00**	<0,0001
	DON[2] (ppm)	0,93	0,87	0,84	0,83	**1,00**
Mean[1]	**FDK (%)**	**1,00**	<0,0001	<0,0001	<0,0001	<0,0001
	SpkFDK (%)	0,86	**1,00**	<0,0001	<0,0001	<0,0001
	AUDPC	0,85	0,84	**1,00**	<0,0001	<0,0001
	SpkAUDPC	0,86	0,83	0,83	**1,00**	<0,0001
	DON[2] (ppm)	0,84	0,78	0,72	0,88	**1,00**

Note. Spearman's correlation coefficients matrix for values of *Fusarium* damaged kernels from whole plots (FDK), from 20 spikelets per plot (SpkFDK), area under diseased progress curves based on Fusarium head blight (FHB) index assessed on whole plots (AUDPC), in 20 spikelets per plot (SpkAUDPC) and deoxynivalenol (DON) content in four experiments (environments) and mean of the three most contrasting environments1

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

[2] Only two repetitions for experiment included, and no data was taken for COL_18_2. NA = Not available.

Table 6.

Percentage of detection of active principles of fungicides (courtesy of Pareja et al., personal communication).

		Prosaro®		Swing® Plus	
		Prothioconazole	**Tebuconazole**	**Epoxiconazole**	**Metconazole**
COL_18_1	**%ND[2]**	100	87.5	100	50
	% <LOQ[3]	0	12.5	0	50
	% D[4]	0	0	0	0
COL_18_2	**%ND**	62.5	87.5	12.5	0
	% <LOQ	37.5	12.5	87.5	100
	% D	0	0	0	0
COL_19_1	**%ND**	100	0	0	69.2
	% <LOQ	0	100	53.8	23.1
	% D	0	0	46.2	7.7
COL_19_2	**%ND**	100	100	0	0
	% <LOQ	0	0	87.5	100
	% D	0	0	12.5	0
Total[1]	**%ND**	90.6	68.8	24.3	35.1
	% <LOQ	9.4	31.3	56.8	62.2
	% D	0	0	18.9	2.7

Note. [1]Total number of samples: 16 samples per essay, corresponding to the late fungicide applications (ZGS71) and five extra samples at COL_19_1 corresponding to the mid-anthesis fungicide application

[2] ND: Not detected

[3] Limit of quantification (LOQ): Prothioconazole 100µg/kg, Tebuconazole 10µg/kg, Epoxiconazole 10µg/kg, Metconazole 10 µg/kg,

[4] D: Detected above LOQ

APP Table 1.

Fusarium graminearum species complex (FGSC) selected isolates for artificial inoculum and chemotype.

Isolate code	**Phylogenetical species[1]**	**Chemotype[1]**	**Plant species[2]**	**Year of collection**	***Inoculum* year**
90-000	*Fusarium graminearum ss.*	15ADON	Wheat	1990	2018 & 2019
00-004	*Fusarium graminearum ss.*	15ADON	Barley	2000	2018 & 2019
02-020	*Fusarium graminearum ss.*	15ADON	Wheat	2002	2018 & 2019
09-005	*Fusarium graminearum ss.*	15ADON	Wheat	2009	2018 & 2019
11-001	*Fusarium graminearum ss.*	15ADON	Barley	2011	2018
12-000	*Fusarium graminearum ss.*	15ADON	Wheat	2012	2018
12-017	*Fusarium graminearum ss.*	15ADON	Wheat	2012	2019
12-035	*Fusarium graminearum ss.*	15ADON	Wheat	2012	2019
13-023	*Fusarium graminearum ss.*	15ADON	Wheat	2013	2018 & 2019
14-003	*Fusarium graminearum ss.*	15ADON	Wheat	2014	2018 & 2019
14-012	*Fusarium graminearum ss.*	15ADON	Wheat	2014	2018
16-000	*Fusarium graminearum ss.*	15ADON	Wheat	2016	2018 & 2019
16-014	*Fusarium graminearum ss.*	15ADON	Wheat	2016	2018
17-003	*Fusarium graminearum ss.*	15ADON	Wheat	2017	2018 & 2019
17-010	*Fusarium graminearum ss.*	15ADON	Wheat	2017	2018 & 2019
18-006	*Fusarium graminearum ss.*	15ADON	Wheat	2018	2019

Note. [1]Phylogenetical species and chemotype were determined by the group of PhD. Silvana Vero and communicated by email through Brancatti G. on January 26th of 2021

[2]Plant species from where the isolate was obtained

APP Table 2.

Average values for the Cultivar (CULT) × moment (MOM) interaction, for area under disease progression curve (AUDPC) ,SpkAUDPC, *Fusarium* damaged kernels (FDK), SpkFDK, and DON content

Environment	CULT× MOM	AUDPC[4]		SpkAUDPC[4]		FDK[5] (%)		SpkFDK[5] (%)		DON[4-6] (ppm)	
		IDA[2]	LE2387[3]	IDA	LE2387	IDA	LE2387	IDA	LE2387	IDA	LE2387
	Z61[7]	42.42^{b}	14.50^{cd}	0.41^{b}	0.07^{b}	2.00	0.50	1.50	0.00	0.81	0.66
COL_18_1	**Z65**[7]	52.51^{b}	8.20^{d}	0.80^{b}	0.05^{b}	2.00	1.00	0.50	0.00	1.09	0.84
	Z71[7]	232.48^{a}	31.05^{bc}	9.14^{a}	0.20^{b}	5.50	2.00	6.00	0.50	1.06	0.98
	Z61	57.32	45.54	1.12	0.80	7.50	3.50	4.50	1.50	NA	NA
COL_18_2	**Z65**	78.81	46.38	2.73	0.37	6.50	4.00	5.00	1.00	NA	NA
	Z71	66.35	39.03	1.93	0.49	8.50	4.00	5.00	0.00	NA	NA
	Z61	234.30	73.47	130.51^{b}	21.01^{c}	16.50	5.00	9.50	5.00	13.24^{bc}	5.29^{c}
COL_19_1	**Z65**	218.09	102.32	129.21^{b}	25.15^{c}	14.50	5.00	10.50	3.00	19.98^{b}	4.53^{c}
	Z71	455.61	125.48	446.48^{a}	55.52^{c}	18.00	6.00	19.50	3.50	29.44^{a}	8.54^{c}
	Z61	145.18^{b}	22.39^{d}	85.70^{b}	7.66^{c}	7.50	2.00	4.00	1.00	7.68^{b}	1.50^{d}
COL_19_2	**Z65**	136.00^{b}	24.97^{d}	169.12^{b}	2.88^{c}	4.50	1.00	6.00	1.00	4.71^{c}	1.67^{d}
	Z71	328.99^{a}	49.57^{c}	297.98^{a}	11.61^{c}	10.50	1.50	11.00	0.00	15.59^{a}	2.26^{cd}
	Z61	126.92^{b}	32.27^{d}	48.88^{b}	6.37^{c}	7.00	2.00	4.00^{b}	1.00^{c}	7.24	2.52
Mean[1]	**Z65**	126.12^{b}	32.40^{d}	46.24^{b}	5.29^{c}	5.50	1.50	5.00^{b}	1.00^{c}	7.60	2.30
	Z71	332.70^{a}	62.95^{c}	190.36^{a}	14.09^{c}	9.50	2.50	10.50^{a}	1.00^{c}	15.37	3.93

Note. Average values for the Cultivar (CULT) × moment (MOM) interaction, for area under disease progression curve (AUDPC) index assessed on whole plots (AUDPC), in 20 spikelets per plot (SpkAUDPC), *Fusarium* damaged kernels (FDK) from whole plots, SpkFDK, and from 20 spikelets per plot (SpkFDK) and deoxynivalenol (DON) content in four experiments (environments) and mean from the general mixed model of the three most contrasting environments[1]

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

[2] INIA Don Alberto (IDA), susceptible to FHB (Castro et al., 2014)

[3] Génesis 6.87 (LE2387), moderately resistant to FHB (Castro, Germán, et al., 2022)

[4] Values transformed from the least square means of the linear general model (√AUDPC, √SpkAUDPC and DON)

[5] Values transformed from the least square means of the generalized linear models (FDK and SpkFDK)

[6] Only two repetitions for experiment included, and no data was taken for COL_18_2. NA = not available.

[7] Moment of application of fungicides, according to Zadoks growth scale (Zadoks et al., 1974). ZGS61 corresponds to early anthesis, ZGS65 to mid-anthesis and ZGS71 to kernel water ripe.

Mean values with the same letter are not significantly different at p = 0.05, within a given variable and environment, according to pairwise comparisons based on *F* test from linear general and mixed models[4] or according to maximum likelihood statistic, χ^2 from generalized linear and mixed models[5].

App Table 3.

Average values for the cultivar (CULT) × moment (MOM) interaction, for grain yield, test weight (TW) and thousand grains weight (TGW).

Environment	CULT×MOM	Grain yield[4] (kg/ha)		TW[4] (kg/hl)		TGW[4] (g)	
		IDA[2]	LE2387[3]	IDA	LE2387	IDA	LE2387
COL_18_1	Z61[5]	4892.76	6050.50	82.83	83.70	33.36	39.20
	Z65[5]	4201.51	6283.26	82.64	83.62	32.46	38.58
	Z71[5]	3982.94	5821.07	81.34	83.35	29.92	39.28
COL_18_2	Z61	2107.40	2796.54	78.85	76.91	37.04	37.70
	Z65	2170.63	3024.53	78.32	77.10	35.92	37.26
	Z71	2161.36	2856.43	78.31	77.28	38.05	39.00
COL_19_1	Z61	4334.31	6277.52	74.54	77.97	30.94	36.13
	Z65	4209.50	5928.07	74.72	78.36	30.63	36.57
	Z71	3341.72	6170.17	72.53	78.30	28.44	36.38
COL_19_2	Z61	5991.42	6422.50	77.07	78.86	32.32^{ab}	35.00^{a}
	Z65	5785.67	6725.84	77.45	78.92	34.63^{a}	32.82^{a}
	Z71	4959.96	6587.76	74.95	78.89	29.88^{b}	33.76^{a}
Mean[1]	Z61	5702.83^{b}	6250.17^{a}	78.18^{b}	80.18^{a}	32.40^{b}	36.78^{a}
	Z65	4732.23^{b}	6314.05^{a}	78.28^{b}	80.30^{a}	32.57^{b}	35.99^{a}
	Z71	4094.87^{c}	6193.00^{a}	76.27^{c}	80.18^{a}	29.41^{c}	36.47^{a}

Note. Average values for the cultivar (CULT) × moment (MOM) interaction, for grain yield, test weight (TW) and thousand grains weight (TGW) on whole plots in four experiments (environments) and mean from the general mixed model of the three most contrasting environments1

[1] Mean corresponds to combined data. General or generalized mixed models include COL_18_1, COL_19_1 and COL_19_2

[2] INIA Don Alberto (IDA), susceptible to FHB (Castro et al., 2014)

[3] Génesis 6.87 (LE2387), moderately resistant to FHB (Castro, Germán, et al., 2022)

[4] Values transformed from the least square means of the linear general model (Grain yield, TW, TGW)

[5] Moment of application of fungicides, according to Zadoks growth scale (Zadoks et al., 1974). ZGS61 corresponds to early anthesis, ZGS65 to mid-anthesis and ZGS71 to kernel water ripe.

Mean values with the same letter are not significantly different at p = 0.05, within a given variable and environment, according to pairwise comparisons based on *F* test from linear general and mixed models[4].

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4. Conclusiones generales y perspectivas

La FE ha sido una enfermedad recurrente en Uruguay, que compromete la inocuidad y calidad del grano de trigo, motivo por el cual fue objeto de esta tesis. Nuestros resultados permiten profundizar en la interacción entre distintas especies locales del complejo de especies de *Fusarium graminearum* y genotipos de trigo con niveles de resistencia contrastantes hacia la FE (capítulo 1: Aggressiveness of *Fusarium graminearum* isolates with 15ADON and NIV chemotypes from wheat in Uruguay), pues demuestran que la agresividad de los aislados varía de acuerdo con la resistencia del genotipo de trigo. También analizamos el efecto de la combinación de distintas estrategias de manejo en el control de FE, respecto a inocuidad en el grano y variables productivas (capítulo 2: Strategies for integrated management of Fusarium Head Blight of wheat in Uruguay): nuestros resultados indican que, cuando hay niveles bajos de FE, la resistencia del genotipo de trigo es suficiente para controlar la FE y obtener bajos niveles de DON, mientras que la aplicación de fungicida es necesaria para alcanzar niveles aceptables de DON y calidad física aceptable del grano únicamente en años epidémicos.

Se observó que los aislados de *F. graminearum sensu stricto* quimiotipo 15ADON fueron más agresivos que cepas con quimiotipo NIV.

Es importante destacar que encontramos una interacción entre genotipo de trigo y aislado a los 14 y 21 días posinfección (dpi). Este resultado mostró que, bajo diferentes niveles de resistencia, los diferentes aislados de *Fusarium* tuvieron comportamientos distintos, destacando que los aislados de *F. graminearum ss.* siempre fueron los primeros en cuanto a infección. En algunos casos, *F. brasilicum* tendía a comportarse como *F. graminearum*; esto sugiere que el genotipo de trigo puede tener un papel importante en el avance de la infección de FE.

A pesar de no observar una interacción a los siete dpi, se verificó variabilidad en los niveles de infección por FE en los diferentes genotipos de trigo y esta respuesta fue acorde a la caracterización de resistencia frente a FE de estos.

Respecto al capítulo 2, todos los tratamientos fueron eficaces en la reducción de la FE, comparados al control susceptible no tratado. Se observó un claro efecto de la resistencia genética en la evaluación a campo (AUDPC), el contenido de deoxinivalenol (DON) y en la calidad física del grano (rendimiento, peso hectolítrico y peso de mil granos).

Nuestros resultados muestran que aplicaciones en ZGS65 son igual de efectivas que en ZGS61, mientras que en ZGS71 se encontraron AUDPC y niveles de DON significativamente más altos y similares a los de los controles sin fungicida. De acuerdo con este resultado, no sería recomendable realizar aplicaciones tardías cuando las condiciones no permitan aplicar en floración.

Nuestros resultados sugieren que, bajo niveles de infección intermedio-altos, fungicidas con metconazol tienden a controlar mejor FE, aunque es necesario profundizar; nuestros resultados apuntan a que en años epidémicos la elección correcta del fungicida puede reducir los niveles de FE.

Encontramos una interacción genotipo por momento, más difícil de detectar a niveles altos de FE. Esta interacción apuntó que no había diferencias entre los momentos de aplicación en el cultivar moderadamente resistente (Génesis 6.87), mientras que sí se observan en el cultivar susceptible INIA Don Alberto.

La comparación de la escala visual (1-10/1-10), utilizada para evaluar FE a campo, con una metodología más exacta, marcando 20 espigas a campo para medir incidencia y severidad de forma más precisa, llevó a la conclusión de que hay una correlación significativa entre ambas y que la evaluación visual ofrece información valiosa de forma rápida a campo.

Por último, en cuanto a residuos de fungicidas, no detectamos residuos de ninguno de los principios activos analizados por encima del límite máximo de residuos (LMR); nuestros resultados son congruentes con los obtenidos anteriormente en el país.

Finalmente, es necesario realizar nuevos estudios que analicen el papel de la resistencia frente a diferentes especies del FGSC y sus quimiotipos potenciales en condiciones de campo o en mayor dimensión con la finalidad de poder analizar también su efecto en la producción de micotoxinas. Mientras que, en cuanto a estrategias de manejo, es necesario analizar en detalle el papel que puede tener la correcta elección del fungicida en años epidémicos. También debemos considerar que este trabajo se centró únicamente en el análisis de DON, pero *Fusarium* es capaz de producir otras micotoxinas y es relevante llevar a cabo el análisis de los derivados glicosilados de DON (15ADON y 3ADON), así como de nivalenol (NIV) y zearalenona (ZEA).

Es importante también hacer un seguimiento a la eficacia de los fungicidas utilizados para tratar FE en Uruguay, ya que Brancatti et al. (2022) han detectado un incremento en la resistencia a tebuconazol, respecto a estudios anteriores, y mayores concentraciones efectivas 50 % (CE50) respecto a protioconazol, metconazol y epoxiconazol. Además, en un análisis preliminar de tres muestras de campo provenientes de estos experimentos, una de ellas apuntó resistencia a protioconazol, por ello es necesario profundizar. Asimismo, detectamos epoxiconazol con frecuencia, por lo que sería importante analizar por qué y sus posibles efectos.

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