USWBSI FHB Integrated Management Coordinated Project (IM-CP) Standard Protocols

Outline of Coordinated Project:

The Objectives of this FHB Integrated Management Coordinated Project (IM-CP) are to:

1) Evaluate the integrated effects of fungicide treatment and genetic resistance on FHB and DON in all major grain classes, with emphasis on new combination fungicides, Prosaro Pro, Sphaerex, and Miravis "Era".
2) Compare the efficacy of Prosaro Pro, Sphaerex, and Miravis "Era" to that of Prosaro and Miravis Ace.
3) Compare the efficacy and economic benefit of FHB forecast-based fungicide programs to that of calendar/growth stage-based programs.
4) Generate data to quantify the economic benefit of FHB and DON management programs.
5) Generate data to validate and advance the development of FHB risk prediction models.

In the first year of this new 4-year funding cycle (2026), we propose to repeat the same protocols and fungicide programs used in the previous funding cycle in an effort to gather more data on the efficacy of the new fungicides Prosaro Pro, Sphaerex, and Miravis "Era" in particular across a wider range of environmental conditions and baseline levels of FHB/DON. Integrated management (IM) and uniform fungicide (UFT) trials in wheat and barley will be repeated to evaluate new DMI-DMI and DMI-SDHI combinations of AIs either as pre-mixtures or as sequentially applied treatments. For the IM trials, PIs will conduct inoculated trails consisting of at least two cultivars with different levels of resistance to FHB, subjected to at least five fungicide treatment programs, plus two nontreated checks. The treatments will be: **1)** an inoculated, nontreated check, **2)** a non-inoculated, nontreated check, **3)** Prosaro, **4)** Miravis Ace, **5)** Prosaro Pro, **6)** Sphaerex, **7**) Miravis Era. All fungicide treated plots will be inoculated. For the UFTs, separate plots of a susceptible cultivar will be subjected to at least nine fungicide treatments plus an untreated check, namely: **1)** the nontreated check, **2)** Prosaro, **3)** Miravis Era, **4)** Miravis Ace, **5)** Prosaro Pro, **6)** Sphaerex, **7)** Miravis Ace fb tebuconazole; **8)** Miravis Ace fb Prosaro Pro, **9)** Miravis Ace fb Sphaerex. All treatments in the IM trials and treatments 2-6 as well as the first applications of treatments 7-9 in the UFT will be made at Feekes 10.5.1 in wheat trials and Feekes 10.5 in barley trials, whereas the second applications in the UFT will be made 4-6 days after the first.

In years 2, 3, and 4 (2027, 2028, and 2029), protocols will be modified to include FHB forecast-based fungicide programs in the UFT and IM trials and new moderately resistant (MR) cultivars and/or advanced breeding lines in the IM trials. For the IM trials, cultivar x fungicide program combinations will consist of at least two MR cultivars, a standard reference MR cultivar and a newly released MR cultivar (or advanced MR breeding line), a moderately susceptible cultivar, and a susceptible cultivar, all subjected to at least five calendar/growth stage-based fungicide treatments, at least one forecast-based fungicide treatment, plus a nontreated checks. The treatments will be: **1)** a nontreated check, **2)** Prosaro, **3)** Miravis Ace, **4)** Prosaro Pro, **5)** Sphaerex, **6**) Miravis Era and **7**) a forecast-based fungicide program. For the UFTs, sequential (two-pass) fungicide application programs will be replaced by FHB forecast-based fungicide programs. Separate plots of a susceptible cultivar will be subjected to at least five calendar/growth stage-based fungicide treatments and at least one forecast-based fungicide program plus a nontreated check, namely: **1)** the nontreated check, **2)** Prosaro, **3)** Miravis Era, **4)** Miravis Ace, **5)** Prosaro Pro, **6)** Sphaerex, **7-9)** a forecast-based fungicide program. All calendar/growth stage-based treatments will be made at Feekes 10.5.1 in wheat trials and at Feekes 10.5 in barley trials, whereas for the forecast-based treatments, the fungicide(s) used, the number of applications and application timing will depend on FHB risk predictions as influence cultivar resistance and local weather conditions. All trials will be naturally infected.

In all trails, FHB, DON, grain yield, and test weight will be quantified. Meta-analysis will be used to estimate and compare the overall efficacy and economic benefit of the tested fungicide programs. Data from the nontreated, non-inoculated checks will be used to validate and refine the web-based FHB risk assessment tool to help guide fungicide application decision-making.

Project Description: Standard Protocol

Title: Individual and Integrated effects Fungicide Combinations and Genetic Resistance for FHB and DON Management

Introduction and Rationale: The demethylation inhibitors (DMI) prothioconazole, metconazole, and tebuconazole have been the most effective, and in the US, the only or most widely recommended active ingredients (AI) for FHB and DON control in wheat and barley. However, mixtures of fungicide AIs from different chemical (and/or FRAC) groups are now being markets for FHB and DON control. One such fungicide, Miravis Ace, a premix of a Succinate Dehydrogenase Inhibitor (SDHI; adepidyn) and a DMI (propiconazole), was shown to be just as, and occasionally more, effective than Prosaro (tebuconazole + prothioconazole) and Caramba (metconazole), old industry standards for FHB and DON management (Luis et al. 2020a, 2020b). During the previous funding cycle (2022-2025), three additional, relatively new fungicide AI mixtures, Prosaro Pro (a premix of prothioconazole, tebuconazole, and the SDHI Fluopyram), Sphaerex (a premix of metconazole and prothioconazole) and Miravis Era (a premix of adepidyn and prothioconazole) were evaluated as part of the management coordinated project (CP) for FHB and DON control in wheat and barley and to determine whether they were of comparable efficacy to Prosaro, Caramba, and Miravis Ace, a new industry standard. The results were promising, with the new fungicides showing comparable efficacy to the standards (**Fig. 1**). However, since Miravis Era was only included in the CP in 2024, the uniform fungicide (UFT) and integrated management (IM) trials will be repeated in 2026 following a protocol similar to that used from 2022 to 2025 to allow for an additional season of comparison among the tested fungicides, and in 2027, 2028, and 2029, with modifications to the protocol to address stakeholder questions related to fungicide decision-making as influenced by FHB risk.

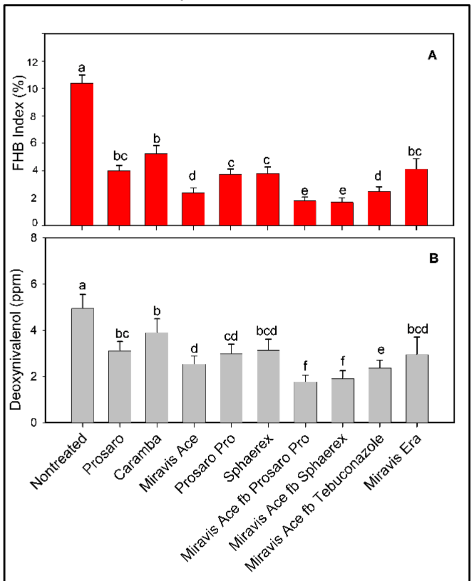


Fig. 1. Comparison of treatment mean for Fusarium head blight index (**A**) and DON contamination of grain (**B**), averaged across blocks and UFT from 2022 to 2024.

With several fungicides of comparable efficacy but vastly different costs to choose from, additional stakeholder questions pertaining to which fungicide to use or application program to follow (from a cost-benefit standpoint) under natural (non-inoculated) field conditions. For instance, when is a slightly more efficacious and often considerably more expensive newer fungicide such a Miravis Ace most warranted for FHB/DON management as opposed to slightly less efficacious but considerably cheaper older (off-patent) product such as Prosaro or tebuconazole? These questions can be addressed by using the FHB Forecasting Tool (www.wheatscab.psu.edu) as a decision support system. This would be the natural next step for the management coordinated project.

Here we propose IM and UFT trials in wheat and barley that would allow us to evaluate and compare the efficacy of new premix fungicides to Miravis Ace, and Prosaro under both artificial inoculation (YR1) and natural infection (YR2, 3 and 4), and to evaluate fungicide application decision-making as influenced by FHB risk. We hypothesize that **a**) the newer fungicides will provide comparable FHB and DON control to Prosaro and Miravis Ace when applied at or shortly after 50% early anthesis, **b**) the highest percent reduction of FHB and DON relative to the nontreated, susceptible check will be observed when Prosaro Pro, Sphaerex or Miravis Era are applied to moderately resistant cultivars, and **3**) risk-based fungicide decision-making will lead to more cost-effective choices of fungicide products and application programs.

This project will address the following **goals in the USWBSI Action Plan**: Goal # "***1)*** *Develop integrated management strategies for FHB and mycotoxins that are robust to conditions experienced in production fields of wheat and barley and Goal #* ***2)*** *Help develop and validate the next generation of management and mitigation tools to reduce FHB and associated mycotoxins*". **The research needs in the USWBSI Action plan** addressed by the proposed research are: "***1)*** *Validating the integrated management strategies with next generation of wheat and barley varieties in multiple production environments;* ***2)*** *Evaluating flexibility of fungicide and/or biocontrol agent application timing within the context of the integrated management strategies;* ***3)*** *Utilizing data generated from integrated management experiments to maintain, improve, and refine current FHB forecasting models; and* ***4)*** *Developing economic analyses of effective integrated management strategies used alone and in combination (i.e., fungicide, biological control, cultivar, residue management, crop rotation)".*

Research Material and Methods

Description and sequence of proposed experiments

Obj. 1. *Inoculated Integrated Management Trial (****YR1:****): Evaluate the integrated effects of fungicide treatment and genetic resistance on FHB and DON in all major grain classes, with emphasis on new combinations fungicides, Prosaro Pro, Sphaerex and Miravis Era*. Field experiments will be conducted in all major grain market classes to investigate the effects of cultivar resistance and fungicide treatment programs on FHB and DON contamination. Plots will be established on university research farms or in farmers' fields in areas previously planted with a crop that is representative of the typical cropping sequence of each location. All trials will be managed according to standard agronomic practices for each grain class and location. For ease of data collection and manipulation and comparison of results across trials, we propose a standard experimental design and treatment arrangement, and suggest that plots be inoculated. **The recommended design will be a randomized complete block (RCBD), with a split-plot arrangement of cultivar as whole-plot and fungicide treatment (Table 1 or 2) as the sub-plot. There will be four to six replicate blocks. An example plot plan is shown in Figure 3.** However, to accommodate differences in the availability of space and other resources among locations/PIs, other experimental designs and treatment structures will be entertained. For instance, a PI may utilize a RCBD, with a factorial arrangement of cultivar and fungicide program. For PIs interested in evaluating other management strategies such as cropping sequence or residue management, other designs may be utilized (for example, a RCBD with a split-split-plot arrangement of the three factors or a RCBD with a split-plot arrangement of one factor as whole-plot and a factorial arrangement of the other two factors as sub-plot). Each cooperator will utilize at least two cultivars; a standard FHB susceptible or moderately susceptible and a resistant or moderately resistant.

Table 1. The following core fungicide treatments will be randomly assigned to experimental units (Wheat)

Trt[a]	Product	Rate (fl oz/A)	Timing
1	Untreated check	…	…
2	Untreated, non-inoculated check		
3	Prosaro	6.5	Feekes 10.5.1 (early anthesis)
4	Miravis Ace	13.7	Feekes 10.5.1 (early anthesis)
5	Prosaro Pro	10.3	Feekes 10.5.1 (early anthesis)
6	Sphaerex	7.3	Feekes 10.5.1 (early anthesis)
7	Miravis Era	10.3	Feekes 10.5.1 (early anthesis)

[a]All treatments will be applied with an NIS @ 0.125 v/v

[b]Early anthesis is defined as when approximately 50% of the tillers have fresh anthesis extruded in the center of the spikes.

Table 2. The following core fungicide treatments will be randomly assigned to experimental units (Barley)

Trt[a]	Product	Rate (fl oz/A)	Timing[b]
1	Untreated check	…	…
2	Prosaro	6.5	Feekes 10.5 (heading)
3	Miravis Ace	13.7	Feekes 10.5 (heading)
4	Prosaro Pro	10.3	Feekes 10.5 (heading)
5	Sphaerex	7.3	Feekes 10.5 (heading)
6	Sphaerex	7.3	4-6 days after heading
7	Miravis Era	10.3	Feekes 10.5 (heading)

[a]All treatments will be applied with an NIS @ 0.125 v/v

[b]Heading is defined as when approximately 100% of the tillers have spikes fully emerged.

***Obj. 2.** Inoculated Uniform fungicide trial (**YR1**): Compare the efficacy of Prosaro Pro, Sphaerex, and Miravis Era to that of Prosaro and Miravis Ace.* Trial establishment, plots dimensions, and general management will be as described under Obj. 1. Plots of a single susceptible cultivar will be planted in a randomized complete block, with 4-6 replicate blocks, and subjected to at least nine fungicide treatments (**Table 3** and **4**):

Table 3. The following core fungicide treatments will be randomly assigned to experimental units (wheat)

Trt[a]	Product	Rate/Acre (fl oz)	Timing
1	Untreated check	…	…
2	Prosaro	6.5	Feekes 10.5.1 (early anthesis)
3	Miravis Era	10.3	Feekes 10.5.1 (early anthesis)
4	Miravis Ace	13.7	Feekes 10.5.1 (early anthesis)
5	Prosaro Pro	10.3	Feekes 10.5.1 (early anthesis)
6	Sphaerex	7.3	Feekes 10.5.1 (early anthesis)
7	Miravis Ace fb Prosaro Pro	13.7/10.3	Early anthesis/4-6 days after early anthesis
8	Miravis Ace fb Sphaerex	13.7/7.3	Early anthesis/4-6 days after early anthesis
9	Miravis Ace fb Tebuconazole	13.7/4	Early anthesis/4-6 days after early anthesis

[a]All treatments will be applied with an NIS @ 0.125 v/v

[b]Early anthesis is defined as when approximately 50% of the tillers have fresh anthesis extruded in the center of the spikes.

Table 4. The following core fungicide treatments will be randomly assigned to experimental units (barley)

Trt[a]	Product	Rate/Acre (fl oz)	Timing[b]
1	Untreated check	…	…
2	Prosaro	6.5	Feekes 10.5 (heading)
3	Miravis Era	10.3	Feekes 10.5 (heading)
4	Miravis Ace	13.7	Feekes 10.5 (heading)
5	Prosaro Pro	10.3	Feekes 10.5 (heading)
6	Sphaerex	7.3	Feekes 10.5 (heading)
7	Miravis Ace fb Prosaro Pro	13.7/10.3	Heading/4-6 days after heading
8	Miravis Ace fb Sphaerex	13.7/7.3	Heading/4-6 days after heading
9	Miravis Ace fb Tebuconazole	13.7 fl oz/4	Heading/4-6 days after heading

[a]All treatments will be applied with an NIS @ 0.125 v/v

[b]Heading is defined as when approximately 100% of the tillers have spikes fully emerged.

PIs will also have the option of including one or more of the following treatments **i)** Prosaro Pro at 10.3 fl oz at 4-6 days after 50% early anthesis (100% heading in the case of barley), **ii)** Sphaerex at 7.3 fl oz at 4-6 days after 50% early anthesis (100% heading in barley), **iii)** Sphaerex at 7.3 fl oz at 50% early anthesis (100% heading in barley) fb tebuconazole at 4 fl oz 4-6 days later (100% heading in barley), **iv)** Prosaro at 8.2 fl oz at 50% early anthesis (100% heading in barley), **v)** Prosaro Pro at 10.3 fl oz at 50% heading in wheat; and **vi)** Sphaerex at 7.3 fl oz at 50% heading in wheat.

Obj. 3. (YR2, 3 and 4)*: Compare the efficacy and economic benefit of FHB forecast-based fungicide programs to that of calendar/growth stage-based programs.*

Obj 3a. In integrated management programs. For this subobjective, the protocol will be similar to that described under Obj. 1., with the primary difference being that plots will not be inoculated, and at least one forecast-based fungicide program (**Table 5**) will be added to the list in Tables 1 (instead of the nontreated, non-inoculated check) and 2. In any given trial (location/environment), the risk scenario will vary with cultivar resistance and maturity, likely triggering different forecast-based treatment programs by cultivar.

Obj. 3b. In fungicide-only management programs. For this subobjective, the protocol will be similar to that described under Obj. 2., with the primary difference again being that plots will not be inoculated, and at least three forecast-based fungicide programs (Table 5) will be added to the list in Tables 3 (replacing the sequentially applied fungicide treatment programs) and 4. For instance, if the risk is high at anthesis, treatment programs 1, 4, and 5 will be triggered, whereas, if the risk is moderate, programs 2, 3, and 6 will be triggered.

Table 5. Core FHB forecast-based fungicide programs (PIs are free to consider other risk scenarios and/or fungicide programs

1	Single Application: Apply Miravis Ace at early anthesis (heading for barley) if FHB risk is high (map is red in the area of the trial).
2	Single Application: Apply Tebuconazole at early anthesis (heading for barley) if FHB risk is moderate (map is orange in the area of the trial)
3	Single Application: Apply Miravis Ace, Prosaro Pro, or Sphaerex at early anthesis (heading for barley) is FHB risk is moderate.
4	Two Applications: Apply Miravis Ace if the risk is high at early anthesis (heading for barley), followed by a second application using Sphaerex or Prosaro Pro 3-4 days later if the risk continues to be high.
5	Two Applications: Apply Miravis Ace if the risk is high at early anthesis (heading for barley), followed by a second application using Prosaro or Tebuconazole 3-4 days later if the risk turns moderate.
6	Two Applications: Apply Prosaro or Tebuconazole if the risk is moderate at early anthesis (heading for barley), followed by a second application using Prosaro or Tebuconazole 3-4 days later if the risk continues to be moderate.

- All treatments will be applied with an NIS @ 0.125 v/v
- Early anthesis is defined as when approximately 50% of the tillers have fresh anthesis extruded in the center of the spikes.
- Heading is defined as when approximately 100% of the tillers have spikes fully emerged.

Treatment application (Obj. 1, 2, and 3): In all trials, applications will be made using a (back or tractor-mounted) sprayer equipped with Twinjet or flat fan XR8001 or XR8002 nozzles, mounted at an angle (30-45^{o} from the horizontal) forward and backward (or forward only) and calibrated to deliver the fungicide mixture at a rate of 10 to 20 gallons of per acre.

Inoculation (Obj. 1 and 2): **In YR1 (2026), all but one plot of each cultivar in the wheat IM and all plots in the UFTs will be spray-inoculated approximately 24-36 hours after the anthesis (full head emergence for barley) treatments are applied with a spore suspension (40,000 – 100,000 spores/ml or based on PIs' experience) consisting of a mixture of local isolates of *Fusarium graminearum*.** Other methods such as grain spawn may also be used to enhance inoculum production and disease development. For cooperators evaluating cropping sequence or in-field residue (inoculum) manipulation (tillage for instance) as a third management strategy in the IM trials, artificial inoculation will not be required. Plots in the UFTs will be mist-irrigated where possible to enhance infection and disease development. **Date from the non-inoculated wheat plots in the IM trials will be used for FHB risk assessment model validation and refinement.**

Data collection: In all trials, FHB incidence (**INC; mean proportion of diseased spikes in a sample**) and index (**IND; mean proportion of diseased spikelets per spike, which is equivalent to the number of diseased spikelets per spike divided by the total number of spikes rated x 100**) will be rated as described (Stack and McMullen 1998) at the soft dough growth stage (Feekes 11.2). Severity, often referred to as diseased head severity (**DHS; mean proportion of diseased spikelets per diseased spike**), will be calculated from INC and IND as described (Paul et al. 2005). **PIs are encouraged to rate at least 100 spikes (5 clusters of 20 spikes) per plot, because smaller sample sizes were shown to give imprecise estimates of plot-level mean IND** (Moraes et al. 2021) The presence and flag leaf severity (as a percentage) of foliar diseases will also be rated. For those IM trials established in fields previously planted with a *F. graminearum* host crops and those with in-field host crop residue management as a treatment factor, the PI will provide an estimate of percent residue on the soil surface across the experiment (or in each whole- or sub-plot, where applicable). Plots will be harvested with a plot combine and yield and test weight determined. Two subsamples of grain from each plot will be pulled - one will be rated to determine the percentage of Fusarium damaged kernels (FDK) and the other will be sent to one of the USWBSI-funded DON testing laboratories for mycotoxin analysis.

Campbell Scientific units (Campbell Sci., Logan UT), other onsite weather stations, or cloud-based source will be used to collect temperature, relative humidity, surface wetness, rainfall, wind speed, and solar radiation data at regular intervals from Feekes GS 7 (stem elongation) to harvest.

Results expected*:* By conducting experiments across multiple locations, we anticipate that different baseline levels of FHB will develop by virtue of differences in local weather and other FHB-influencing factors. We will be able to determine the degree to which baseline FHB and DON levels influence the efficacy of the tested growth-stage- and forecast-based fungicide programs and fungicide programs x cultivar combinations across locations and grain classes. We anticipate that for Prosaro Pro, Sphaerex or Miravis Era, application at or shortly after 50% early anthesis, will provide comparable levels of FHB and DON reduction to Prosaro or Miravis Ace. However, due to interactions among cultivar maturity (time of anthesis), genetic resistance, and weather conditions at the time of application (the location effect) in the IM trials, we expect to see variations in the absolute levels of efficacy for any given management program. We anticipate that percent FHB/DON reduction for a given program may be comparable among cultivars with different resistance classifications or different among cultivars in a similar resistance class in some situations. These are desired effects that would allow us to quantitatively evaluate different permutations in the associations among cultivar trails, fungicide programs, weather conditions, FHB, and DON.

Analytic methods*:* Mixed models (Littell et al. 2006; Schabenberger and Pierce 2002) will be fitted to the data to quantify and compare treatments effects on INC, DHS and IND, yield, test weight, FDK, and DON. Each PI will use his/her own data locally to demonstrate the value of fungicide treatment and integrated management programs on FHB and DON. In addition, the Paul lab at OSU will compile data from all trials and use meta-analysis (Hedges et al. 1999; Madden and Paul 2011; Madden et al. 2016) or some other method of research synthesis to quantify the effects of fungicide treatments and treatment x cultivar combinations on all measured response variables. Study-specific characteristics such as grain market class, date of anthesis, percent surface residue, and severity of foliar diseases will be used as moderator variables in the meta-analyses to evaluate their influence on the overall efficacy/responses. Summary results will be used along with grain prices, price discounts, fungicide application costs, and estimates of yield loss due to wheel tracks to estimate and compare the economic benefit of fungicide programs (Salgado et al. 2014, Moraes et al. 2022), both alone and in combination with genetic resistance, for FHB and DON management.

Application of results/technology transfer: Results from these experiments will allow us to determine whether Prosaro Pro, Sphaerex, and Miravis Era are just as effective as or more effective than Prosaro and Miravis Ace against FHB and DON and whether the performance of these new fungicides is consistent across combinations of locations/environments, cultivar resistance classes, and grain market classes. We will also gain valuable information about the cost-benefits of using different fungicide products and application programs as influenced by FHB risk. This will provide producers with additional options for managing FHB and DON, as well as guidelines for evidence/risk-based approaches to fungicide decision-making. Summary results from these studies will be published as annual IM and UFT report on the USWBSI website and delivered to stakeholders, county extension educators, and others in the wheat and barley industry by extension specialists. Data from these trials will also be used to advance the development and validations of FHB and DON risk assessment models.

Possible pitfalls and limitations: The success of this project is heavily dependent on the weather. If weather conditions are extremely unfavorable for FHB development and DON contamination of grain (hot and dry during anthesis and early grain fill), little or no FHB will develop, making it impossible to compare treatments factors and implement forecast-based treatment programs. Conversely, if weather is extremely favorable for infection (consistently wet and humid with warm temperatures during anthesis), there may be no significant treatment effect. However, based on results from previous IM and UFT trials, conducting the experiments at multiple locations (weather conditions; **Obj. 1, 2** and **3**), with artificial inoculation and mist-irrigation (**Obj. 1** and **2**) and using cultivars with different maturities and resistance responses (**Obj. 1**), minimizes the probabilities of either of these extreme eventualities occurring across all trials.

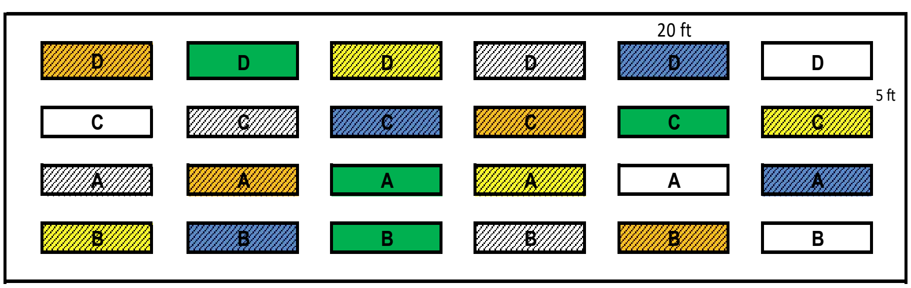
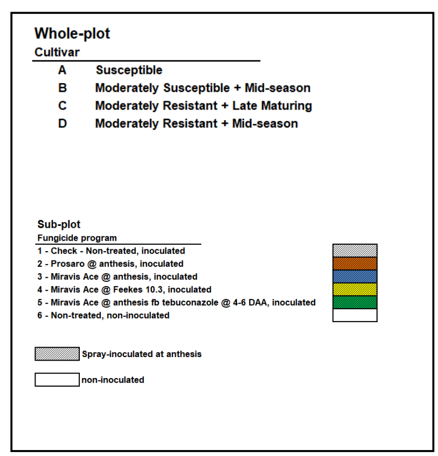


Fig. 2 - An example block of the multistate FHB IM trial: Letters represent cultivars (whole-plot) and colors fungicide x inoculation treatment (sub-plot). Shades represent inoculation. The size of the plots and position of the blocks relative to each other may vary depending on local terrain and available space. In this example cultivars A = Hopewell (standard susceptible, mid-season), B = Bromfield (new moderately susceptible, mid-season), C = Truman (standard moderately resistant, late-maturing), and D = Malabar (new moderately resistant, mid-season)



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