



**JHONATA CANTUÁRIA MEDEIROS**

**INDUSTRIAL PROCESSES OF TREATED CORN SEEDS  
FOR MAINTENANCE OF PHYSIOLOGICAL QUALITY  
AND THEIR IMPACT ON STORAGE**

**LAVRAS – MG  
2024**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitotecnia, área de concentração em Produção Vegetal para obtenção do título de Doutor.

Prof. Dr. Everson Reis Carvalho  
Orientador

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**Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca  
Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).**

Cantuária Medeiros, Jhonata.

Industrial processes of treated corn seeds for maintenance of  
physiological quality and their impact on storage / Jhonata  
Cantuária Medeiros. - 2024.

106 p.

Orientador(a): Everson Reis Carvalho.

Tese (doutorado) - Universidade Federal de Lavras, 2024.  
Bibliografia.

1. análise ultraestrutural. 2. armazenamento seguro. 3.  
fitotoxidez sementes tratadas. I. Reis Carvalho, Everson. II. Título.

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**PROCESSOS INDUSTRIAIS EM SEMENTES DE MILHO TRATADAS PARA  
MANUTENÇÃO DA QUALIDADE FISIOLÓGICA E SEU IMPACTO NO  
ARMAZENAMENTO**

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APROVADA em 28 de Maio de 2024

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**LAVRAS – MG  
2024**

## **AGRADECIMENTOS**

À Deus, fonte inesgotável de força, sabedoria, proteção, por me guiar e iluminar meus passos.

À minha mãe Neuza, por todo o amor, carinho e apoio. Ao meu irmão Jeferson pelo companheirismo.

Em memória a minhas falecidas avós Maria e Cirila.

Ao meu orientador na Universidade Federal de Lavras, professor Dr. Everson Reis Carvalho, pela orientação, confiança, cumplicidade, comprometimento e exemplo de profissional.

Ao meu orientador na Ohio State University, professor Dr. Osler Ortez por todos os ensinamentos, profissionalismo e oportunidades.

A minha namorada Ana Paula pelo amor e companheirismo.

Aos docentes do setor de sementes da UFLA Édila Von Pinho, Heloisa Santos e Raquel Pires pela inspiração e conhecimentos compartilhados.

Aos atuais e ex-colegas de pós-graduação Amanda Penido, Danilo Maciel, Dayliane Andrade, Debora Rocha, Juliana Espíndola, Leandro Reis, Luiz Moraes, Nasma Cossa e Venicius Reis pela prontidão, conselhos, apoio, dedicação durante a execução do projeto, amizade e troca de experiência.

Aos colegas do Laboratório Central de Sementes da Escola de Ciências Agrárias da Universidade Federal de Lavras (ESAL-UFLA) os bolsistas de Iniciação científica Ariela Mesquita, Bárbara Sabino, Elias Costa, Hellen Assis, Larissa Carvalho e Luan Gusmão. Aos técnicos de laboratório Geraldo e Jaqueline, ao assistente de UBS senhor Itamar, pelo auxílio e contribuição nos trabalhos e análises. A secretaria da Pós-Graduação Agronomia/Fitotecnia Marli pelo suporte.

A equipe de pesquisa e extensão em Corn & Emerging Crops da OSU Andres Velasco, Bishal Dhakal, Jonah VanRoekel e Mohsen Shahrokhi pelo companheirismo e suporte.

Aos colegas dos grupos de estudo NeSem e G-Cia pelo aprendizado e experiências compartilhadas.

Aos familiares e amigos de Minas Gerais, Goiás, São Paulo no Brasil e do estado de Ohio nos Estados Unidos pelo apoio, amizade, carinho, incentivo e auxílio.

À todos os ex-tutores da Unimontes, Helix Sementes, Advanta Seeds, IF-Goiano, USP-Esalq pelos ensinamentos e amadurecimento profissional proporcionados.

À Universidade Federal de Lavras (UFLA), ao Programa de Pós-graduação em Agronomia/Fitotecnia e The Ohio State University (OSU), Department of Horticulture and Crop Science (HCS) pela oportunidade de fazer parte do corpo discente e aprimorar meus conhecimentos.

Aos órgãos de fomento, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), à Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG). Em especial à Fundação de Desenvolvimento Científico e Cultural (FUNDECC).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Minha eterna gratidão a todos vocês.

## RESUMO

O tratamento de sementes é uma técnica importante utilizada na cultura do milho por proporcionar proteção às sementes e plântulas, entretanto, existem relatos de fitotoxidez por inseticidas em plântulas e sementes, sobretudo armazenadas, o que demanda pesquisas para fornecer informações para o setor sementeiro, desde a produção das sementes até os processos e produtos utilizados para o tratamento fitossanitário. Assim, foram realizados três estudos com os objetivos de 1) avaliar diferentes processos operacionais de tratamento de sementes de milho sobre a qualidade fisiológica da sementes e funcional do tratamento ao longo do armazenamento; 2) avaliar características fisiológicas e morfoanatômicas de diferentes lotes de sementes tratadas com inseticidas neonicotinóides e armazenadas que possam contribuir para a manutenção do vigor e potencial de armazenamento em sementes tratadas; e 3) caracterizar como melhorias nos últimos 50 anos de características agronômicas associadas à qualidade, tecnologia e genética de sementes híbridas de milho reportados nos ensaios do Ohio Corn Performance Test (OCPT) foram relevantes para o aumento de rendimento no milho. Os processos de tratamento e disposição dos ingredientes ativos junto às sementes afetaram a manutenção da qualidade das sementes de milho. O uso do fungicida mais polímero (FP) em primeiro estágio auxilia na preservação do vigor e mitiga a fitotoxidez junto às sementes e plântulas, principalmente após 12 meses de armazenamento. Nessa linha, o procedimento industrial de tratamento estratificado FP/Inseticida/Pó secante é indicado por favorecer a manutenção do vigor até 12 meses de armazenamento, sendo semelhante ao tratamento somente com FP. A qualidade funcional do tratamento, desprendimento de poeira, plantabilidade, recobrimento não é um impedimento para o uso dos processos industriais de tratamentos de sementes com estratificação dos componentes. Os princípios ativos Imidacloprido + Tiodicarbe e Tiametoxam apresentaram maior efeito fitotóxico, maior impacto na germinação e no vigor, principalmente em genótipos sensíveis e de baixo vigor inicial. O vigor inicial do lote de sementes afeta a tolerância ao tratamento e armazenamento com inseticidas neonicotinóides. Há efeito dos genótipos de milho sobre a tolerância ao tratamento e armazenamento de sementes com neonicotinóides. Há relação entre análises ultraestruturais, como maior espessura do pericarpo e tamanho dos grânulos de amido, com tendência de maior vigor e tolerância ao armazenamento de sementes tratadas com inseticidas. Através dos 50 anos de ensaios do OCPT foram constatados aumentos de 10,3% (ou 0,66 ano<sup>-1</sup>) na emergência de plântulas e aumento do estande final de plantas em 66% ou 34 mil plantas ha<sup>-1</sup>, com correlação positiva de r= 0,64 e r= 0,82 de ambas as variáveis com rendimento, além da evolução do número de eventos transgênicos, produtos fitossanitários para sementes e tecnologias de plantio que corroboram para o aumento de 84,7% no rendimento de grãos, saindo de 7,91 Mg ha<sup>-1</sup> em 1972 para 14,6 Mg ha<sup>-1</sup> em 2021 por meio da resposta sinérgica a novos genótipos, sementes, tecnologias e práticas de manejo.

Palavras-chave: análise ultraestrutural; armazenamento seguro; fitotoxidez sementes tratadas; morfoanatomia de sementes; produtividade; vigor; *Zea mays*.

## ABSTRACT

The seed treatment is an important technique used in corn crops as it provides protection to seeds and seedlings. However, there have been reports of phytotoxicity caused by insecticides in seedlings, mainly stored, which promotes interest in research to provide information for the production sector, from the seed production to the processes and products used for phytosanitary treatments. Thus, three studies were carried out with the objectives of 1) assess different operational processes for corn seed treatment in terms of seed physiological and treatment functional quality during storage; 2) evaluating physiological and morphoanatomical traits in different seed treated with neonicotinoid insecticides and stored, that contributes to the maintenance vigor and storage potential.; and 3) to characterize how improvements in the last 50 years of agronomic traits associated with the quality, technology, and genetics of corn seeds reported in the Ohio Corn Performance Test (OCPT) were relevant to increasing corn yield. The treatment processes and the arrangement of the active ingredients with the seeds affected the maintenance of the treated corn seeds quality. The use of the fungicide plus polymer (FP) in the first stage aid to maintain vigor and mitigate phytotoxicity in the seeds and seedlings, especially during 12 months of storage. In these lines, the stratified process of FP/Insecticide/Dry powder is indicated for favoring the maintenance of vigor for up to 12 months of storage, being similar to the FP treatment. Functional quality of treatment dust release, plantability and coating is not an impediment to the use of industrial seed treatment processes with component stratification. Seed lots not treated with neonicotinoid insecticides showed superior performance compared to treated and stored lots. The active ingredients imidacloprid + thiodicarb and thiamethoxam showed a greater phytotoxic effect, greater negative impact on germination and vigor, especially in the sensitive and low initial vigor genotypes. The initial seed lot vigor affects tolerance to neonicotinoid insecticide treatment and storage. The corn genotypes vary in their tolerance to neonicotinoid seed treatment and storage. There is relationship between ultrastructural analyses, such as greater pericarp thickness and starch granule size, with a trend toward greater vigor and storage tolerance of insecticide-treated seeds. Over the past 50 years, the OCPT an increase of 10.3% (or 0.66% year<sup>-1</sup>) in seedling emergence and an increase of 66% or 34 thousand plants ha<sup>-1</sup> (equivalent to 694 plants year<sup>-1</sup>) in final stand population, with positive correlations  $r= 0.64$  and  $r= 0.82$ , both variables with yield, in addition to the evolution of the number of transgenic events, phytosanitary products for seeds and planting technologies, contributed to an 84.7% increase in grain yield, from 7.91 Mg ha<sup>-1</sup> in 1972 to 14.6 Mg ha<sup>-1</sup> in 2021 provided by synergistic responses to new genotypes, technologies, seeds and management practices.

Keywords: phytotoxicity treated seeds; productivity; seed morphoanatomy; storage tolerance; ultrastructural analysis; vigor; *Zea mays*.

## INDICADORES DE IMPACTO

O desenvolvimento da pesquisa exerce impacto sobre o setor de produção e tecnologia de sementes híbridas de milho e conseqüentemente impactos sobre a produção agrícola, visto que o milho é umas das culturas mais importantes no Brasil e no mundo como fonte de alimento, matéria-prima e combustível. A pesquisa conduzida, traz a proposta de implementação de novos processos industriais de tratamento de sementes de milho, bem como demonstra a influência e a relação de estruturas morfoanatomicas, vigor e tolerância de híbridos quanto à manutenção da qualidade após o tratamento químico e armazenamento. Algumas classes de produtos fitossanitários como inseticidas neonicotinóides, apesar de importantes no manejo de pragas da cultura podem ocasionar fitotoxidez às sementes e plântulas. Os novos processos de tratamento que favorecem a manutenção da qualidade fisiológica de sementes de milho tratadas, o que impacta de forma positiva toda a cadeia de produção, pesquisa-desenvolvimento de sementes de milho até o agricultor que irá utilizar a sementes tratada. Foi abordado ainda como a qualidade fisiológica e novos híbridos, tecnologias de tratamento fitossanitário, *traits* transgênicos, técnicas de plantio e população de plântulas são fundamentais e estão relacionados para a obtenção de altas produtividades, otimizando assim a utilização dos recursos naturais em prol de uma agricultura de alto desempenho e mais sustentável. A pesquisa também tem proporcionado o desenvolvimento e compartilhamento de conhecimento técnico-científico com parte dos resultados obtidos já publicados como artigo científico “*Quality of corn seed industrial seed treatment (IST) and on-farm treatment (OFT) in Brazilian agribusiness*” (<https://doi.org/10.1590/2317-1545v45268856>) pela Journal of Seed Science, sete resumos publicados, duas orientações de iniciação científica e palestras e reuniões sobre o tema. Além disso, o desenvolvimento e condução do trabalho contou com a parceria e/ou colaboração de empresas privadas como Instituto Seedcare - Syngenta, e universidade internacional como The Ohio State University. Dessa forma, os resultados obtidos no trabalho contribuem com os objetivos do desenvolvimento sustentável (ODS) das Nações Unidas (ONU), contribuindo com a produção de um dos principais cereais do mundo, milho, com impactos sobre a erradicação da pobreza, fome, agricultura sustentável além da indústria, inovação e infraestrutura.

## IMPACT INDICATORS

The development of research has an impact on the corn hybrid seed production and technology sector and consequently impacts on agricultural production, since corn is one of the most important crops in Brazil and in the world as a source of food, raw material and fuel. The research conducted proposes the implementation of new industrial corn seed treatment processes, as well as demonstrate the influence and relationship of morphoanatomical structures, vigor and tolerance of hybrids in terms of maintaining quality after chemical treatment and storage. Some classes of crop protection products, such as neonicotinoid insecticides, despite being important in managing crop pests, can cause phytotoxicity to seeds and seedlings. New treatment processes that promote the maintenance of the physiological quality of treated corn seed, which has a positive impact on the entire production chain, from corn seed research and development to the farmer who will use the treated seed. It was also discussed how physiological quality and new hybrids, phytosanitary treatment technologies, transgenic traits, planting techniques and seedling population are fundamental and related to obtaining high productivity, thus optimizing the use of natural resources in favor of agriculture high performance and more sustainable. The research has also provided the development and sharing of technical-scientific knowledge with part of the results obtained already published as a scientific article “Quality of corn seed industrial seed treatment (IST) and on-farm treatment (OFT) in Brazilian agribusiness” (<https://doi.org/10.1590/2317-1545v45268856>) by the Journal of Seed Science, seven published abstracts, two scientific initiation guidelines and lectures and meetings on the topic. In addition, the development and execution of the work involved partnership and/or collaboration with private companies, such as Instituto Seedcare - Syngenta, and international universities, such as The Ohio State University. In this way, the results obtained in the work contribute to the Sustainable Development Goals (SDGs) of the United Nations (UN), contributing to the production of one of the most important cereals in the world, corn, with impact on poverty eradication, hunger, sustainable agriculture beyond industry, innovation and infrastructure.

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## 1 INTRODUÇÃO GERAL

O milho (*Zea mays* L.) é considerado uma das culturas mais difundidas e produzidas no mundo (Dowd; Johnson, 2018). Com produção mundial em torno de 1.230 milhões de toneladas, os Estados Unidos ocupam o posto de maior produtor com 390 milhões de toneladas e 40% do mercado mundial, seguido da China com 299 milhões de toneladas e 23%. O Brasil assume o posto de terceiro maior produtor do cereal com 124 milhões de toneladas e 10% do mercado mundial (USDA FAS, 2024). No cenário nacional, o milho representa 41% de toda produção de grãos no país, com área cultivada de 22,2 milhões de hectares (CONAB, 2023), sendo a segunda cultura mais produzida e plantada no país, atrás apenas da soja.

A utilização de sementes de milho de alta qualidade é primordial para o bom desenvolvimento inicial das plantas (Marcos-Filho, 2015; Reis *et al.*, 2022). Processos considerados vitais como a germinação de sementes, emergência e crescimento inicial de plântulas são investigados com o objetivo de estabelecer métodos que assegurem a proteção de tecidos e estruturas como sistema radicular e parte aérea contra pragas e patógenos na fase inicial, uma vez que, por ainda serem pequenos e pouco desenvolvidos, são mais vulneráveis e propensos ao ataque de agentes fitopatogênicos (Antonello *et al.*, 2009; Carvalho *et al.*, 2022; Delian *et al.*, 2016).

Uma alternativa para mitigar e proteger contra os possíveis efeitos deletérios de pragas e patógenos na semente e na fase inicial de desenvolvimento, é o tratamento de sementes (Cherry *et al.*, 2017). Essa e outras importantes técnicas de proteção de cultivos vem crescendo e se destacando no setor, pois consiste na aplicação de defensivos pesticidas, corantes entre outros aditivos específicos que preservam as sementes e o desempenho das plântulas, utilizando quantidades menores de produtos, se comparado a outros métodos de aplicação e tratamento. Dessa forma, permite que as culturas expressem seu potencial genético voltados para obtenção de altos rendimentos (Aguiar *et al.*, 2018; Mariucci *et al.*, 2018), pois, sementes de alta qualidade fisiológica são associadas a alto potencial produtivo (Egli; Rucker, 2012; Ebone *et al.*, 2020; Reis *et al.*, 2022).

Com o avanço das tecnologias de aplicação, as empresas produtoras de sementes vêm adotando técnicas que buscam otimizar o beneficiamento e a logística de comercialização, como o uso do tratamento industrial de sementes (TSI) (Medeiros *et al.*, 2023), assim, permite que lotes de sementes estejam com o seu processo de pós-colheita concluído armazenadas e já tratadas, prontas para expedição e comercialização no período de semeadura, o que otimiza o trabalho na empresa sementeira e facilita a vida do produtor ao adquirir um produto acabado

pronto para o uso, isto torna o tratamento de sementes com produtos fitossanitários uma técnica muito utilizada e importante para o cultivo da cultura do milho (Vojvodić *et al.*, 2021).

Todavia, há relatos que alguns produtos utilizados no tratamento de sementes, principalmente alguns importantes ingredientes ativos do grupo de moléculas inseticidas neonicotinóides, podem ocasionar fitotoxidez ao longo do armazenamento, o que prejudica a germinação e vigor das sementes de milho (Bittencourt *et al.*, 2000; Deuner *et al.*, 2014; Moraes *et al.*, 2022). Essas moléculas são relevantes no manejo de pragas iniciais da cultura (North *et al.*, 2018), por isso demandam maior atenção em sua utilização e pesquisas para aperfeiçoamento de seu uso via tratamento de sementes sem que afetem a qualidade fisiológica.

Apesar do conhecimento acerca dos benefícios do tratamento de sementes, ainda faltam informações quanto ao melhor processo de tratamento para os lotes de sementes, bem como uma melhor compreensão de quais características estão envolvidas nos genótipos para tolerância ao tratamento, quais procedimentos realizados no tratamento podem impactar no vigor de sementes tratadas, qual o período ideal para o armazenamento das sementes tratadas, e quais os possíveis impactos que o tratamento pode gerar à qualidade fisiológica das sementes em função da sua natureza genética, fisiológica e/ou morfoanatômicas.

Assim, o objetivo principal no trabalho foi estudar a relação da fitotoxidez causada por inseticidas em lotes de sementes híbridas por meio de características fisiológicas e morfoanatômicas de sementes, além de investigar tecnologias de aplicação, posicionamento de produtos, moléculas, processos de tratamento e estratificação das aplicações, no intuito de identificar e desenvolver estratégias que proporcionem menor impacto sobre a qualidade fisiológica em sementes e preservem ao máximo o vigor ao longo do armazenamento. Também foi estudada a evolução e a relação da qualidade genética, fisiológica, física e sanitária de sementes híbridas de milho nas últimas décadas no estabelecimento e na produtividade do milho.

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**ARTIGO 1 - Processos industriais de tratamento de sementes de milho e os impactos sobre a qualidade fisiológica ao longo do armazenamento**

**Industrial corn seed treatment processes and impacts on physiological quality during storage**

(Normas de acordo com a revista Pesquisa Agropecuária Brasileira)

**Abstract** - Seed treatment is a widely used technique in agriculture to protect seeds and seedlings, but reports of phytotoxicity caused by insecticides in seedlings has aroused the interest for alternatives to mitigate this effect. Thus, the aim in this work was to evaluate different operational processes for corn seeds treatment in terms of physiological and functional quality throughout storage. Hybrid corn seeds from the same batch were subjected to 11 different industrial treatments process including the application of phytosanitary products, such as the insecticide, in different orders/layers/stages in the process, in addition to the treatment with no insecticide application. Seed quality was assessed by germination and vigor tests over four storage periods of 0, 6, 12 and 21 months, and treatment quality by a functional test. There was a decrease in vigor of the treated seeds during storage. The treatment processes and the arrangement of the active ingredients with the seeds affected the maintenance of the treated corn seeds quality. The use of the fungicide + polymer (FP) in the first stage creates a layer of protection that helping to maintain vigor and mitigate phytotoxicity in seeds and seedlings, especially during 12 months of storage. In these lines, the stratified process of FP/Insecticide/Dry powder is indicated for favoring the maintenance of vigor for up to 12 months of storage, being similar to the FP treatment alone. Functional quality of the treatments: dust release, plantability and coating is not an impediment to the use of industrial seed treatment processes with component stratification.

**Index terms:** Phytotoxicity, seed shelf life, storage tolerance, *Zea mays*.

**Resumo** - O tratamento de sementes é uma técnica muito utilizada na agricultura por proporcionar proteção às sementes e plântulas, entretanto, relatos de fitotoxidez por inseticidas em plântulas vem despertando o interesse por alternativas no processo para mitigar tal efeito. Assim, o objetivo nesse trabalho foi avaliar os efeitos de diferentes processos industriais de tratamento de semente de milho sobre a qualidade fisiológica das sementes e funcional do tratamento ao longo do armazenamento. Sementes híbridas de milho, de um mesmo lote, foram submetidas a 11 diferentes processos industriais de tratamento envolvendo a aplicação dos produtos fitossanitários, como inseticidas, fúngica + polímero (FP) em diferentes ordens/estágios/camadas do processo, além do tratamento sem uso de inseticidas. A qualidade das sementes foi avaliada por meio de testes de germinação e vigor ao longo de quatro períodos de armazenamento 0, 6, 12 e 21 meses e a qualidade do tratamento por meio de testes de qualidade funcional. Ocorreu redução do vigor das sementes tratadas ao longo do armazenamento. Os processos de tratamento e disposição dos ingredientes ativos afetaram a manutenção da qualidade das sementes de milho tratadas. O uso do fungicida + polímero (FP) em primeiro estágio auxilia na preservação do vigor e mitiga a fitotoxidez junto às sementes e plântulas, principalmente após 12 meses de armazenamento. Nessa linha, o procedimento industrial de tratamento estratificado FP/Inseticida/Pó é indicado por favorecer a manutenção do vigor das sementes até 12 meses de armazenamento, sendo semelhante ao tratamento somente com FP apenas. A qualidade funcional dos tratamentos: desprendimento de poeira, plantabilidade, recobrimento, não é um impedimento para o uso dos processos industriais de tratamentos de sementes com estratificação dos componentes.

**Termos para indexação:** Armazenamento seguro, fitotoxidez, tratamento seguro de semente, *Zea mays*.

## Introdução

A utilização de sementes de milho (*Zea mays* L.) de alta qualidade é primordial para o bom desenvolvimento inicial das plantas, o que contribui para a obtenção de altas produtividades (Egli et al., 2012; Reis et al., 2022). Com isso, processos considerados vitais como a germinação de sementes, emergência e crescimento inicial de plântulas são investigados com o objetivo de estabelecer métodos que assegurem a proteção de tecidos e estruturas como sistema radicular e parte aérea contra pragas e patógenos na fase inicial (Delian et al., 2016).

Uma alternativa para mitigar e proteger sementes e plântulas contra os possíveis efeitos deletérios de pragas e patógenos é o uso do tratamento químico de sementes com produtos fitossanitários. Essa técnica tem se destacado na área de produção e tecnologia de sementes pois consiste na aplicação de defensivos agrícolas, corantes, películas, polímeros ou outros aditivos que preservem o desempenho das sementes, permitindo que as culturas expressem todo seu potencial genético em condições de campo (Aguiar et al., 2018; Carvalho et al., 2022).

Os processos de tratamento de sementes podem ocorrer basicamente na própria fazenda, próximo ao momento da semeadura ou na unidade de beneficiamento de sementes das empresas produtoras (Reis et al., 2023). Esse último processo, conhecido como tratamento de semente industrial (TSI) tem aumentado de forma significativa, pois proporciona ganhos logísticos ao produtor, além de fornecer um tratamento com melhor recobrimento e distribuição do ingrediente ativo de produtos fitossanitários pelo uso de tecnologias específicas para essa finalidade (Medeiros et al., 2023).

Por questões logísticas e técnicas, é comum que a produção, beneficiamento e tratamento de sementes de milho híbrido ocorra alguns meses antes do período de semeadura, fazendo com que os lotes de sementes tratadas passem obrigatoriamente por determinados períodos armazenados. Todavia, existem relatos que produtos utilizados no tratamento de

sementes, como algumas importantes moléculas inseticidas usadas no manejo de pragas nos estádios iniciais (Utono & Adamu, 2023), podem ocasionar fitotoxidez ao longo do armazenamento, prejudicando a germinação e vigor das sementes de milho (Deuner et al., 2014; Moraes et al., 2022).

Apesar do conhecimento acerca dos benefícios do tratamento de sementes, ainda faltam informações quanto ao melhor processo de tratamento, o período de tolerância para o armazenamento das sementes tratadas, bem como possíveis danos que o tratamento possa gerar à qualidade fisiológica e as propriedades de funcionalidade do tratamento das sementes. Situações como essa, tem gerado demanda pelo desenvolvimento de inovações em produtos e processos de aplicação dos produtos fitossanitários no tratamento de sementes, como segmentar o tratamento em estágios para garantir que os produtos sejam utilizados da maneira mais segura e eficaz, mitigando o risco de fitotoxidez (Ma, 2019; Oliveira et al. 2021).

Neste sentido, o objetivo no trabalho foi avaliar processos industriais de tratamentos químicos com estratificação dos componentes junto a semente de milho, envolvendo fungicidas, inseticidas, polímero e pó secante, sobre a manutenção da qualidade fisiológica e funcional ao longo do armazenamento.

## **Material e Métodos**

O experimento foi conduzido no Laboratório Central de Pesquisa em Sementes (LCPS) da Escola de Ciências Agrárias de Lavras (ESAL) da Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brasil e no Seedcare Institute Syngenta, Holambra, São Paulo, Brasil.

As sementes de milho da cultivar híbrida Syn555 VIP3 da empresa Syngenta foram submetidas aos processos industriais de tratamento, envolvendo variações na ordem de aplicação dos produtos, formando camadas de aplicação, estágios, com aplicação simultânea ou segregada dos produtos (estratificação), com 1 a 3 estágios de aplicação dos produtos com

duração de 10 segundos cada processo (Tabela 1). Em todos os processos foram utilizados o produto fungicida com os ingredientes ativos azoxistrobina (1,5%), tiabendazol (30,0%), fludioxonil (3,75%), metalaxil-m (3,0%), e os inseticidas com ingrediente ativo ciantraniliprole (60%) e tiametoxam (60%) e polímero Disco AG Red L-450 Incotec, doses especificadas na Tabela 1. Em alguns processos foi utilizado o pó secante *Biogloss* e/ou inserida a secagem com ventilação mecânica entre os estágios de aplicação dos produtos líquidos. A descrição dos 12 processos de tratamento de sementes está apresentada na Tabela 1. No tratamento FP foi utilizado somente fungicida e polímero em mistura com aplicação única, sem aplicação de inseticida.

**Tabela 1.** Protocolos operacionais de Tratamento de Sementes Industrial.

Tratamento	1° estágio <sup>(1)</sup>	2° estágio <sup>(1)</sup>	3° estágio <sup>(1)</sup>	Resumo
T1	Fun <sup>(2)</sup> + Pol <sup>(3)</sup>			FP
T2	Fun + Ins <sup>(4)</sup> + Pol (mix)	-	-	Mix
T3	Fun + Ins + Pol (mix)	Pó secante <sup>(5)</sup>	-	Mix/Pó
T4	Fun + Ins + Pol (mix)	Secagem <sup>(6)</sup>	-	Mix/Secagem
T5	Fun + Pol	Inseticida	-	FP/Inseticida
T6	Fun + Pol	Pó secante	Inseticida	FP/Pó/Inseticida
T7	Fun + Pol	Secagem	Inseticida	FP/Secagem/Inseticida
T8	Fun + Pol	Inseticida	Pó secante	FP/Inseticida/Pó
T9	Fun + Pol	Inseticida	Secagem	FP/Inseticida/Secagem
T10	Inseticida	Fun + Pol	-	Inseticida/FP
T11	Inseticida	Pó secante	Fun + Pol	Inseticida/Pó/FP
T12	Inseticida	Secagem	Fun + Pol	Inseticida/Secagem/FP

Abreviações: Fun: fungicida; Ins: Inseticida; Pol: polímero.

<sup>(1)</sup>Configuração do tratamento: velocidade 304 rpm, duração de cada estágio de 10 segundos.

<sup>(2)</sup>Fungicida: Maxim Quattro® (20 mL/60.000 sementes, ingredientes ativos azoxistrobina (1,5 %), tiabendazol (30,0 %), fludioxonil (3,75 %), metalaxil-m (3,0 %)).

<sup>(3)</sup>Polímero: Disco L-450 AG Red *Incotec* (110 mL/60.000 sementes).

<sup>(4)</sup>Inseticida: Fortenza 600® (40 mL/60.000 sementes, ingrediente ativo ciantraniliprole (60%)) + Cruiser 600® (70 mL/60.000 sementes, ingrediente ativo tiametoxam (60 %)).

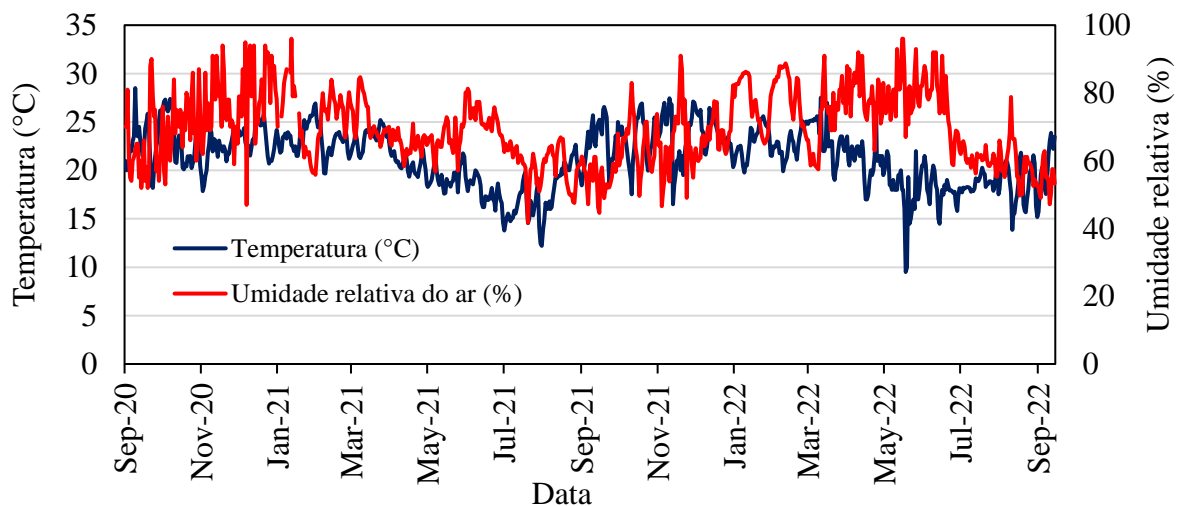
<sup>(5)</sup>Pó secante: *Biogloss* (20 g/60.000 sementes).

<sup>(6)</sup>Velocidade do vento de secagem 17,51 nós kg<sup>-1</sup>, duração de 10 segundos.

Todas as sementes foram tratadas ainda com a mistura dos inseticidas deltametrina 2,5% e pirimifós-metílico 50% de nome comercial K-obiol 25 EC e Actellic 500 EC nas doses de 1,62 e 0,32 mL/60.000 de sementes, respectivamente, para controle de pragas de

armazenamento. Para a realização dos tratamentos foi utilizada a máquina de tratamento do tipo batelada, modelo Arktos L-5k da marca Momesso, em cada processo de tratamento a batelada foi de 2,5 kg, os processos foram conduzidos no Laboratório de Processamento Pós-colheita de sementes do LCPS/UFLA.

Logo após os processos de tratamentos as sementes foram acondicionadas em sacos de papel multifoliado e armazenadas a temperatura e umidade relativa ambiente no Laboratório de Processamento Pós-colheita de Sementes do LCPS/UFLA. Durante o armazenamento, as temperaturas e umidades relativas foram registradas diariamente com uso de *Data Logger*, sendo constatado temperatura média de 21,3 °C e umidade relativa do ar de 69,3%, apresentados na Figura 1.



**Figura 1.** Temperatura e umidade relativa do ar durante o período de armazenamento ambiente.

Com intuito de avaliar o desempenho fisiológico logo após o tratamento e em períodos avançados de armazenamento, a qualidade das sementes foi avaliada aos 0 (logo após o tratamento), 6, 12 e 21 meses de armazenamento. A cada época, as sementes foram analisadas por meio dos seguintes testes:

*O teor de água das sementes* determinado conforme metodologia prescrita nas Regras para Análise de Sementes - RAS (Brasil, 2009), utilizando o método da estufa a  $105 \pm 3^\circ\text{C}$ , durante 24 horas, com três repetições, sendo os resultados expressos em porcentagem.

Para avaliação da *germinação*, as sementes foram semeadas em folhas de papel toalha, germitest<sup>®</sup>, umedecidas com água destilada, em quantidade equivalente a 2,5 vezes o peso do substrato seco, com quatro repetições de 50 sementes. Os rolos foram acondicionados em germinador tipo EL006 Mangelsdorf (Eletrolab Ltda., São Paulo, Brazil) regulado à temperatura de  $25^\circ\text{C}$ . As avaliações foram realizadas na primeira contagem ao 4º dia, e final ao 7º dia seguindo os critérios das RAS, considerando o número de plântulas normais (Brasil, 2009). Os resultados foram expressos em porcentagem.

*Germinação em rolo de papel mais vermiculita*, em quatro repetições de 50 sementes, foram semeadas em rolos de papel toalha, umedecidas com água destilada em quantidade equivalente a 3,0 vezes o peso do papel seco, sobre 2 folhas do papel úmido. Antes da semeadura, foi adicionada uma fina camada de vermiculita úmida (proporção vermiculita/água destilada de 1:1), distribuída uniformemente na quantidade de 100 mL (Rocha et al., 2020, 2023) e então realizada a semeadura, cobertura com outra folha de papel úmido e confecção dos rolos. Em seguida, foram mantidos em germinador Mangelsdorf a  $25^\circ\text{C}$  e as plântulas normais foram computadas aos 4 e 7 dias após a semeadura conforme as RAS (2009).

*Emergência de plântulas*, foi realizada a semeadura em bandejas plásticas contendo como substrato areia + solo na proporção 2:1. Foram utilizadas quatro repetições de 50 sementes. Após a semeadura, as bandejas foram mantidas sob condições controladas em câmara de crescimento vegetal à temperatura de  $25^\circ\text{C}$ , em regime alternado de luz e escuro (12 horas), e o substrato hidratado a 60% da capacidade de retenção de água. O número de plântulas emergidas foi contada diariamente por sete dias para o cálculo do índice de velocidade de

emergência (Maguire, 1962), e também foi registrado o número total de plântulas emergidas ao sétimo dia após a semeadura, sendo os resultados expressos em porcentagem.

*Comprimento de plântulas*, 20 sementes foram submetidas ao teste de germinação, seguindo a metodologia descrita anteriormente com quatro repetições. No terceiro dia, após a semeadura, foram realizadas a captura e análise do comprimento de plântulas. As avaliações foram feitas no GroundEye<sup>®</sup>, versão S120, do módulo de captação para a obtenção de imagens de alta resolução. Subsequentemente, o equipamento foi calibrado utilizando o parâmetro de cor YCbCr com índice de luma de 0,09 a 0,66, índice de azul 0,00 a 0,50 e índice de vermelho -0,30 a 0,11, o que permitiu a extração dos valores médios do tamanho total das plântulas (parte aérea + raiz primária) em centímetros.

O teste de frio foi realizado utilizando um substrato constituído por 2:1 de areia e terra em bandejas. As sementes foram distribuídas uniformemente sobre o substrato e cobertas com uma camada de aproximadamente 3,0 cm do substrato, que foi irrigado até atingir 60% de sua capacidade de retenção de água. Foram utilizadas quatro repetições de 50 sementes. As sementes permaneceram em câmara fria por sete dias a 10°C e em seguida, foram mantidos, durante sete dias, em câmara de crescimento de plantas com temperatura de 25°C e fotoperíodo de 12 horas; no sétimo dia foram contabilizadas as plântulas normais que emergiram e os resultados foram expressos em porcentagem (Cicero & Vieira, 2020; Caseiro & Marcos Filho, 2002).

O teste de envelhecimento acelerado foi realizado com uso de caixas adaptadas de poliestireno transparente “gerbox”, contendo 40 mL de água destilada e camada única de sementes cobrindo toda a tela suspensa. Posteriormente, as caixas gerbox com tampa foram mantidas em câmara BOD a 41°C por 96 horas (Marcos-Filho, 2020). Em seguida, 50 sementes foram semeadas em substrato papel em quatro repetições, conforme metodologia descrita para o teste de germinação, e outras quatro repetições de 50 sementes foram semeadas em substrato

como descrito no teste de emergência de plântulas, e as avaliações foram realizadas quatro e sete dias após a semeadura, com resultados expressos em porcentagem de plântulas normais (Brasil, 2009) e emergência de plântulas normais em substrato.

De modo geral, as análises realizadas foram divididas em duas categorias, análises de qualidade fisiológica realizadas ao longo do período de armazenamento no Laboratório Central de Pesquisa em Sementes da UFLA descritas acima, submetidas a análise estatística entre tratamentos e épocas. E a análises exploratória/descritiva das características das propriedades físicas-funcionais dos tratamentos/procedimentos no qual as sementes foram realizadas no Seedcare Institute, somente uma única vez, logo após o processo de tratamento, por meio dos seguintes testes:

*Desprendimento de partículas (Dust-off)* as sementes foram acondicionadas por 48 horas a 20°C e 50% de umidade relativa. Após este período, amostras de 100 gramas de sementes dos tratamentos foram avaliadas com uso de um medidor de poeira, modelo Heubach D.38679 Langelsheim. Um filtro de microfibras de 6 cm de diâmetro foi colocado no porta-filtro e o conjunto foi pesado em balança de precisão com cinco casas decimais. O equipamento foi acionado e o movimento giratório do tambor com defletores e o atrito das sementes durante 2 minutos resultaram na liberação de partículas de poeira. Essas partículas foram aspiradas pelo fluxo de 20 litros de ar por minuto durante de os 2 minutos através do filtro, e o pó desprendido ficou retido no filtro. Ao final do tempo estabelecido, o conjunto do filtro e o porta-filtro foram pesados novamente e a massa de partículas de poeira destacadas foi estimada pela diferença de peso (Euroseeds, 2011; Reis et al., 2023). Os resultados foram apresentados em g de poeira por 60.000 sementes, conforme equação:

$$\text{Desprendimento de partículas (g 60.000 sementes}^{-1}) = ((W_1 - W_0) * 20.285) / W_s$$

$W_1$ : peso do porta-filtro com filtro de fibra de vidro após teste (g);

$W_0$ : peso do porta filtro com filtro de fibra de vidro antes do teste (g);

$W_s$ : peso das sementes tratadas (g);

*Para o teste de plantabilidade* amostras de 1 kg de sementes por tratamento foi utilizado em um simulador de semeadura da marca Momesso, acoplado ao sensor a laser de captura Corn Counter MW (Syneltro, Suíça) para medir a distribuição de sementes durante a semeadura em condições controladas. O equipamento possui uma cavidade de micro-ondas, oscilando a 1,6 GHz e um loop de bloqueio de fase (PLL) usado para detectar mudanças de frequência se a semente cair através do sensor. A tensão de controle do oscilador controlado por tensão (VCO) é amplificada e digitalizada por um microcontrolador a 2,5 kSPS. O fluxo de dados é enviado para um computador que gera um registro de dados de passagem no sensor. Os dados foram analisados com o Software que gera um relatório de quantificação do índice de semeadura correto, percentual de falhas, duplas e múltiplas (Syneltro, 2024).

*Resistência a abrasão (Rub-off) com quantificação do recobrimento por meio de análise de imagem de alta resolução* foi utilizado o aparelho modelo PTF 20E/ER (Pharma Test) para a simulação de abrasão, composto por dois cilindros rotativos de polímero transparente com faces internas polidas, o qual gira em torno de seu eixo. Uma amostra de 300 gramas de sementes foi pesada e submetida a ação do aparelho por 6 minutos, 50 rotações por minuto de atrito, com total de 300 giros (Pharma Test, 2023). Após o teste de abrasão, amostras de cada lote, antes e após a simulação foram submetidas a captura e processamento do recobrimento no LCPS-ESAL-UFLA por meio do sistema de análise de imagens GroundEye® versão S800 com quatro amostras de 50 sementes por tratamento. Depois da aquisição das imagens foram realizadas a configuração, processamento e quantificação da porcentagem do recobrimento do tratamento da cor vermelho e rosa das sementes de acordo com Medeiros et al. (2023).

O delineamento estatístico utilizado foi o de blocos inteiramente casualizados (DIC) em arranjo fatorial 12x4, sendo 12 (Processos industriais de tratamento) x 4 (períodos de armazenamento). Quando necessário, os dados foram transformados em  $(x+1)^{0,5}$  para atender o teste de normalidade dos resíduos de Shapiro and Wilks (1965) para posterior realização das análises. Os dados foram submetidos à análise de variância a 5% de probabilidade, pelo teste F, e quando significativos, os processos ao longo do armazenamento foram analisados por meio de regressão linear ou polinomial, com a escolha de modelos matemáticos significativos a 5%, com maior coeficiente de determinação. Já para a comparação entre os processos de tratamento foi utilizado o teste de agrupamento de médias Scott-Knott ( $p < 0.05$ ).

Para a análise de cluster foi realizada a média das quatro repetições de cada tratamento para cada variável fisiológica em cada período de armazenamento, e escolhido o modelo hierárquico de distância "maximum", com método para análises dos agrupamentos hierárquicos "centroid" devido ao alto coeficiente de correlação cofenética (Saraçlı et al., 2013).

O software computacional R versão 4.1.2 e os pacotes MVar.pt e ExpDes foram utilizados para realizar todas as análises (Ferreira et al., 2014; R Development Core Team, 2016; Ossani et al., 2023).

## **Resultados e Discussão**

Por meio da análise de variância foi observado diferenças significativas ( $p < 0,05$ ) entre os processos de tratamento e períodos de armazenamento, como também a interação entre os fatores foi significativa para todas as variáveis analisadas, os coeficientes de variação foram entre 1,2% e 6,0% (Tabela 1). Para análises fisiológicas dos tratamentos foram utilizadas as variáveis: germinação, germinação mais vermiculita, comprimento de plântulas, teste de frio e envelhecimento acelerado (Tabela 2), porém, todas as variáveis descritas no material e métodos foram utilizadas para performar a análise de cluster.

**Tabela 2.** Analysis of variance of the variable of corn seeds subjected to seed treatment processes and storage.

Variable	DF	Mean square values					
		MC	G	G+V	SL	CT	AA
Process (P)	11	0.3	18.8***	9.4***	1.1***	37.0***	142.0***
Storage (S)	3	7.4***	1095.0***	976.7***	159.8***	2051.1***	62612.0***
P x S	33	0.3**	12.8***	4.4***	0.3***	17.7***	87.0***
Residuals	144 <sup>(1)</sup>	0.2	1.6	1.6	0.1	3.7	6.0
CV (%)		4.1	1.3	1.2	6.1	2.0	3.3

MC: Moisture Content; G: Germination; G+V: Germination + Vermiculite; SL: Seedling length; CT: Cold test; AA: Accelerated aging.

Significance code: 0 '\*\*\*' 0.001, '\*\*' 0.01, '\*' 0.05.

<sup>(1)</sup>Degree freedom Residual moisture content  $n = 96$ .

O teor de água das sementes foram todos próximos, com variação máxima de teor de água de 0,3 a 1,7 pontos percentuais entre os processos de tratamento, indicando que as variações nos processos não afetaram essa variável. Timóteo & Marcos-Filho (2013) ao avaliar o desempenho de diferentes genótipos de milho durante o armazenamento, também observaram lotes de sementes de híbridos de milho com diferenças de 1,0 a 1,7 pontos percentuais no teor de água, considerando essa variação uniforme e adequada, pois não interfere no comportamento das sementes durante os testes fisiológicos no o armazenamento, uma vez que as variações ficam dentro dos limites de tolerância, 2 a 3 pontos percentuais de acordo com Marcos-Filho (2020).

Foi observada também tendência de aumento de 1% em média do teor de água ao longo do armazenamento, de 9,8% logo após o tratamento para 10,8% aos 21 meses de armazenamento. As variações estão relacionadas ao equilíbrio higroscópico dos lotes de sementes em função da umidade e temperatura do local de armazenamento, que oscilaram ao longo do tempo em função das características das estações climáticas (Figura 1). Os valores médios foram temperatura de 21,3 °C e umidade relativa média de 69,3%. Efeito similar foi constatado por Angelovič et al. (2018) em que o teor de água de sementes de milho diferiu com a mudança da umidade relativa e temperatura do ar às quais foram submetidas durante a

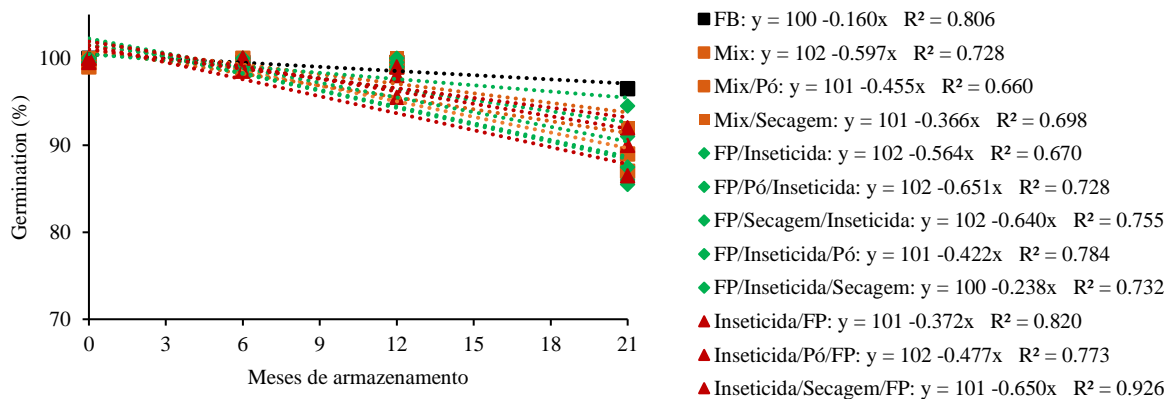
secagem e armazenamento. O aumento da umidade relativa do ar, estão associadas a maiores teores de água nos grãos de milho (Santos et al., 2020).

A porcentagem de germinação das sementes também foi influenciada pelo período de armazenamento, sendo observada germinação igual ou superior a 99% em todos os lotes na primeira e segunda época, e com 21 meses de armazenamento no tratamento apenas com fungicida e polímero foi observado valor de 97%, compondo de forma isolada o grupo de maior média para a germinação, e os demais lotes que receberam de alguma forma o tratamento inseticida, compuseram os demais grupos de “b” a “e”, com os valores de 87% a 95%, e média de 89% de germinação (Tabela 3), o que demonstra a alta qualidade inicial das sementes do híbrido utilizado e adequação para uso em tratamento industrial de sementes. Todos os lotes assim atendem ainda mesmo após 21 meses de armazenamento, após o tratamento, o padrão mínimo de 85% de germinação para comercialização exigidos pela legislação brasileira (Brasil, 2013), sabe-se que é tendência no setor sementeiro a seleção e/ou escolha de lotes com alta qualidade inicial para o tratamento de sementes, em função da importância do uso de sementes de alta qualidade e tratadas para o estabelecimento de plântulas em campo, além do elevado valor agregado na produção de sementes híbridas e a alta tecnologia no tratamento industrial de sementes de milho (Baldini et al., 2018; Pereira et al., 2019; Medeiros et al., 2023). Todavia, foi constatada germinação menor das sementes com algum tipo de tratamento químico inseticida após 21 meses em relação ao tratamento FP, que apresentou uma redução mensal na germinação ao longo do armazenamento 0,16% (Figura 2), enquanto sementes com tratamento inseticida apresentaram maior redução na germinação em média de 0,49%, exceto para o tratamento FP/Inseticida/Secagem com redução mensal 0,23% (Figura 2), indicando a necessidade de estudos em processos de tratamento e armazenamento de sementes tratadas com essas relevantes moléculas no manejo de pragas iniciais da cultura do milho para que não afetem a qualidade das sementes, mesmo quando com alta qualidade inicial.

**Tabela 3.** Germinação final (%) de sementes de milho submetido a procedimentos industriais de tratamento de sementes e períodos de armazenamento de 0, 6, 12 e 21 meses.

Tratamento	Armazenamento (meses)			
	0	6	12	21
FP	100 <sup>ns</sup>	100 <sup>ns</sup>	100 a	97 a
Mix	100	100	99 a	87 e
Mix/Pó	99	99	100 a	89 d
Mix/Secagem	100	100	100 a	92 c
FP/Inseticida	100	100	100 a	88 e
FP/Pó/Inseticida	100	99	99 a	86 e
FP/Secagem/Inseticida	100	100	99 a	86 e
FP/Inseticida/Pó	100	100	99 a	91 c
FP/Inseticida/Secagem	100	99	100 a	95 b
Inseticida/FP	100	100	98 a	92 c
Inseticida/Pó/FP	100	100	99 a	90 d
Inseticida/Secagem/FP	100	99	96 b	87 e

Médias seguidas de letras diferentes na coluna, existe diferença entre os procedimentos de tratamento de sementes para a época de armazenamento segundo o teste de agrupamento de médias Scott-Knott ( $p < 0.05$ ), <sup>ns</sup>: não há diferença entre os procedimentos.



**Figura 2.** Germinação final (%) em rolo papel de sementes de milho submetido a procedimentos industriais de tratamento de sementes em função dos períodos de armazenamento de 0, 6, 12 e 21 meses.

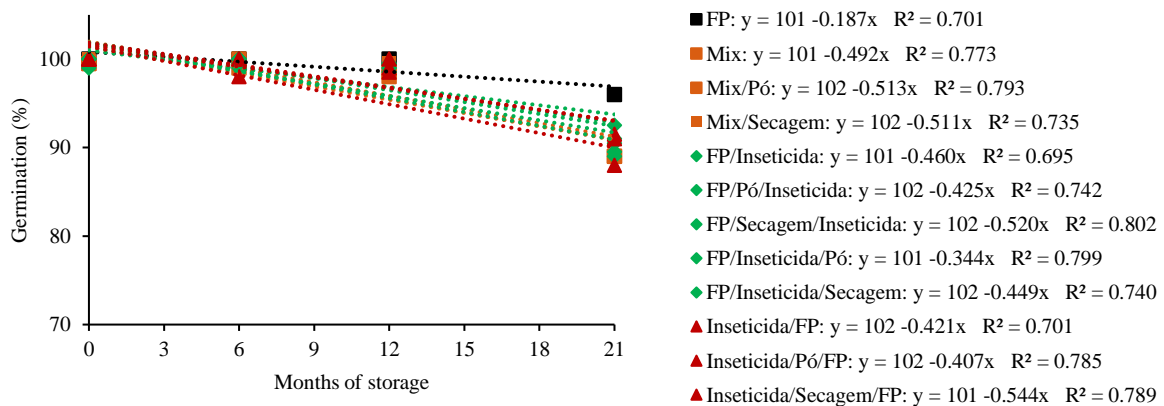
Com o intuito de mitigar a fitotoxidez observada no teste de germinação em rolo de papel para sementes tratadas com produtos químicos, sobretudo inseticidas, foi realizado o teste de germinação com uso de vermiculita entre papel, no qual foram observados valores mais elevados de modo geral, Tabela 4 e Figura 3, em relação ao constatado para a germinação em rolo de papel (Tabela 3 e Figura 2) para o mesmo período de armazenamento. Teste de germinação com uso da vermiculita entre papel, diminui a concentração de produtos fitossanitários em contato direto com as sementes podem auxiliar na expressão da real qualidade

fisiológica de sementes tratadas minimizando a possível fitotoxidez por inseticidas (Rocha et al., 2020; 2023).

**Tabela 4.** Germinação final (%) em rolo papel mais vermiculita de milho submetido a procedimentos industriais de tratamento de sementes e períodos de armazenamento de 0, 6, 12 e 21 meses.

Tratamento	Armazenamento (meses)			
	0	6	12	21
FP	100 <sup>ns</sup>	100 <sup>ns</sup>	100 <sup>ns</sup>	96 a
Mix	100	99	99	89 d
Mix/Pó	100	100	98	89 d
Mix/Secagem	100	100	100	89 d
FP/Inseticida	99	100	99	90 d
FP/Pó/Inseticida	100	100	100	91 c
FP/Secagem/Inseticida	100	100	99	89 d
FP/Inseticida/Pó	100	99	99	93 b
FP/Inseticida/Secagem	100	100	100	91 c
Inseticida/FP	100	100	100	91 c
Inseticida/Pó/FP	100	100	99	92 c
Inseticida/Secagem/FP	100	98	99	88 d

Médias seguidas de letras diferentes na coluna, existe diferença entre os procedimentos de tratamento de sementes para a época de armazenamento segundo o teste de agrupamento de médias Scott-Knott ( $p < 0.05$ ), <sup>ns</sup>: não há diferença entre os procedimentos.



**Figura 3.** Germinação final (%) em rolo papel mais vermiculita de sementes de milho submetido a procedimentos industriais de tratamento de sementes em função dos períodos de armazenamento de 0, 6, 12 e 21 meses.

Foi constatado para a germinação em vermiculita diferenças entre os tratamentos somente com 21 meses de armazenamento, com desempenho menor entre os lotes com tratamento químico, sobretudo para o processo com Tiametoxam Inseticida/Secagem/FP (Tabela 4), assim como na germinação em papel (Tabela 3), redução de 8 a 10 pontos

percentuais em relação ao tratamento somente com FP. Entre os lotes com tratamentos com inseticidas, os procedimentos envolvendo o tratamento fungicida mais polímero em primeiro estágio FP/Inseticida/Pó (no teste de germinação mais vermiculita – Tabela 4) e FP/Inseticida/Secagem (no teste de germinação em rolo de papel – Tabela 3) aos 21 meses de armazenamento demonstraram estar entre os melhores processos de tratamento químico (Tabela 3 e 4). Dessa forma, observa-se que a estratificação dos produtos no processo de tratamento das sementes exerce influência sobre a qualidade.

Lorenzetti et al. (2014) também observaram redução mais acentuada na germinação de sementes de milho sobre efeito dos inseticidas neonicotinóides, imidacloprid + thiodicarb e principalmente Tiametoxam armazenadas em temperaturas entre 20 a 30 °C e UR  $\pm$  60%, com redução de 19,6 e 32,4 pontos percentuais da germinação respectivamente, enquanto em sementes submetidas ao tratamento apenas com fungicida mais polímero houve redução de 10,4 pontos percentuais ao longo do armazenamento. Em outros estudos foi observado que o armazenamento fora das condições ideais intensificam o processo de deterioração, com o acúmulo de espécies reativas de oxigênio - EROs decorrentes do estresse oxidativo, alterações metabólicas, como desestruturação das membranas celulares, inativação de enzimas, degradação de proteínas perda de suas funções biológicas culminando com a morte celular, prejudicando a germinação e o vigor (Kumar et al., 2015); condições essas que são intensificadas principalmente em sementes tratadas com inseticida (Oliveira et al., 2020; Moraes et al, 2022).

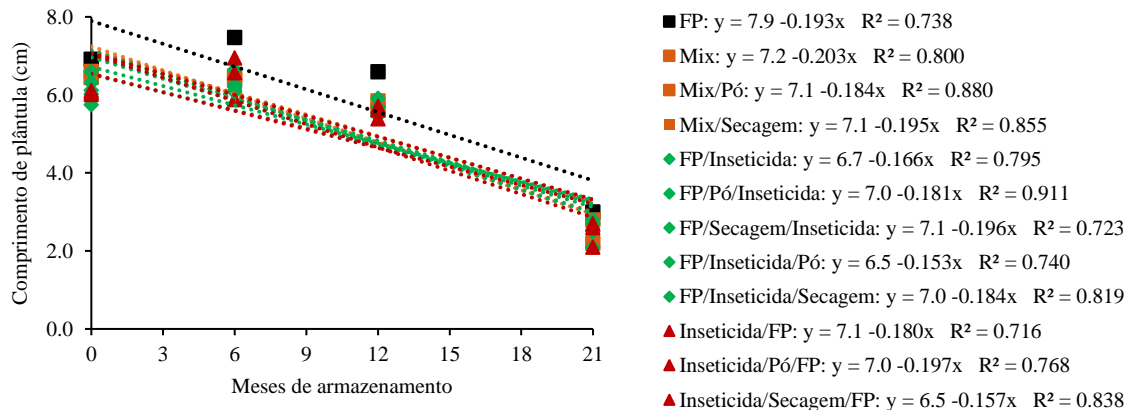
Para os testes de vigor, como comprimento de plântulas, em todos os lotes houve redução linear do comprimento ao longo do armazenamento (Figura 4). As sementes do tratamento apenas com fungicida e polímero foram de 0,5 a 1,0 cm superior em média (Tabela 5, Figura 4), reforçando que o comprimento de plântulas pode ser um indicativo de verificação de fitotoxidez no substrato papel. O tratamento com alguns inseticidas como Tiametoxam

podem diminuir o desenvolvimento inicial das plântulas, conforme demonstrado na Figura 5, imagens capturadas das plântulas ao terceiro dia após a semeadura em substrato papel.

**Tabela 5.** Comprimento de plântulas de milho (cm) ao terceiro dia em rolo papel submetidos a procedimentos industriais de tratamento de sementes e períodos de armazenamento de 0, 6, 12 e 21 meses.

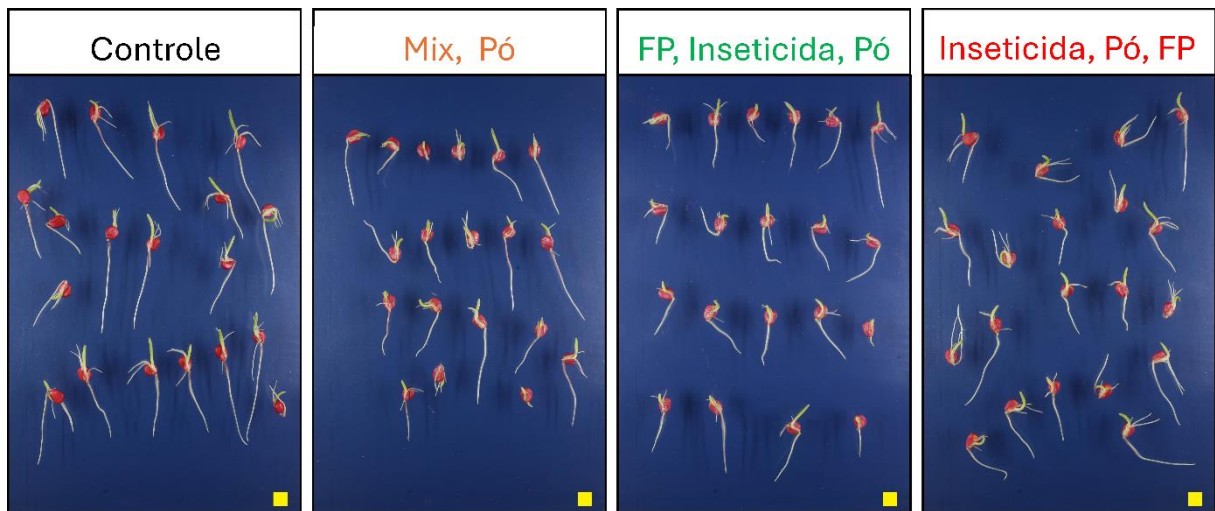
Tratamento	Armazenamento (meses)			
	0	6	12	21
FP	6.9 a	7.5 a	6.6 a	3.0 a
Mix	6.5 a	6.5 c	5.9 b	2.2 b
Mix/Pó	6.6 a	6.4 c	5.6 b	2.8 a
Mix/Secagem	6.5 a	6.2 d	5.7 b	2.4 b
FP/Inseticida	6.0 b	6.3 d	5.5 b	2.7 a
FP/Pó/Inseticida	6.6 a	6.1 d	5.5 b	2.8 a
FP/Secagem/Inseticida	6.1 b	6.6 c	5.9 b	2.1 b
FP/Inseticida/Pó	5.8 b	6.2 d	5.5 b	2.7 a
FP/Inseticida/Secagem	6.3 a	6.2 d	5.7 b	2.5 b
Inseticida/FP	6.1 b	6.9 b	5.7 b	2.6 a
Inseticida/Pó/FP	6.1 b	6.6 c	5.6 b	2.1 b
Inseticida/Secagem/FP	6.0 b	5.9 d	5.4 b	2.7 a

Médias seguidas de letras diferentes na coluna, existe diferença entre os procedimentos de tratamento de sementes para a época de armazenamento segundo o teste de agrupamento de médias Scott-Knott ( $p < 0.05$ ).



**Figura 4.** Comprimento de plântulas de milho (cm) ao terceiro dia em rolo papel submetidos a procedimentos industriais de tratamento de sementes em função dos períodos de armazenamento de 0, 6, 12 e 21 meses.

Entre as sementes tratadas, ao final de 21 meses de armazenamento, nos tratamentos com posicionamento de fungicida mais polímero em primeiro estágio houve o maior número de tratamentos com comprimento de plântulas superior, destaque para as sementes tratadas com FP/Inseticida com 2,7 cm, FP/Pó/Inseticida com 2,8 cm, e FP/Inseticida/Pó com 2,7 cm de comprimento de plântulas, não diferendo do tratamento FP (Tabela 5).



**Figura 5.** Amostras dos processos de aplicação de produtos fitossanitários, tratamento fungicida mais polímero (sem aplicação de inseticida), posicionamento padrão (mix: aplicação simultâneo de produtos inseticida, fungicida, polímero e pó secante), posicionamento fungicida mais polímero em primeiro estágio (fungicida mais polímero, seguido de inseticida e por último pó secante) e posicionamento contraprova com inseticida em primeiro estágio (inseticida, seguido de pó secante e por último fungicida mais polímero), no teste de comprimento de plântulas ao terceiro dia de germinação com 6 meses de armazenamento capturadas pelo equipamento GroundEye. Escala de tamanho representada no canto inferior direito pelo quadrado amarelo de 1 cm<sup>2</sup>.

O vigor por meio do teste de frio proporcionou estratificação entre os processos industriais de tratamento somente com 21 meses de armazenamento (Tabela 6). Em geral, a exemplo do constatado para os demais testes de germinação e vigor, o tratamento apenas com FP e quatro dos cinco tratamentos com o fungicida e polímero em primeiro estágio foi observada uma menor deterioração ao final do armazenamento, 21 meses, 88 a 92%, indica que esse processo pode amenizar os efeitos fitotóxicos ao longo do armazenamento quando da utilização de inseticidas no controle de pragas. Já os menores valores, 80 a 82%, foram constados com uso dos produtos em mistura ou com inseticida em primeiro estágio de aplicação (Tabela 6).

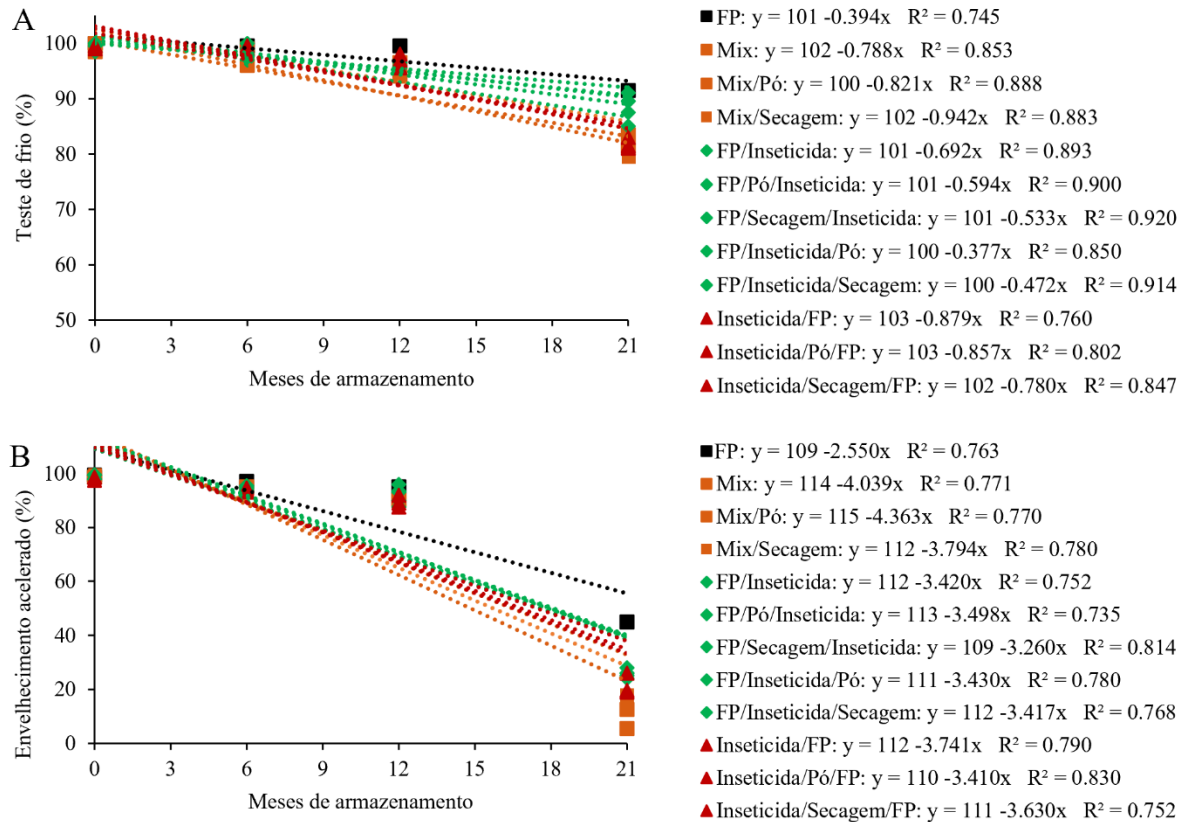
A deterioração ao longo do armazenamento para todos os processos ocorreu de forma linear, porém com diferentes intensidades, para o tratamento somente FP a diminuição da emergência no teste de frio foi de 0,39% ao mês (Figura 6A). Em sementes com a aplicação de

fungicida mais polímero em primeiro estágio houve redução média do vigor de 0,53% por mês. A menor taxa de deterioração de sementes neste grupo de processos, 0,377% ao mês foi observada, com uso do processo FP/Inseticida/Pó, semelhante ao observado no tratamento somente com fungicida mais polímero (FP), o que é desejável (Figura 6A). Já entre os processos de tratamento com aplicação dos defensivos agrícolas na forma de misturas (mix) ou com uso inseticida em primeiro estágio, houve redução média de 0,85% por mês em média (Figura 6A), com os piores resultados para os processos Mix/secagem e Inseticida/FP com 0,94% e 0,87% ao mês, indicando que o uso do inseticida em primeiro estágio pode otimizar a deterioração das sementes, esta informação é importante para o controle de qualidade de sementes visto que o teste de frio é amplamente difundido para avaliação do vigor de lotes de milho e em estudos tem sido observada relação do bom desenvolvimento inicial e rendimento em campo da cultura para lotes de alto vigor com base no teste (Mondo et al., 2013; Reis et al., 2022).

**Tabela 6.** Porcentagem de plântulas normais emergidas após teste de frio em substrato de sementes de milho submetido a procedimentos industriais de tratamento de sementes e períodos de armazenamento de 0, 6, 12 e 21 meses.

Tratamento	Armazenamento (meses)			
	0	6	12	21
FP	100 <sup>ns</sup>	100 <sup>ns</sup>	100 <sup>ns</sup>	92 a
Mix	100	99	97	84 b
Mix/Pó	99	96	94	81 c
Mix/Secagem	100	97	95	80 c
FP/Inseticida	100	97	96	85 b
FP/Pó/Inseticida	100	99	97	88 a
FP/Secagem/Inseticida	100	100	96	90 a
FP/Inseticida/Pó	99	99	97	91 a
FP/Inseticida/Secagem	100	98	97	90 a
Inseticida/FP	100	100	98	81 c
Inseticida/Pó/FP	100	99	97	82 c
Inseticida/Secagem/FP	99	99	96	83 b

Médias seguidas de letras diferentes na coluna, existe diferença entre os procedimentos de tratamento de sementes para a época de armazenamento segundo o teste de agrupamento de médias Scott-Knott ( $p < 0.05$ ), <sup>ns</sup>: não há diferença entre os procedimentos.



**Figura 6.** Testes de vigor, porcentagem de plântulas normais emergidas após teste de frio (A) e envelhecimento acelerado (B) de sementes de milho submetido a procedimentos industriais de tratamento de sementes em função dos períodos de armazenamento de 0, 6, 12 e 21 meses.

Já para o vigor avaliado por meio do teste de envelhecimento acelerado a estratificação do vigor em função dos processos seguiu as mesmas tendências do teste frio, com diferenças significativas aos 12 meses, em que o tratamento com apenas fungicida mais polímero não diferiu de três tratamentos com FP em primeiro estágio e excepcionalmente do processo Inseticida/Secagem/FP, que foram superiores aos demais. Porém essa tendência não se manteve ao final do armazenamento, em que o tratamento com apenas fungicida mais polímero foi superior a todos os demais, 45% (Tabela 7). Aos 21 meses de armazenamento, abaixo do tratamento FP, todos os lotes tratados com fungicida mais polímero em primeiro estágio, apresentaram vigor superior, 24% a 28%, aos demais se comparado com a maioria dos demais tratamentos com inseticidas em primeiro estágio, com alguns valores abaixo de 20% (Tabela 7).

**Tabela 7.** Porcentagem de plântulas normais emergidas após envelhecimento acelerado de sementes de milho submetido a procedimentos industriais de tratamento de sementes e períodos de armazenamento de 0, 6, 12 e 21 meses.

Tratamento	Armazenamento (meses)			
	0	6	12	21
FP	100 <sup>ns</sup>	97 <sup>ns</sup>	95 a	45 a
Mix	99	95	91 b	13 d
Mix/Pó	99	95	90 b	06 e
Mix/Secagem	100	94	91 b	18 c
FP/Inseticida	100	95	94 a	26 b
FP/Pó/Inseticida	100	96	96 a	24 b
FP/Secagem/Inseticida	98	94	88 b	28 b
FP/Inseticida/Pó	98	95	90 b	25 b
FP/Inseticida/Secagem	100	95	93 a	26 b
Inseticida/FP	99	95	89 b	19 c
Inseticida/Pó/FP	99	94	88 b	26 b
Inseticida/Secagem/FP	98	93	92 a	20 c

Médias seguidas de letras diferentes na coluna, existe diferença entre os procedimentos de tratamento de sementes para a época de armazenamento segundo o teste de agrupamento de médias Scott-Knott ( $p < 0.05$ ), <sup>ns</sup>: não há diferença entre os procedimentos.

Ao longo do armazenamento, todos os processos industriais apresentaram deteriorações lineares ao longo do período de armazenamento, o controle somente com FP, houve redução de 2,55% ao mês, já os processos industriais em que o fungicida mais polímero foram em primeiro estágio, 3,41% por mês em média, para o uso do inseticida em primeiro estágio 3,59% ao mês em média, e posicionamento da mistura de todos os produtos fitossanitários em primeiro estágio 4,07% por mês em média (Figura 6B), reiterando que o uso do inseticida no primeiro estágio de aplicação ou seja primeira camada acentua a deterioração ao longo do armazenamento, isso reforça a importância do estudo e implementação do tratamento estratificando com fungicida mais polímero em primeiro estágio para a manutenção da qualidade fisiológica.

Comportamento semelhante também foi observado no estudo sobre a fitotoxidez em sementes tratadas com inseticida Tiametoxam e armazenadas se comparado com o controle para o vigor de sementes de milho desenvolvido por Bittencourt et al. (2001) e em sementes de soja por Junior et al. (2020). Mariucci et al. (2018) também observaram redução do vigor de

sementes de milho tratadas com a combinação de produtos como o mix de inseticida Neonicotinóides mais inoculantes e armazenadas. Quando o processo de deterioração está mais avançado as estruturas mais afetadas são as membranas celulares e as regiões de crescimento da plântula como a extremidade da radícula nas monocotiledôneas comprometendo o desempenho fisiológico (Marcos-Filho, 2015). Carvalho et al. (2020) também identificaram em sementes de soja ocorrência de fitotoxidez, com redução no comprimento de plântulas em sementes tratadas com inseticida como Imidacloprid + Thiodicarb, Acetamiprid e Thiamethoxam em relação ao tratamento controle, concluíram que características de comprimento de plântulas, sobretudo raízes, é um parâmetro a ser considerado na avaliação de ocorrência de fitotoxidez em sementes tratadas.

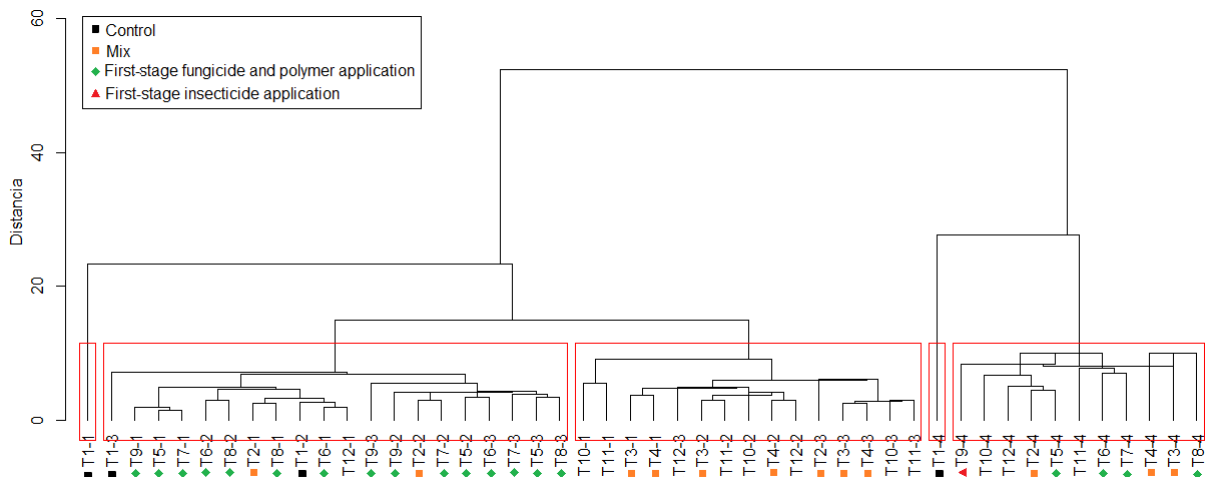
Uma possível explicação para esse efeito é de que os inseticidas sistêmicos do grupo químico Diamida antranflica apresenta alta seletividade e pouca fitotoxidez como o Ciantraniliprole (Brasil, 2018a; Li et al., 2022), contudo, o mesmo não é observado no grupo Neonicotinóide, como o Tiametoxan (Brasil, 2018b), usados para o tratamento de sementes que frequentemente são associados à redução do vigor (Deuner et al., 2014).

Desse modo, pode se inferir que o processo de tratamento e disposição dos ingredientes ativos junto às sementes afetaram a manutenção da qualidade das sementes de milho tratadas. O uso do polímero mais fungicida em primeiro estágio pode ter criado uma camada de proteção que evitou o contato direto da molécula inseticida Neonicotinóides com a semente, o que auxilia na manutenção do vigor e mitiga a fitotoxidez junto às sementes e posteriormente às plântulas no processo de germinação. Porém, outros trabalhos ainda são necessários para comprovar tal hipótese.

Em função dos resultados dos testes de germinação e vigor, o processo de tratamento com FP/Inseticida/Pó demonstrou ser uma alternativa adequada para a manutenção da qualidade fisiológica de sementes de milho tratadas e armazenadas, além de ser um

procedimento operacional relativamente mais simples de ser implementado se comparado com outros procedimento de estratificação e aplicação também testados em nosso trabalho. A utilização de secagem, ventilação mecânica, entre camadas, não demonstrou efeito sobre a formação de camada de proteção.

Com uso da análise de cluster performada por meio das médias de todas as variáveis fisiológicas utilizadas para a avaliação dos tratamentos ao longo do armazenamento, ficou claro o comportamento geral dos processos industriais sobre a qualidade fisiológica das sementes (Figura 7), foram representados os materiais com maior vigor na extrema esquerda, com o primeiro grupo composto pela tratamento apenas com FP sem armazenamento, em seguida o segundo grupo com 21 tratamentos (processos de tratamento x épocas de armazenamento), em que 85% desses foram de processos com o tratamento com fungicida mais polímero em primeiro estágio com até 12 meses de armazenamento (Figura 7). Isso reforça que a não utilização de inseticidas neonicotinóides em primeiro estágio/camada favorece a manutenção do vigor e reduz a intensidade de deterioração por fitotoxidez a esses produtos.



**Figura 7.** Dendrograma de sementes de milho submetidos a diferentes processos de tratamento industrial ao longo do armazenamento, através da métrica das distancias dos agrupamentos hierárquicos "maximum" e método de análise dos agrupamentos "centroid".

Tratamentos: T1: Controle, fungicida mais polímero; T2: Mix; T3: Mix/Pó; T4: Mix/Secagem; T5: FP/Inseticida; T6: FP/Pó/Inseticida; T7: FP/Secagem/Inseticida; T8: FP/Inseticida/Pó; T9: FP/Inseticida/Secagem; T10: Inseticida/FP; T11: Inseticida/Pó/FP; T12: Inseticida/Secagem/F. Período de armazenamento: -1: sem armazenamento; -2: 6 meses; -3: 12 meses; -4: 21 meses.

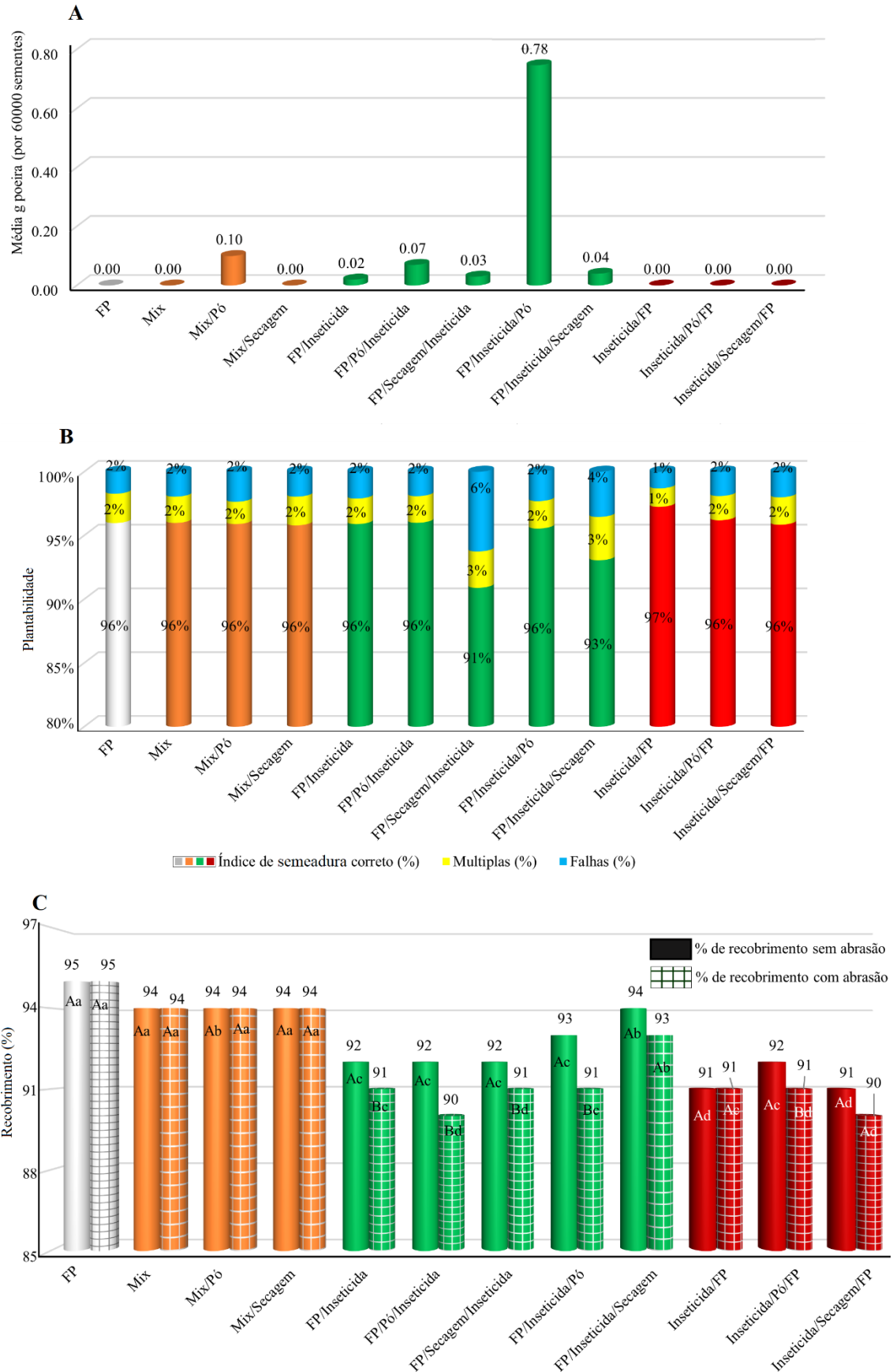
O terceiro grupo foi composto 100% por amostras de sementes que passaram pelo processo de tratamento com mix de produtos ou inseticida em primeiro estágio, reiterando que o uso de inseticida neonicotinóide em primeiro estágio é prejudicial à qualidade. Já o quarto grupo foi constituído novamente pelo tratamento somente uso de fungicidas e polímero, porém, já com período de armazenamento avançado que ocasionou a deterioração, contudo superior em relação ao 5º agrupamento, representado na extrema direita pelos lotes de menor vigor, constituído pelos demais tratamentos com uso de inseticida com 21 meses de armazenamento. Isso caracteriza a deterioração acentuada devido a combinação do período avançado de armazenamento e uso do inseticida neonicotinóide, indicando que esse período de armazenamento em condições não controladas, independente do processo de tratamento, não é seguro para sementes de milho.

Medeiros et al. (2021) também por meio de organização hierárquica de dendrograma em híbridos de milho com diferentes níveis de severidade do complexo do enfezamento-do-milho, agrupou os lotes em função do seu nível de vigor, permitindo assim distinguir lotes de alta qualidade fisiológica. Esta técnica foi importante para a segregação de lotes de milho doce em grupos de alto, intermediário e baixo vigor por Marinho et al. (2019).

Além da qualidade fisiológica das sementes de milho, avaliações das características funcionais dos tratamentos/procedimentos foram consideradas, estas características interferem em questões técnicas como segurança ambiental e distribuição e manutenção dos produtos junto às sementes até no processo de semeadura em si, avaliados por meio dos testes de desprendimento de poeira, recobrimento das sementes e plantabilidade.

Os processos de tratamento de sementes afetaram os parâmetros de qualidade funcional avaliados. Para o desprendimento de poeira, determinado pelo teste *dust-off*, nos tratamentos com pó secante na última camada de estratificação foram observados os maiores valores, com ênfase para o fungicida com polímero/inseticida/pó secante com 0,78 g de poeira para 60.000

sementes seguido do Mix/pó secante com 0,10 g de poeira para 60.000 sementes (Figura 8A). A Euroseeds STAT Dust Working Group sugere quantidade máxima de 0,75 g de poeira por 100.000 sementes (Euroseeds, 2011), logo em 60.000 sementes o recomendável seria até 0,45 g. Nesse caso, apenas o primeiro tratamento citado ultrapassaria esse limite, todavia, em sua grande maioria, a poeira foi constituída do pó secante e não do desprendimento dos produtos fitossanitário, que seria o mais prejudicial por perda de produto e por questões ambientais. O desprendimento do pó secante pode ser explicado pelo seu baixo peso e sua aplicação isolada no último estágio, diminuindo seu contato e mistura com a calda, facilitando assim, seu desprendimento da semente durante a realização do teste.



**Figura 8.** Teste de desprendimento de poeira *Dust-off* (A), plantabilidade (B), e teste de abração *Rub-off* e recobrimento (C) em sementes de milho submetidas a diferentes procedimentos industriais de tratamento de sementes.

O índice de semeadura correta das sementes ficou em torno de 96% por meio do teste de plantabilidade, apenas para o tratamento com FP/secagem/inseticida e para o tratamento FP/inseticida/secagem houve valores inferiores a 96%, sendo 91% e 93% respectivamente (Figura 8B). Uma das possíveis causas para esses resultados nos dois tratamentos pode ser a aplicação do polímero em primeiro estágio somado ao uso da secagem por ventilação que pode ter tornado a superfície mais áspera comprometendo o índice de semeadura correta. Mas mesmo assim com índices satisfatórios, acima de 90%. A quantidade de sementes com espaçamentos uniforme por unidade de área é fundamental para que a cultura possa expressar todo seu potencial (Rontani et al., 2020).

Para a análise do recobrimento das sementes antes e após o teste de abrasão das sementes apresentou leve redução no recobrimento, máxima de 2% (Figura 8C). As principais reduções, entre 1 e 2% ocorreram nos tratamentos com fungicida mais polímero em primeiro estágio, recebendo assim em última camada inseticida, pó ou secagem, que não exercem a mesma finalidade de acabamento proporcionada pelo polímero, pois sabe-se que o uso de polímero melhora a cobertura, acabamento e a aderência dos produtos nas sementes (Ludwig et al., 2011). Todavia, como mencionado em nosso estudo, a perda da eficiência do polímero em primeiro estágio na manutenção do recobrimento são indícios, pois em dois tratamentos com aplicação de fungicida mais polímero em último estágio também houve redução de 1% no recobrimento, assim mais estudos quanto a essa variável são necessários.

A realização desse teste é importante pois durante os processos de manuseio das sementes como tratamento químico, ensaque, armazenamento, transporte e semeadura ocorrem atrito entre as sementes e entre elas e a embalagem o que pode ocasionar diminuição do recobrimento e da dose do ingrediente ativo por semente diminuindo sua eficiência devido ao desprendimento de produtos (Medeiros et al., 2023).

Tal comprometimento proveniente da abrasão foi constatado de forma muito sutil em nosso estudo, pois a média geral no recobrimento antes do teste foi de 93% e após de 92%, o que demonstra boa adequação dos processos de tratamento. Segundo critério proposto por Medeiros et al. (2023) para sementes de milho, todos os tratamentos apresentam recobrimento satisfatório-bom por apresentarem recobrimento  $\geq 90\%$ , ou nota 5, melhor nota para o recobrimento do tratamento com 91-100% segundo Andrade et al. (2023) para sementes de soja. De forma geral, nenhuma variável utilizada para a avaliação da qualidade funcional dos procedimentos adotados seriam impedimento para a utilização dos processos de tratamento estratificado.

### **Conclusões**

A disposição dos ingredientes ativos junto às sementes no processo de tratamento químico afeta a manutenção da qualidade das sementes de milho durante o armazenamento.

O procedimento operacional de tratamento estratificado com uso de fungicida e polímero (FP) em primeiro estágio e inseticida neonicotinóide posteriormente, ameniza a fitotoxidez em sementes de milho armazenadas. O processo industrial FP/Inseticida/Pó é indicado por favorecer a manutenção do vigor até 12 meses de armazenamento, sendo semelhante ao tratamento somente com FP.

O uso de inseticidas neonicotinóides em primeiro estágio de aplicação no processo de tratamento, isolados ou em mistura, acentua o processo de fitotoxidez e deterioração das sementes de milho armazenadas, sobretudo após 21 meses de armazenamento.

A qualidade funcional dos tratamentos, desprendimento de poeira, plantabilidade, recobrimento, não é influenciada pelos processos industriais de tratamentos de sementes com estratificação dos componentes.

## Agradecimentos

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), à Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), à Fundação de Desenvolvimento Científico e Cultural (FUNDECC), e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelas bolsas concedidas, iniciação científica, pós-graduação e de produtividade em pesquisa (CNPq) e apoio financeiro.

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## **ARTIGO 2 - Effect neonicotinoids treatment seed on physiological quality, storage, and morpho-anatomy corn seeds**

(Standards according to the *Agronomy Journal*)

### **ABSTRACT**

The use of hybrid seeds with high production potential and physiological quality, combined with efficient phytosanitary treatment, is essential to obtain the ideal plant stand and high yield performance. The neonicotinoid seed treatments are an effective control option for early season insect pests in corn, currently common practice to apply phytosanitary treatments to seeds prior to storage, but records indicate a reduction in germination and/or vigor of seeds treated over storage with systemic neonicotinoid insecticides, in some situations. Faced with this challenge intrinsic investigations of seeds to identify and quantify these structures and compounds, that can be related to the vigor and storage potential of treated seeds are required. Therefore, the objective in this work was to evaluate through the physiological and ultrastructural morphoanatomical characteristics of different seed lots treated with neonicotinoid insecticides that contributes to the maintenance vigor and storage potential. The lots 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, and 2LV: hybrid sensitive to seed treatment and low vigor were subjected to insecticide treatments clothianidin, imidacloprid + thiodicarb or thiamethoxam, and control, insecticide free, and storage periods 0, 3, 6 and 12 months, and evaluated through germination and vigor tests, and ultrastructural analysis such as pericarp thickness and starch granule diameter. Seed lots not treated with neonicotinoid insecticides show superior performance compared to treated and stored lots. The active ingredients imidacloprid + thiodicarb and thiamethoxam showed a greater phytotoxic effect, greater impact on germination and vigor, especially in the sensitive and low initial vigor lots. The initial seed lot vigor affects tolerance to neonicotinoid insecticide treatment and storage, with lower vigor lots more susceptible to deterioration. The insecticide-tolerant and high vigor seed lots showed superior vigor performance and morphoanatomical development. Even with high initial vigor, corn genotypes vary in their tolerance to neonicotinoid seed treatment and storage. There is evidence of a relationship between ultrastructural analyses, such as greater pericarp thickness and starch granule size, with a trend toward greater vigor and storage tolerance of insecticide-treated seeds.

### **Core Ideas**

- The active ingredients imidacloprid + thiodicarb and thiamethoxam showed a greater phytotoxic effect, greater impact on germination and vigor in stored corn seeds.
- Low vigor lots are more susceptible to phytotoxicity with neonicotinoid insecticide treatment, mainly when stored.
- Lots of seeds from high vigor and insecticide-tolerant hybrids showed superior performance and morphoanatomical development.

- Thickness, formation, diameter and arrangement of pericarp and starch granules can be used as important tools in investigating the vigor and tolerance of seeds treated with neonicotinoid insecticides and stored.

## 1. INTRODUCTION

The intensified investment in research and development worldwide to improve corn crop production contributes to sustainable livelihood roles, as corn is one of the most important food crops in the world, also a key ingredient in animal feed and is used extensively in industrial products, including the production of biofuels (Shiferaw et al., 2011; Erenstein et al., 2022). Therefore, the use of hybrid seeds with high production potential and physiological quality, combined with efficient phytosanitary treatment, correct management of the treatment process and the selection of appropriate insecticides, is essential to obtain the ideal plant stand and high productive performance (Beckett et al., 2017; Vojvodić & Bažok, 2021; Reis et al., 2022).

Thus, neonicotinoid seed treatments are an effective control option for early season insect pests in corn by activating nicotinic receptors (nAChR) found in the central nervous system of insects, inducing ion flux across the cell membrane, resulting in ionic imbalance and death of a wide range of insect pests (Zhang et al, 2010), improving crop protection resulting in higher yields and net returns in pest-infected areas (North et al., 2018; Ding et al., 2018). And for technical and logistical reasons, it is currently common practice to apply phytosanitary treatments to seeds prior to storage, but records indicate a reduction in germination and/or vigor of seeds treated with systemic neonicotinoid insecticides such as clothianidin (Moraes et al., 2022; Oliveira et al., 2020), imidacloprid + thiodicarb (Pereira et al., 2019) and thiamethoxam (Deuner et al., 2014; Oliveira et al., 2020). This effect is intensified during storage, as shown by Dan et al. (2010) and Silva et al. (2020). And despite the introduction of pesticides with

reduced toxicity and improved biodegradability, neonicotinoids are currently the most important group of chemicals used in seed treatment for pest control (Vojvodić & Bažok, 2021).

Faced with the challenge of providing high potential materials, it is known that seeds with well-formed embryos and protective structures, usually have greater potential for storage and production of more vigorous seedlings (Finch-Savage & Bassel, 2015). However, various characteristics can influence the formation of embryo, starch reserve, and protective tissues of a seed, such as genotype, weather conditions, nutrients, and water availability (Serna-Saldivar, 2019). Furthermore, there are reports on the influence of genotypes on the tolerance and maintenance of quality after seed treatment, such as in corn (Oliveira et al., 2020; Moraes et al., 2022) and soybean (Carvalho et al., 2014).

This highlights the need for more intrinsic investigations of seeds to identify and quantify these morphoanatomical structures and compounds, and their relationship to the vigor and storage potential of treated seeds.

For instance, studies such as that of García-Lara et al. (2004) found a positive relationship in genotypes with pericarp, mature ovary wall with the function of protecting the embryo and endosperm, stronger and harder for resistance to storage pests. Lopes et al. (2018) showed that pericarp thickness does not represent resistance to germination of hybrid corn seeds and does not affect seedling growth. Therefore, thicker pericarps need to be studied as it may be related to storage tolerance after treatment with neonicotinoid insecticides and could be used to help select genotypes.

Thus, despite the knowledge of the benefits of seed treatment, there is still a lack of information on the relationship between chemical treatments, storage, genotypes and ultrastructural analysis. This has created a need to elucidate the relationship between phytotoxicity caused by neonicotinoid insecticides in different genotypes and lots, which can assist in the development of strategies that provide less impact on the physiological quality of

treated seeds throughout storage ("Shelf Life") in post-harvest processes, both in breeding programs with phenotyping for this trait. Therefore, the objective of this work was to evaluate through the physiological and ultrastructural morphoanatomical characteristics of different seed lots treated with neonicotinoid insecticides that contributes to the maintenance vigor and storage potential.

## 2. MATERIALS AND METHODS

The experiment was conducted at the Central Seed Laboratory and the Electron Microscopy and Ultrastructural Analysis Laboratory of the School of Agricultural Sciences of Lavras of the Federal University of Lavras (*Universidade Federal de Lavras – UFLA*), Lavras, Minas Gerais, Brazil.

Were used in the study two hybrids of corn seeds, with tolerance to phytosanitary treatment (hybrid 1) and without tolerance to phytosanitary treatment (hybrid 2), data provided by the seed supplier, each hybrid consisted of two lots, one with high vigor (HV) and the other with low vigor (LV). For the initial characterization of the seed lots quality, the germination and cold test was used prior to insecticide treatment (Rocha et al., 2023).

The seed lots were 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, and 2LV: hybrid sensitive to seed treatment and low vigor. The lots were treated with a mixture insecticides with the commercial product (c.p.) K-obiol<sup>®</sup> and Actellic<sup>®</sup> (dose 1.60 and 0.32 ml c.p. /60,000 seeds, 2.5% deltamethrin and 50% pirimiphos-methyl active ingredient), and the fungicide commercial product Maxim Advanced<sup>®</sup> (dose 30 mL c.p. /60,000 seeds, active ingredient metalaxyl-m 2.0%, thiabendazole 15%, and fludioxonil 2.5%) for pest and fungal storage control.

The seeds were subjected to different insecticide treatments, which included the application of 100% of the recommended dose of the commercial product A: Poncho® (dose 80 ml c.p. / 60,000 seeds, 60% clothianidin active ingredient), B: Cropstar® (dose 350 ml c.p. /60,000 seeds, 15% imidacloprid and 45% thiodicarb active ingredient), or C: Cruiser® (dose 120 ml c.p. /60,000 seeds, 35% thiamethoxam active ingredient) in addition to Polymer Disco AG Red L-450 Incotec (110 ml/60,000 seeds), Biogloss drying powder (20 g/60,000 seeds) or D: control (no additional insecticide treatment) (Table 1). To carry out the treatments the treatment machine model Arktos L-5k, brand Momesso, was used per 2.5 kg sample. And physiologic quality of lots was evaluated at time 0 (immediately after treatment), 3, 6 and 12 months of storage (Table 1).

**Table 1.** Coding used and description of experimental factors and treatments.

Lot	Code	Hybrid	ST sensitivity	Vigor	Active ingredient	Storage (months)
1	1LV	1	Tolerant	Low	A - Clothianidin	0, 3, 6 and 12
2	1LV	1	Tolerant	Low	B - Imidacloprid + Thiodicarb	0, 3, 6 and 12
3	1LV	1	Tolerant	Low	C - Thiamethoxam	0, 3, 6 and 12
4	1LV	1	Tolerant	Low	D - Control	0, 3, 6 and 12
5	1HV	1	Tolerant	High	A - Clothianidin	0, 3, 6 and 12
6	1HV	1	Tolerant	High	B - Imidacloprid + Thiodicarb	0, 3, 6 and 12
7	1HV	1	Tolerant	High	C - Thiamethoxam	0, 3, 6 and 12
8	1HV	1	Tolerant	High	D - Control	0, 3, 6 and 12
9	2LV	2	Sensitive	Low	A - Clothianidin	0, 3, 6 and 12
10	2LV	2	Sensitive	Low	B - Imidacloprid + Thiodicarb	0, 3, 6 and 12
11	2LV	2	Sensitive	Low	C - Thiamethoxam	0, 3, 6 and 12
12	2LV	2	Sensitive	Low	D - Control	0, 3, 6 and 12
13	2HV	2	Sensitive	High	A - Clothianidin	0, 3, 6 and 12
14	2HV	2	Sensitive	High	B - Imidacloprid + Thiodicarb	0, 3, 6 and 12
15	2HV	2	Sensitive	High	C - Thiamethoxam	0, 3, 6 and 12
16	2HV	2	Sensitive	High	D - Control	0, 3, 6 and 12

The seeds submitted to the different insecticide treatment protocols were packaged in multi-wall paper bags and stored in a cold chamber with controlled temperature (10°C) and humidity (50%). The quality of the lots was evaluated through the following analyses:

Germination test, seeds were sown on paper towel moistened with distilled water, equivalent to 2.5 times the weight of the dry paper (Brazil, 2009), with four replicates of 50 seeds. The rolls were placed in an EL006 Mangelsdorft type germinator (Eletrolab Ltda., São Paulo, Brazil), set at a temperature of 25°C. The evaluations was carried out in the 7th day, according to the criteria of the Rule for Seed Analysis (RSA) (Brazil, 2009), considering the percentage number of normal seedlings.

Germination in a paper roll plus vermiculite (PR+V), with four replications of 50 seeds, paper towel moistened with distilled water in an amount equivalent to 3.0 times the weight of the dry paper, on wet paper, a thin layer of moist vermiculite was added (thin vermiculite/distilled water ratio of 1:1), evenly distributed in an amount of 100 mL, and then sown with the aid of a seeding plate (Rocha et al., 2023). Then, they were kept in a Mangelsdorf germinator at 25 °C, the evaluations were considering the percentage number of normal seedlings in the last count on the 7th day according (Brazil, 2009).

Seedling length, 20 seeds were placed to germinate, following the methodology previously described for the germination in paper roll test with four replications. On the third day after germination, seedling length was recorded and analyzed (Nakagawa, 1999). The analyses were performed using GroundEye<sup>®</sup>, version S120, of the capture module to obtain high resolution images. Subsequently, the equipment was calibrated using the CIEL\*a\*b color model with a luminosity index from 0 to 100, dimension "a" and dimension "b", which allowed the extraction of average values of total seedling size, shoot and root length.

The cold test was conducted using a 2:1 sand and soil substrate placed in trays. The seeds were evenly distributed and covered with a layer of approximately 3.0 cm of substrate

irrigated until 60% of the retention capacity. With four replications of 50 seeds per lot. The seeds were kept in a cold room at 10°C for seven days and then in a plant growth chamber at 25°C with a 12 h photoperiod for seven days; on the seventh day, the normal seedlings that emerged were counted on the 7th day and the results were expressed as percentage of emerged seedlings (Cicero & Vieira, 2020; Caseiro & Marcos-Filho, 2002).

The accelerated aging test was conducted using the adapted plastic box "Gerbox" method. Four replicates of 50 seeds were placed in a single layer of seeds in gerbox boxes on a stainless-steel screen, and 40 mL of distilled water was placed at the bottom of the gerbox. Later were placed in Biochemical Oxygen Demand B.O.D. regulated at a temperature of 41°C, where they remained for 96 hours (Marcos-Filho, 2020). After this period, they were subjected to the germination paper roll test (Brazil, 2009) on the seventh day.

The eight seed lots more discrepant: sensitive and tolerant seed treatment, high and low vigor, and treated with imidacloprid + thiodicarb and control, were selected for investigation, for Scanning Electron Microscopy (SEM) analysis. The SEM analysis was performed on ten random seeds from each of the selected lots, sectioned transversely to the embryonic axis, standardizing the region, then mounted on an aluminum stub and coated with gold using the Balzers SCD 050 sputtering device (Silva et al., 2017). To examine the samples, the observation region, the upper part of the seeds, was standardized. The images generated were used to measure total pericarp thickness and starch granule diameter, with 8 measurements per lot. ImageJ<sup>®</sup> software was used for the measurements (Abramoff et al., 2004).

## **2.1 Statistical analyzes**

The statistical design used was completely randomized blocks (DIC) in a three-factorial scheme, 4 x 4 x 4, lots (1HV, 1LV, 2HV and 2LV) x products (clothianidin, imidacloprid + thiodicarb, thiamethoxam and control) and storage periods (0, 3, 6 and 12

months). The data were subjected to analysis of variance at 5% probability using the F test, and when significant, the means were compared using the Scott-Knott test at 5% significance applied in a box plot, and when significant, the storage time was analyzed using linear or polynomial regression, with the choice of mathematical models significant at 5% with a higher coefficient of determination. The computational software R and the ExpDes package were used to perform the analyses, and graphs were generated using Ggplot2 (Ferreira et al., 2014; R Development Core Team, 2016).

### 3. RESULTS AND DISCUSSION

#### 3.1 Physiological Quality *versus* Neonicotinoid Seed Treatment

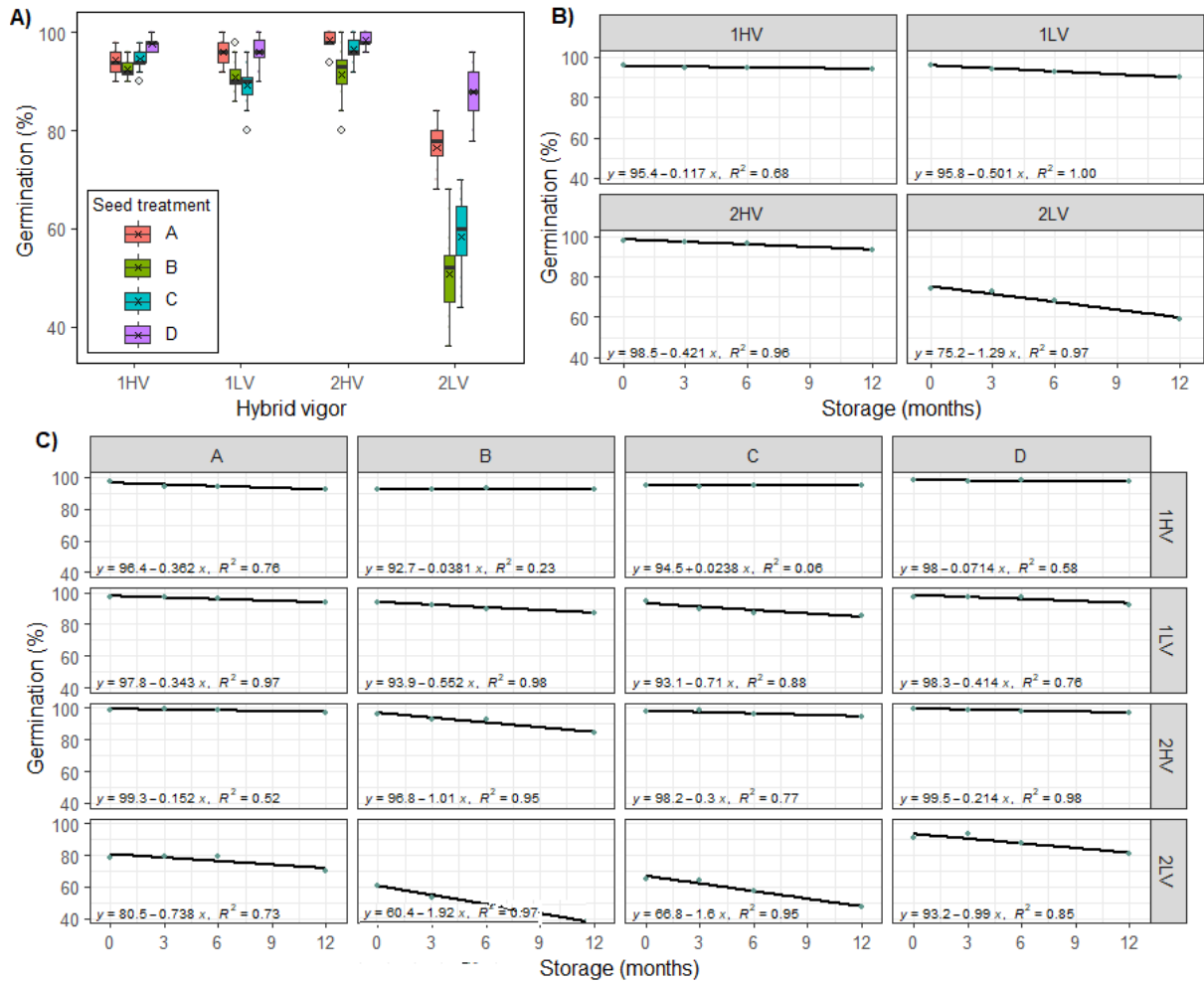
Analysis of variance showed significant differences ( $p < 0.05$ ) between lots (with different tolerance-vigor levels), seed treatments with neonicotinoids and storage periods, and in the interaction between factors for the variables analyzed.

The germination on paper substrate, the controls lots, without neonicotinoid insecticide treatment presented the best performance for the final germination count, with lots 1HV, 2HV and 1LV with 96% to 98%, followed by low vigor 2LV with 88% without insecticide treatment (Figure 1a). In addition, the lot with hybrid sensitive to seed treatment and low vigor (2LV) a decrease in germination was observed in seed lots with insecticide treatment clothianidin (A), followed by thiamethoxam (B) and imidacloprid + thiodicarb (C) with the worst averages (Figure 1a). This shows that the high initial vigor of the lot is essential for greater storage tolerance after treatment with neonicotinoid insecticides. This was also clearly observed during storage for the hybrid lot with higher sensitivity and low initial vigor 2LV, with an initial average germination of 75% and an average monthly reduction of -1.29%, showed the greatest decrease in germination among the four lots (Figure 1b), it was also found that the 2LV lots did not meet the minimum germination standard established for commercialization of corn seeds in

Brazil (85%) (Brasil, 2013), while the others 1HV, 1LV and 2HV showed an initial germination greater than 95% and a maximum average monthly reduction of -0.5%, mainly in high vigor lots, the phytotoxic effect of neonicotinoids was lower (Figure 1b).

When analyzing the triple interaction of factors, the importance of selecting high vigor lots to face possible stress conditions such as insecticide treatment with neonicotinoids and storage becomes evident. Lots 1HV and 2HV showed better performance under the conditions tested, followed by 1LV, thus also demonstrating the importance of selecting hybrids with tolerance to chemical treatment. And with the worst performance was 2LV, mainly under the treatment imidacloprid + thiodicarb and thiamethoxam (Figure 1c).

Silva et al. (2020) also found a reduction -7.3% and -5% in the germination percentage of seeds of the corn hybrid over 9 months of storage with chemical treatment clothianidin and thiamethoxam insecticide, while the control maintained 97% germination in the same period. Germination of corn seed stored in an uncontrolled environment treated with thiamethoxam also showed a 16 percentage points reduction in 12 months of storage by Deuner et al. (2014).



**Figure 1.** Germination (%) on paper substrate of lots corn seeds with neonicotinoid insecticides and stored. A) Interaction of seed lots with neonicotinoid insecticide treatment. B) Interaction of seed lots during storage. C) Interaction of seed lots with neonicotinoid insecticide treatment during storage.

Lots 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, 2LV: hybrid sensitive to seed treatment and low vigor.

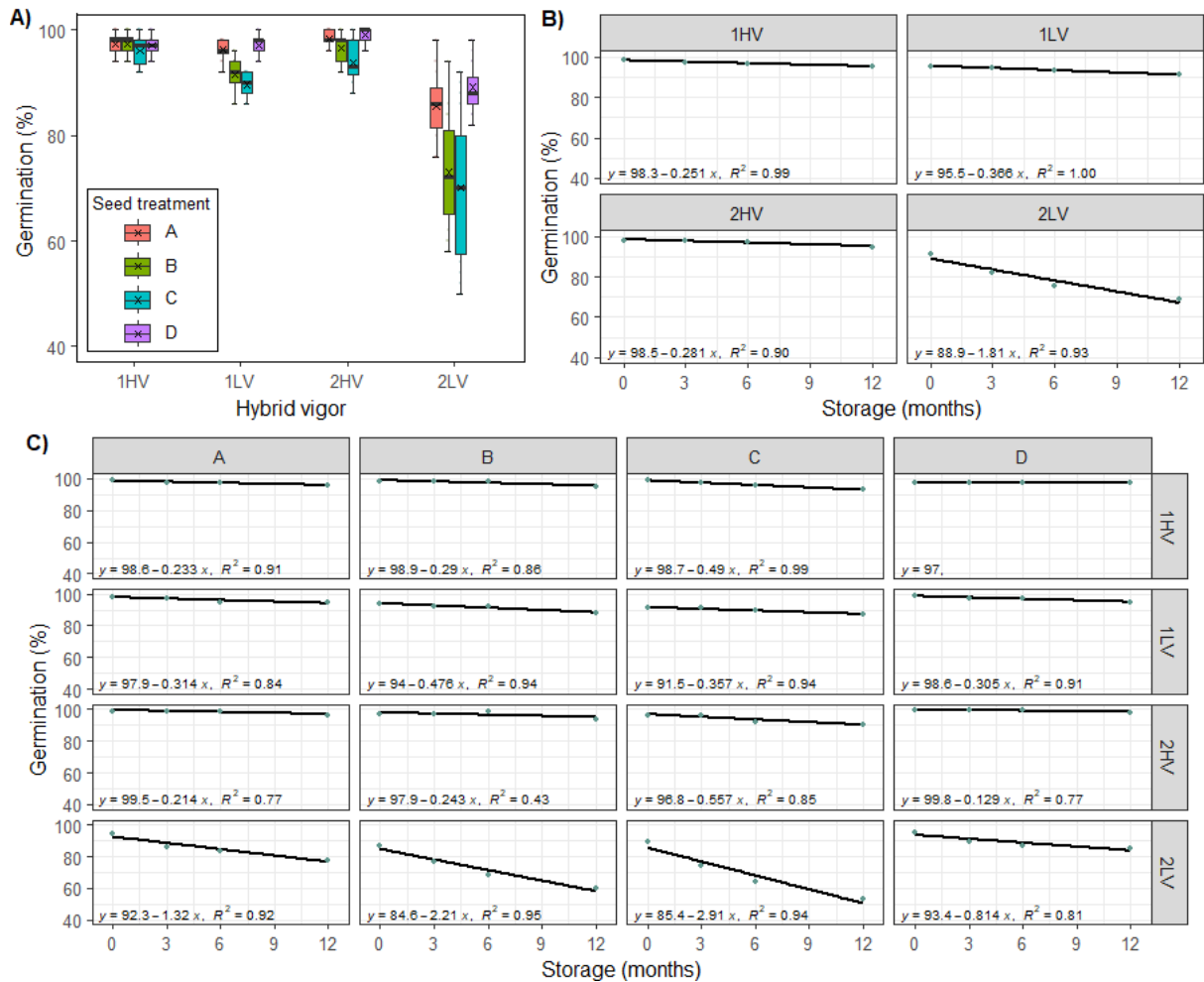
Products A: clothianidin, B: imidacloprid + thiodicarb, C: thiamethoxam and D: control, and storage periods: 0, 3, 6 and 12 months. Boxplot description: The horizontal line in the center of the box indicates the median, the boxes range represents the 50% dispersion of the data covered by the first and third quartiles, Non-overlapping of boxes indicates statistical difference according to Scott-Knott test ( $p < 0.05$ ), and the vertical lines characterize the tails; (x) Represent the mean; (o) Empty dots represent the values outliers. Regression description: green points represent the mean.

A behavior similar to that observed in the germination paper test was observed in the germination in paper roll plus vermiculite test, highlighting the best performance 1HV, 2HV, and 1LV lots, both with a performance greater than 90% even under the effect of insecticide treatment, followed by 2LV with the worst performance (Figure 2 a,b), and again, during storage, only 2LV showed a sharp decline in germination (Figure 2b), demonstrate that it is not

viable in the production process which lot storage after treatment is required, as in industrial seed treatment (IST), the combination of hybrids with sensitivity to the use of neonicotinoids and low initial vigor.

As observed for germination on substrate paper, the intensity of deterioration during storage varied depending on the insecticide molecule used, although they all belong to the neonicotinoid chemical group, in general the most aggressive were imidacloprid + thiodicarb and thiamethoxam, especially in seeds of the most sensitive hybrid and/or in lots with low initial vigor (Figure 2c). In addition to the molecules, the type of substrate used to evaluate seed germination also influenced the observed values, especially for lot 2LV at the beginning of storage, which was from an overall average initial germination in paper of 75% (Figure 1b) to 89% (Figure 2b) with the use of vermiculite in the test, an important improvement also observed for 2LV treated with imidacloprid + thiodicarb and thiamethoxam, which went from 60% and 67% (Figure 1c) to 85% (Figure 2c).

The better performance of the seeds treated in the germination test adapted with vermiculite can be explained due to the possible slower absorption of water and products during the imbibition process (Rocha et al., 2020; Bersch et al. 2021), while sown between rollers paper, there are reports of seeds treated with phytotoxicity due to the high concentration of rapidly absorbed active ingredients, harming percentages of normal seedlings (Rocha et al., 2023; Rossetti et al., 2021), due to the high number of treated seeds placed per area during the paper test, which can result in a high concentration of active ingredient compared to substrate or field conditions that have a greater capacity to dilute the active ingredient.



**Figure 2.** Germination in a paper roll plus vermiculite (%) of lots corn seeds with neonicotinoid insecticides and stored. A) Interaction of seed lots with neonicotinoid insecticide treatment. B) Interaction of seed lots during storage. C) Interaction of seed lots with neonicotinoid insecticide treatment during storage.

Lots 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, 2LV: hybrid sensitive to seed treatment and low vigor.

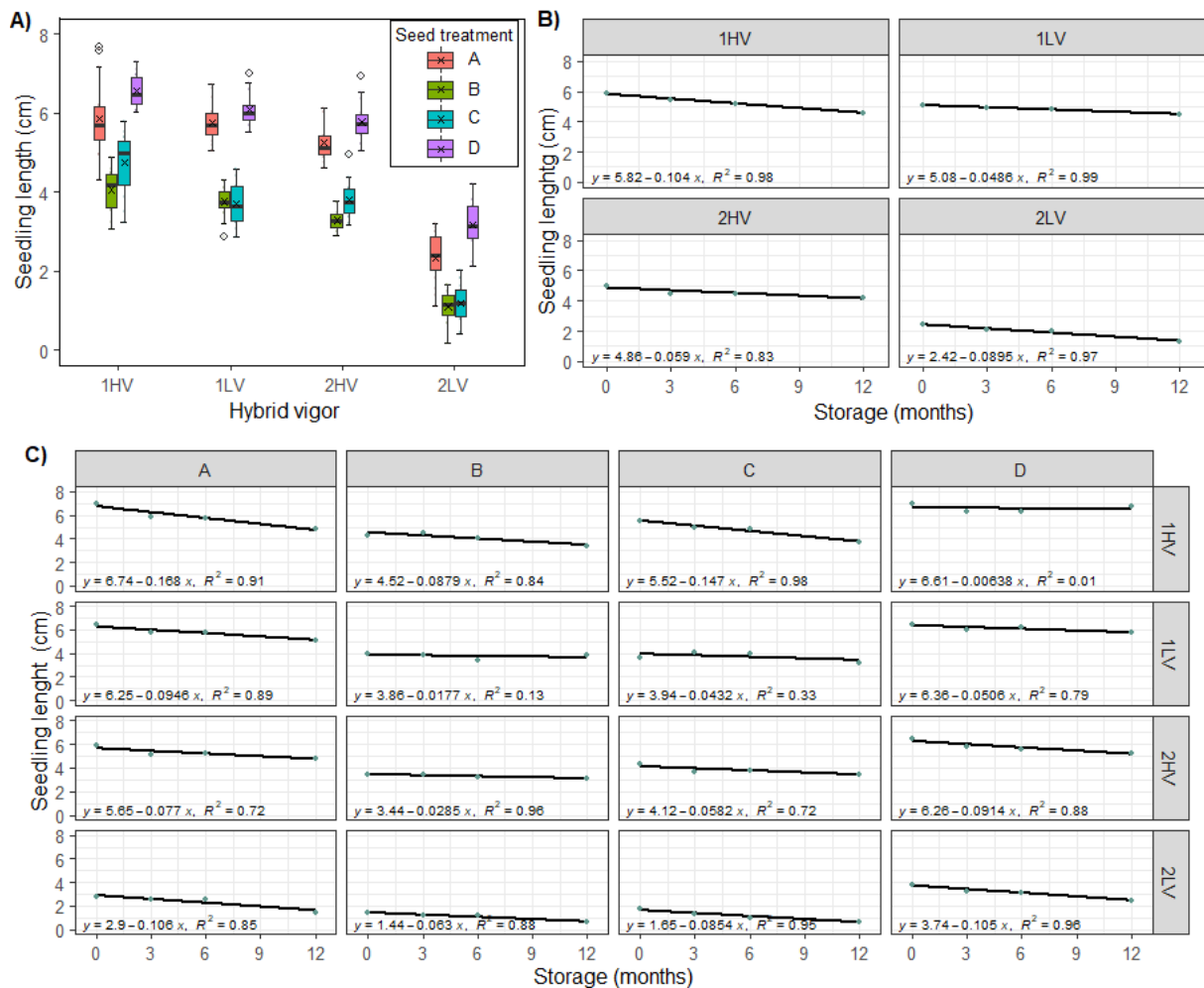
Products A: clothianidin, B: imidacloprid + thiodicarb, C: thiamethoxam and D: control, and storage periods: 0, 3, 6 and 12 months. Boxplot description: The horizontal line in the center of the box indicates the median, the boxes range represents the 50% dispersion of the data covered by the first and third quartiles, Non-overlapping of boxes indicates statistical difference according to Scott-Knott test ( $p < 0.05$ ), and the vertical lines characterize the tails; (x) Represent the mean; (o) Empty dots represent the values outliers. Regression description: green points represent the mean.

### *Vigor test*

### *Seedling length*

The overall average among the products tested, the control was superior at 5.4 cm to overall seedling length, followed by clotianidin at 4.8 cm, and thiamethoxam and imidacloprid

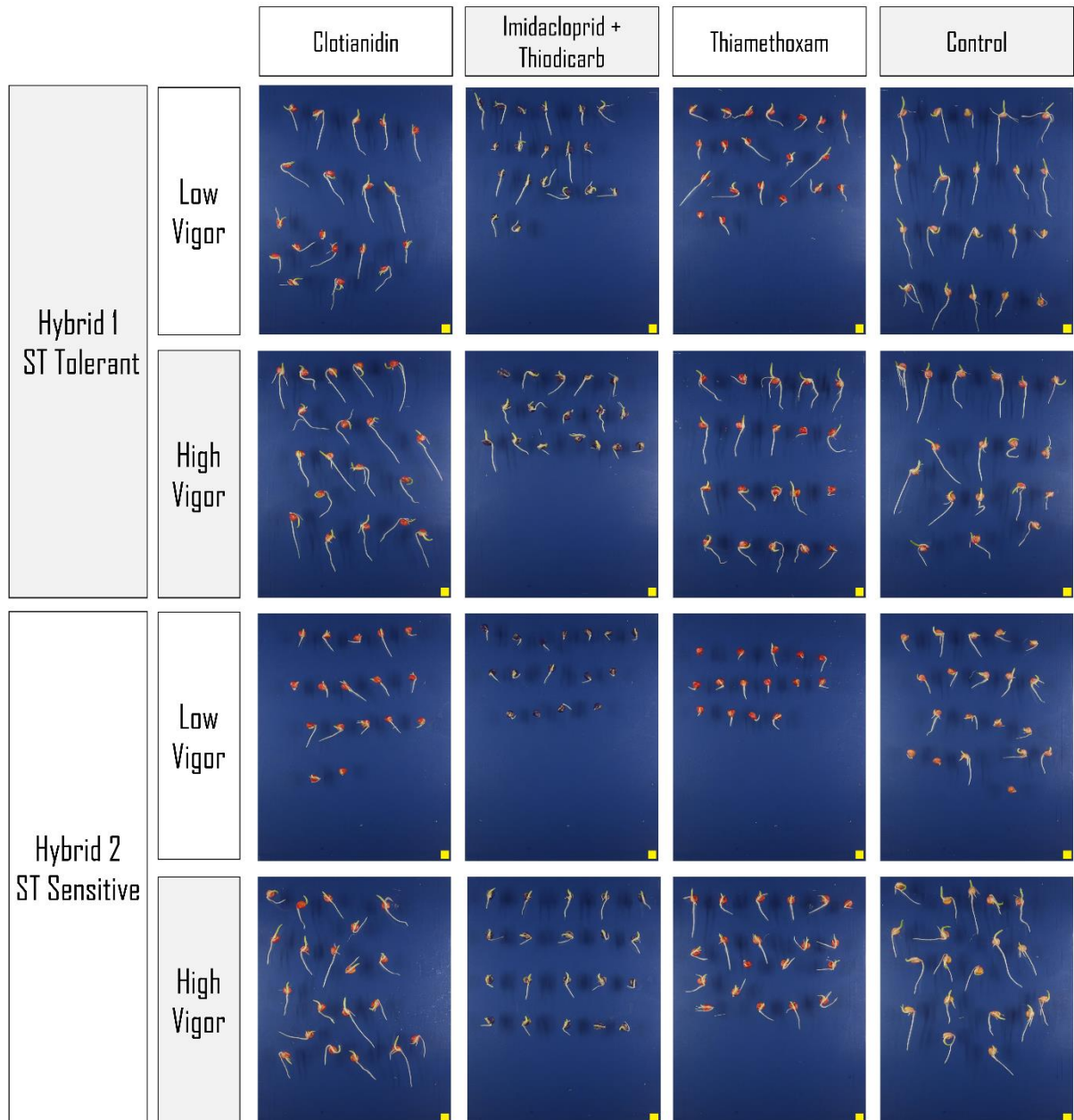
+ thiodicarb at 3.4 cm and 3.1 cm, respectively. In all lots, the tendency to group was similar but with different ranges of values, with superior control, followed by clothianidin, and thiamethoxam and imidacloprid + thiodicarb which did not differ (Figure 3a). For lots, the seedling length ranged from 1.1 cm to 6.6 cm, with a higher length for lot 1HV with an overall average of 5.3 cm, followed by 1LV and 2HV with 4.8 cm and 4.5 cm, respectively, and with the lowest overall average for lot 2LV with 2.0 cm. Throughout storage, all seed lots showed a decrease in seedling length (Figure 3b), as did the unfolding of the triple interaction, with different intensities depending on the insecticide molecule and the initial quality of the lot, demonstrated how vigor and tolerance to insecticide treatment affect seedling length throughout storage (Figure 3c). The combination of factors such as seed treatment tolerance level, initial vigor and neonicotinoid products can be seen in the images taken of seedling length a third day (Figure 4).



**Figure 3.** Seedling length (cm) of lots corn seeds with neonicotinoid insecticides and stored. A) Interaction of seed lots with neonicotinoid insecticide treatment. B) Interaction of seed lots during storage. C) Interaction of seed lots with neonicotinoid insecticide treatment during storage.

Lots 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, 2LV: hybrid sensitive to seed treatment and low vigor.

Products A: clothianidin, B: imidacloprid + thiodicarb, C: thiamethoxam and D: control, and storage periods: 0, 3, 6 and 12 months. Boxplot description: The horizontal line in the center of the box indicates the median, the boxes range represents the 50% dispersion of the data covered by the first and third quartiles, Non-overlapping of boxes indicates statistical difference according to Scott-Knott test ( $p < 0.05$ ), and the vertical lines characterize the tails; (x) Represent the mean; (o) Empty dots represent the values outliers. Regression description: green points represent the mean.



**Figure 4.** Capture of seedling length on the third day of lots corn seeds with neonicotinoid insecticides. Lots: hybrid tolerant to seed treatment and high vigor (1HV), hybrid tolerant to seed treatment and low vigor (1LV), hybrid sensitive to seed treatment and high vigor (2HV), hybrid sensitive to seed treatment and low vigor (2LV). Products: clothianidin, imidacloprid + thiodicarb, thiamethoxam and control. Yellow scale square 1cm<sup>2</sup>.

Huang et al. (2015) also investigating the effects of imidacloprid and thiamethoxam in seed treatments of *Brassica napus* on the early seedling characteristics and aphid control, found that the seedlings of seeds with neonicotinoid treatment showed significant anti-aphid control, however, the seedlings with imidacloprid as seeds treatment agent, showed a significantly decreased shoot/root ratio over at the late two-leaf stage, that control treatment. A reduction of

up to 2.5 cm in shoot length in maize seedlings was also observed in seed maize treated with thiamethoxam according to Battistus et al. (2014). A similar effect was also observed in soybean seeds treated with Imidacloprid + Thiodicarb, which showed a decrease in seedling length of 3.4 cm to 5.3 cm compared to seeds from the same untreated lot in the accelerated aging test (Oliveira et al., 2021). According to Carvalho et al. (2020), phytotoxicity caused by neonicotinoid insecticide treatment of seeds is evident in characteristics such as root length.

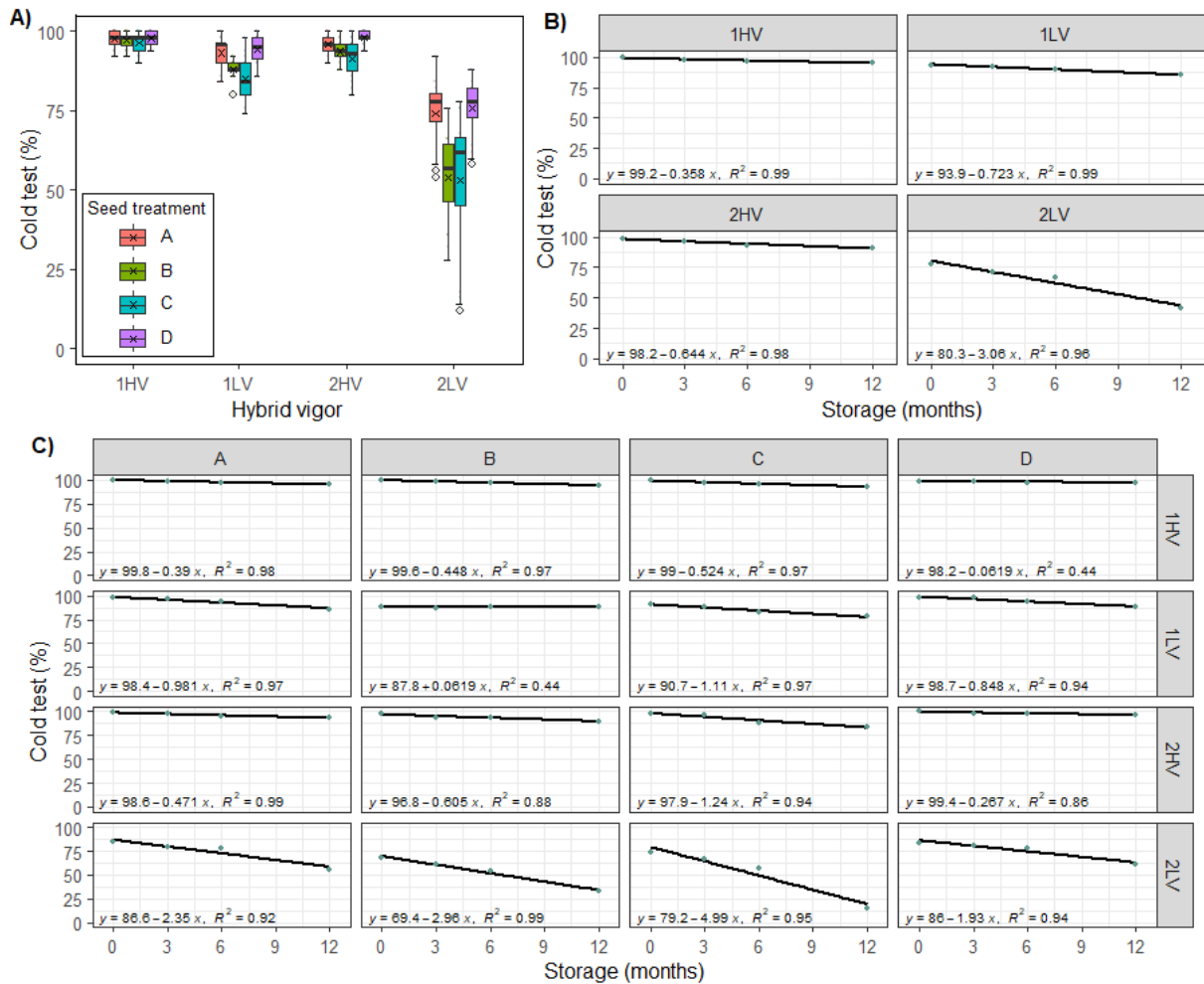
#### *Cold test*

The average cold test vigor ranged from 41% to 99%, with a higher vigor for lots 1HV and 2HV with an overall average of 97% and 95%, followed by 1LV at 90%, and with the lowest overall average for lot 2LV with 64%. In the tolerant high initial vigor lot 1HV and the sensitive high initial vigor lot 2HV there was no difference between insecticide treatments, which is the same as the control (Figure 5a). In general, when there was a difference between seed treatments, it was either in low vigor lots and/or in lots with seeds of a genotype sensitive to the treatment, for example, lot 2LV, where control and clothianidin were superior, followed by thiamethoxam and imidacloprid + thiodicarb (Figure 5a).

With an initial vigor of 99% and a reduction of 0.359 per month or 4.3 percentage points during the 12 months of storage, lot 1HV proved to be the best lot, and lot 2HV with an initial vigor of 98% showed a reduction of 0.646 per month or 7.8 percentage points during storage. This indicates that initial vigor is relevant, but the sensitivity of the genotype must also be considered in the production process of treated seed, since even a lot with high initial vigor, but with sensitive to treatment and storage, show a more pronounced decrease in vigor over time, so this sensitivity must be studied and considered in breeding programs. The factor that becomes more apparent in lots with medium/low initial vigor, where lot 1LV with an initial vigor of 94%, showing a reduction of 0.719 per month or 8.4 percentage points during storage,

and with worse performance and higher deterioration, lot 2LV with 80% initial vigor and reduction of 3.06 per month or 36.5 percentage points during storage (Figure 5b). The most deleterious effect was especially more evident in the 2LV hybrid on the effect of imidacloprid + thiodicarb and thiamethoxan treatment (Figure 5c).

Kuhar et al. (2002) also observed the effect of imidacloprid seed treatment on vigor through the cold test of high and low vigor (aged seed) lots of sweet corn varieties, a decrease of 4 to 7 percentage points in cold test in seeds treated with imidacloprid compared to untreated seeds. A reduction of 9 percentage points in low vigor lots was observed in seeds treated with thiamethoxan for untreated seeds, while in high vigor lots there was a reduction of 2 percentage points in thiamethoxan treated and untreated seeds (Egli & Rucker, 2012). Studies such as Oliveira et al. (2020) also showed a reduction in vigor in the cold test of 7 to 19 percentage points in corn hybrids more susceptible to treatment with thiamethoxam stored for 6 to 9 months, compared with seeds untreated with insecticide or most tolerant hybrid in the same period, the hybrid most sensitive to treatment with the neonicotinoid insecticides clothianidin and thiamethoxam showed reduced expression of  $\alpha$ -amylase, superoxide dismutase, and catalase enzymes after nine months of storage. Therefore, genotypes that is more sensitive to seed treatment when treated with neonicotinoids and stored has a biochemical imbalance that contributes to more pronounced deterioration and loss of vigor. Such studies of corn seed vigor based on the cold test are relevant because of its high relationship with field emergence and yield (Baldini et al., 2018; Reis et al., 2022).



**Figure 5.** Cold test (%) on soil substrate of lots corn seeds with neonicotinoid insecticides and stored. A) Interaction of seed lots with neonicotinoid insecticide treatment. B) Interaction of seed lots during storage. C) Interaction of seed lots with neonicotinoid insecticide treatment during storage.

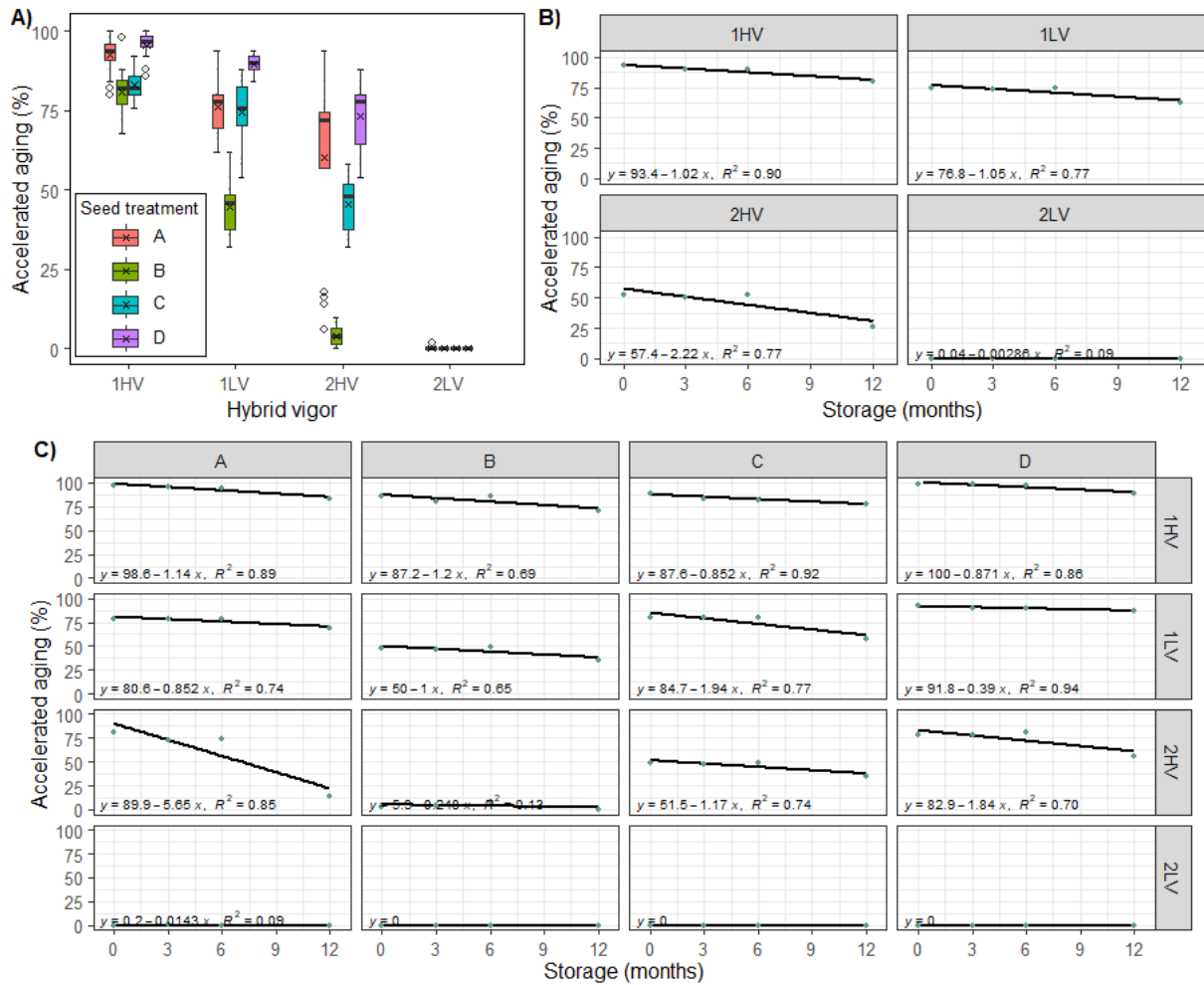
Lots 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, 2LV: hybrid sensitive to seed treatment and low vigor.

Products A: clothianidin, B: imidacloprid + thiodicarb, C: thiamethoxam and D: control, and storage periods: 0, 3, 6 and 12 months. Boxplot description: The horizontal line in the center of the box indicates the median, the boxes range represents the 50% dispersion of the data covered by the first and third quartiles, Non-overlapping of boxes indicates statistical difference according to Scott-Knott test ( $p < 0.05$ ), and the vertical lines characterize the tails; (x) Represent the mean; (o) Empty dots represent the values outliers. Regression description: green points represent the mean.

#### *Accelerated aging*

The average accelerated aging ranged showed the greatest variability, from 0% to 96%, the accelerated aging test proved to be the most powerful in distinguish the physiological potential of the lots, demonstrating Imidacloprid + Thiodicarb to be the most harmful,

phytotoxic (Figure 7a, c), however, many null or very low values were found, which can often compromise statistical evaluations and technical inferences. As well as genotype 2, in which both the 2HV lot showed low values since the beginning of storage, and the 2LV lot, the most sensitive to the test, with an average vigor of 0% since the first evaluation period (Figure 7b), making it inferior to this genotype, in addition to being sensitive to treatment and storage and also sensitive to the stress conditions used in this test, high temperature and high moisture. With the aggravating factor of germination being carried out after aging in a paper roll. Accelerated aging was efficient to segregate vigor levels of hybrid maize seeds Andrade et al. (2019). Moraes et al. (2022) identified a reduction in vigor through the accelerated aging test in hybrids treated with Clothianidin compared to the control, without insecticide. A similar effect was also observed in soybean seeds treated with Imidacloprid + Thiodicarb by Oliveira et al. (2021), which showed a decrease of 23 percentage points in the accelerated aging test compared to seeds from the same untreated lot.



**Figure 6.** Accelerated aging (%) on paper substrate of lots corn seeds with neonicotinoid insecticides and stored. A) Interaction of seed lots with neonicotinoid insecticide treatment. B) Interaction of seed lots during storage. C) Interaction of seed lots with neonicotinoid insecticide treatment during storage.

Lots 1HV: hybrid tolerant to seed treatment and high vigor, 1LV: hybrid tolerant to seed treatment and low vigor, 2HV: hybrid sensitive to seed treatment and high vigor, 2LV: hybrid sensitive to seed treatment and low vigor.

Products A: clothianidin, B: imidacloprid + thiodicarb, C: thiamethoxam and D: control, and storage periods: 0, 3, 6 and 12 months. Boxplot description: The horizontal line in the center of the box indicates the median, the boxes range represents the 50% dispersion of the data covered by the first and third quartiles, Non-overlapping of boxes indicates statistical difference according to Scott-Knott test ( $p < 0.05$ ), and the vertical lines characterize the tails; (x) Represent the mean; (o) Empty dots represent the values outliers. Regression description: green points represent the mean.

Similar behavior to that observed in the germination tests were also identified in the vigor tests with different intensities depending on the test method seedling length, cold test and accelerated aging. The high vigor lots with superior performance, followed by the 1LV and with much lower performance the ST sensitive and low vigor, 2LV. In addition to initial vigor,

the genotypes sensitivities are directly linked to deterioration after insecticide treatment and storage. And again, as observed in germination, the controls, without insecticide treatment with the best performance, followed by the treatment with Clothianidin, and with the worst performance and variability, the lots subjected to the treatment Imidacloprid + Thiodicarb and Thiamethoxan, mainly for 2LV. It is also important to highlight that through the unfolding of the triple interaction, low vigor lots presented to be more sensitive to insecticide treatment, especially under treatment with the molecules Imidacloprid + Thiodicarb and Thiamethoxan.

Despite the proven efficacy of neonicotinoids in seed treatment for pest control, studies indicate greater toxicity of Imidacloprid than thiamethoxam to control *Bemisia tabaci* (Naveed et al., 2010), which may be an indication that this greater toxicity may extend to the seed, affecting the vigor of seeds treated and stored with Imidacloprid.

This may be explained by the fact that Imidacloprid molecules was the first neonicotinoid seed treatment insecticide used commercially to protect seeds and seedlings (Pike et al., 1993), and thiamethoxam is a second-generation neonicotinoid belonging to the thianicotinyl subclass of nicotinoid chemistry with replacement by 2-chloro-5-thiazolyl moiety provides strong increase in activity against chewing insects, and introduction of a methyl group substituent increased activity against sucking pests (Maienfisch et al., 2001; Sâmia et al., 2018). This helps to understand why these two molecules exhibited greater phytotoxicity in our study due to their older, broad-spectrum systemic nature.

While the clothianidin the most recent of the neonicotinoids tested in our study, following the premise of developing new highly effective and more selective molecules is a trend in the current market. As reported by Rocha et al. (2020), the selectivity and low toxicity of insecticides for the physiological quality of stored seeds is an important characteristic of the products targeted for seed treatment. As shown in studies of vegetative growth, final plant densities, dry matter, and yield indicate that clothianidin seed treatments have no phytotoxic

effect on corn (Cox et al., 2007), which corroborates the data observed in our work of lower phytotoxic effect on seed lots subjected to treatment with the insecticide clothianidin.

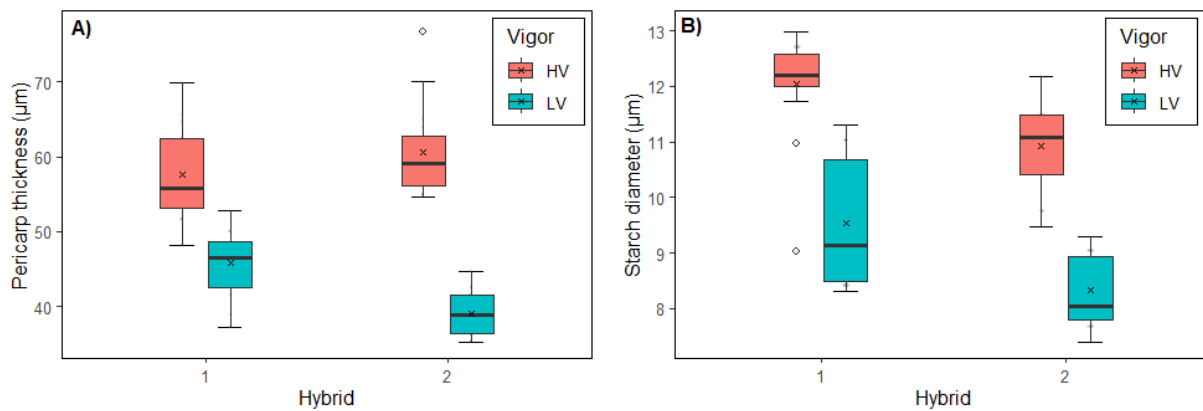
In addition to changes in physiological performance, studies reports the physiological and biochemical stress induced by the neonicotinoid insecticide imidacloprid in rice seeds (*Oryza sativa*) by Sharma et al. (2013) showed that imidacloprid treatment resulted in an adverse effect on seedling growth, with a halving of shoot and root length and biomass, a decrease in seedling protein content, while an increase in malondialdehyde (MDA) and proline content was detected at the highest concentrations of imidacloprid. According to Xiong et al. (2007) and Yang et al. (2018) the decrease in protein content is often associated with protein degradation due to increased activity of proteases under stress conditions, and proline accumulation under stress acts as an osmoprotectant and helps stabilize enzymes and membrane structures (Hosseinifard et al., 2022), MDA is the end product of lipid peroxidation serves as an indicator of the level of oxidative damage in plant tissues (Wang et al., 2011; Parveen et al., 2019), under imidacloprid treatment indicate induced membrane damage in the cell. Still according to Sharma et al. (2013) the activities of antioxidant enzymes such as increased superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) also increased after imidacloprid treatment revealing its phytotoxic effects induced oxidative stress.

### **3.3 Morphoanatomical traits**

The investigation of lots corn hybrids with different levels of tolerance to insecticide seed treatment and initial vigor showed an effect on the morphoanatomical characteristics analyzed, but the application of seed treatment did not affect them, which was already expected given that the formation of tissues-organ and seed vigor are constituted during the development and formation of seeds in the field and are related to environmental conditions and lines genetics

(Finch-Savage & Bassel, 2015; Marcos-Filho, 2015), and not in seed treatment stage at the post-harvest.

Thus, in our study it was possible to identify that there was a difference between hybrids and seed vigor for the thickness of the pericarp, lots 1 and 2 in high vigor, presented greater thickness, 1HV 58  $\mu\text{m}$  and 2HV 60  $\mu\text{m}$ , being statistically similar, however, in lots 1 and 2 of low vigor, a difference was identified, with 1LV a thickness 46  $\mu\text{m}$  higher than compared to 2LV with 39  $\mu\text{m}$  (Figure 7a), and the thickness of the pericarp could therefore represent a possible physical barrier to direct contact of the other internal structures such as the endosperm and especially the embryo during seed treatment with agricultural pesticides, thus helping to maintain the physiological quality of the lots. Confirmation that the selection of hybrids and lots of high initial quality are fundamental for the good establishment of seed treatment with insecticides of phytotoxic potential.



**Figure 7.** Means followed without overlap of first and third quartiles, box plot, there is a difference between high and low vigor and/or hybrid with and without tolerance to seed treatment by test ( $p < 0.05$ ), and Scott-Knott mean cluster test ( $p < 0.05$ ). A) Pericarp thickness, B) Starch granule diameter. 1: Hybrid 1 – ST tolerant, 2: Hybrid 2 – ST sensitive, HV: High vigor, LV: Low vigor.

Boxplot description: The horizontal line in the center of the box indicates the median, the boxes range represents the 50% dispersion of the data covered by the first and third quartiles, Non-overlapping of boxes indicates statistical difference according to Scott-Knott test ( $p < 0.05$ ), and the vertical lines characterize the tails; (x) Represent the mean; (o) Empty dots represent the values outliers.

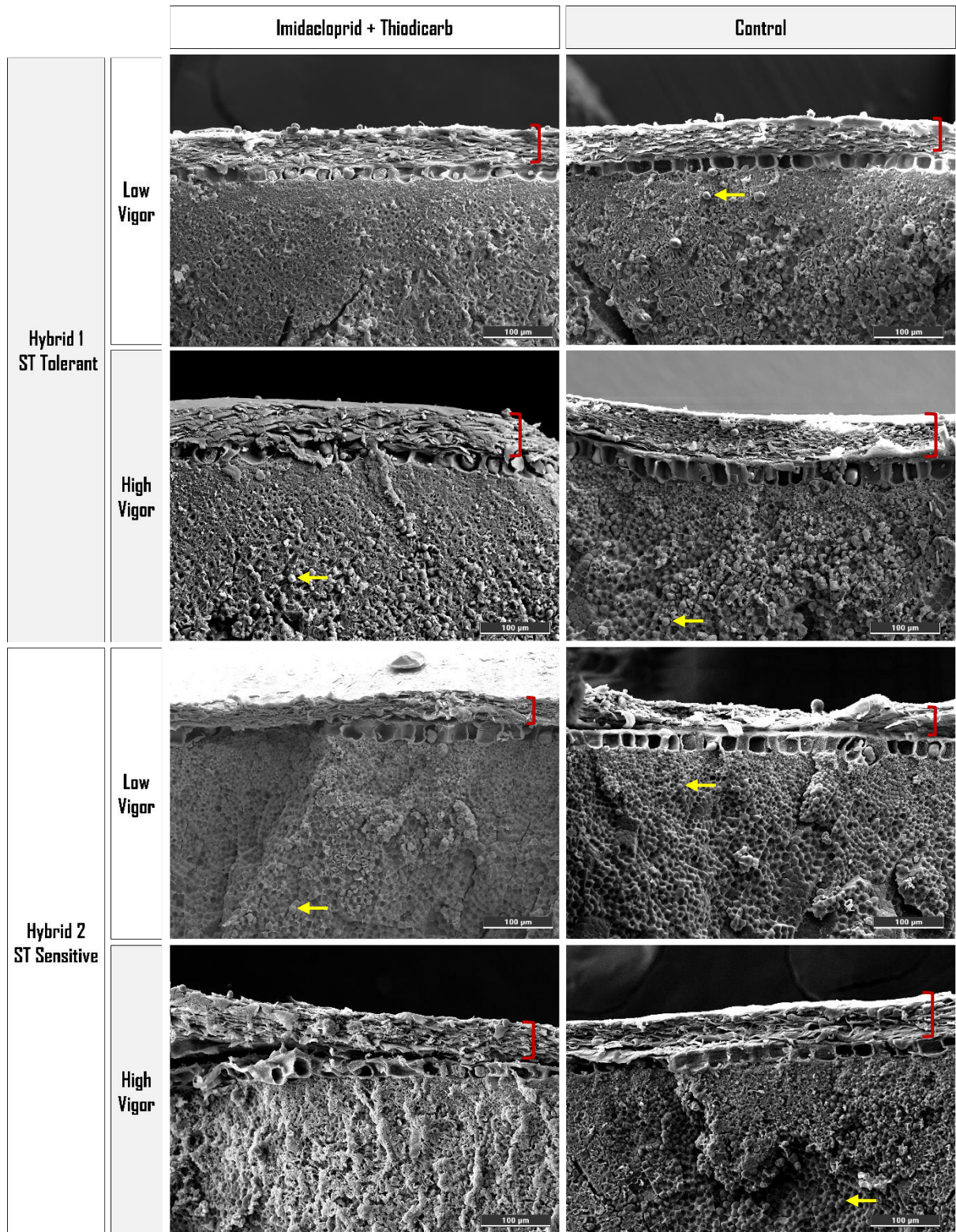
The mature ovary wall, the pericarp, is 100% female and subdivided into epicarp, mesocarp, and endocarp, and these tissues are conformed by fibrous cells rich in

phytochemicals, as mesocarp consists of cross and tube cells that play a crucial role for water distribution within the kernel, in addition, more important function the pericarp is barriers against external biotic agents, protects the germ and the endosperm (Scanlon & Takacs, 2009; García-Lara et al., 2019). Therefore, studies on the identification and development of female progeny with thicker pericarp should be encouraged. As shown by José et al. (2005) found difference in corn seed tolerance to high drying temperatures in seeds from simple hybrids and their reciprocal simple hybrids related pericarp structure. Costa et al. (2021) also report on maternal effect, parental line, in physiological quality in corn seeds for tolerance to delay drying and temperature.

Another important characteristic identified in our study through scanning electron microscopy analysis in addition to thickness was an apparent largest organizational structure, resistance and integrity of the membrane of high vigor lots compared to low vigor ones as observed in Figure 8. The thickness of the pericarp depends on the maize variety (Singh et al., 2013), and according to Xiong et al. (2022), during kernel development, hundreds of genes are transcribed and the pericarp cells stop growing, a process that involves programmed cell death, the volume remains unchanged, and the cells compress against each other and become more densely arranged, thickening the pericarp.

A similar result to our study was found by Salvador-Reyes et al. (2021), in morphological characteristics in Peruvian Andean corn varieties, found pericarp thicknesses ranging from 31.0  $\mu\text{m}$  to 96.8  $\mu\text{m}$ . Freire et al. (2020) investigating the micromorphology in popcorn progenies identified progenies with low popping expansion volume showed a less compact endosperm and pericarp thickness between 40.94 and 38.99  $\mu\text{m}$ , in contrast, progenies with high popping expansion volume a vitreous endosperm, more-compact starch granules without empty spaces between and pericarp thickness between 107.6 and 107.8  $\mu\text{m}$ . This

demonstrates that this structure influences the permeability and physical properties of the kernel.



**Figure 8.** Scanning electron micrographs of the pericarp and endosperm in a longitudinal section of the upper region, in corn seeds with and without tolerance to insecticide treatment, high and low vigor. Left side seeds treated with imidacloprid + thiodicarb, right side seeds

without treatment with neonicotinoid insecticide, control. Bars = 100  $\mu\text{m}$ . Red line indicate pericarp, and yellow arrows indicate starch granules.

The diameter of the starch granules varied from 8.3  $\mu\text{m}$  to 12.0  $\mu\text{m}$ , there was no interaction between hybrids and vigor, but a difference was observed between the isolated factors, the hybrids 1 with 10.7  $\mu\text{m}$  starch diameter was higher, than hybrid 2 with 9.6  $\mu\text{m}$ , as well as the high vigor lot had an average of 11.5  $\mu\text{m}$  while the low vigor lots had an average of 8.9  $\mu\text{m}$  (Figure 7b).

Our results are consistent with those reported by Penfield and Campbell (1990), where normal corn has starch granules with diameters range from 5 to 25  $\mu\text{m}$ , which can be classified by size into small granules, range from 1 to 7  $\mu\text{m}$ , and large granules, from 15 to 20  $\mu\text{m}$  (Singh et al., 2013). Furthermore, the physicochemical and morphological properties of normal, waxy, and high amylose corn starch granules differ and influence the functional properties for food use (Weber et al., 2009). However, their association with seed quality is still scarce.

The starch granules present in the endosperm are essential for germination, where through the hydrolysis of the bonds of starch molecules, amylose and amylopectin polysaccharides are converted into smaller molecules by the action of  $\alpha$ -amylase producing dextrose and  $\beta$ -amylase producing maltose, essential for energy supply in the germination process and initial growth of seedlings (Tester et al., 2004). As pointed out by Andrade et al. (2019), genotypes with higher seed reserve utilization efficiency, reserve mobilization rate to the seedling, have higher vigor, produce seedlings with higher dry matter and higher total length.

Thus, larger and well-formed starch granules may indicate adequate environmental and nutritional conditions during the seed formation phase, contributing to the production of vigorous seeds, since environmental conditions during starch granule formation and development have a direct influence on starch granule size, as pointed out by Li et al. (2007), The size of starch granules up to 14 days after pollination showed a diameter of 4-7  $\mu\text{m}$  to a

diameter of 10-23  $\mu\text{m}$ , 14 days after pollination, thus conditions how drought stress in post-pollination can affect the development of starch granules, compromising vigor, which helps explain the possible difference in starch diameter and vigor between lots of the same hybrid.

High vigor lots had larger starch diameters, which may be synonymous with superior development that contributes to vigor and storage potential. In addition to the greater thickness of the pericarp, should be studied as we agree it can serve as a physical protection mechanism against contact of tissues and internal organs with insecticidal molecules. Therefore, the assessment of thickness, formation, constitution and arrangement of morphoanatomical characteristics, as pericarp and starch granules can be used as important tools in investigating the vigor and tolerance of seeds treated with neonicotinoid insecticides.

#### **4. CONCLUSIONS**

The active ingredients imidacloprid + thiodicarb and thiamethoxam showed a greater phytotoxic effect, greater impact on germination and vigor, especially in the sensitive and low initial vigor lots.

The initial seed lot vigor affects tolerance to neonicotinoid insecticide treatment and storage, low vigor seed lots are more susceptible to deterioration.

Corn genotypes vary in their tolerance to neonicotinoid seed treatment and storage. The insecticide-tolerant and high vigor seed lots showed superior vigor performance and morphoanatomical development.

There is evidence of a relationship between ultrastructural analyses, such as greater pericarp thickness and starch granule size, with a trend toward greater vigor and storage tolerance of insecticide-treated seeds.

## ACKNOWLEDGEMENT

To the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), e à *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Capes) for the scholarships granted, scientific initiation, postgraduate studies and research productivity (CNPq) and financial support.

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### **ARTIGO 3 - Evolution Over the Last 50-years of Quality and Technology of Hybrid Corn Seeds in Establishment and Yield in the Ohio Corn Performance Test (OCPT)**

(Standards according to the *Crop Science*)

#### **Core Ideas**

- The OCPT tested more than 52,000 hybrid combinations (hybrids × environments × years) across Ohio over 50 years.
- Grain yields increased by 0.134 Mg ha<sup>-1</sup> year<sup>-1</sup>, with a mean yield of 7.91 Mg ha<sup>-1</sup> in 1972 and 14.6 Mg ha<sup>-1</sup> in 2021.
- Seeding emergence, seeding rate and final plant stands showed the strong correlations with yield ( $r = .64$ ,  $r = .81$  and  $r = .82$ , respectively).

#### **ABSTRACT**

The Ohio Corn Performance Test (OCPT) aims to provide information to aid growers in making decisions for hybrid selection and increase overall yield. For more than 50 years the OCPT has evaluated the agronomic performance of commercially available corn hybrids across the state. The objective of this study was to highlight trends of seed technologies and quality as they relate to agronomic performance and grain yields. The program has been sowed annually at about ten distinct sites grouped in three regions: Southwest/West Central Region, Northwest Region, and North Central/Northeast Region. Data collection and measurements include seedling emergence percentages, seeding rate, final plant stands and grain yields. Over the the last 50 years, more than 52,000 hybrid combinations (i.e., hybrids × environments × years) were tested in the OCPT. From 1972 to 2021, seedling emergence increased from 86.3% to 94.9% (10.3% increase), and plant populations increased from 52,181 to 86,872 plants per hectare (66% increase). The same period saw a grain yield gain of 0.134 Mg ha<sup>-1</sup> year<sup>-1</sup> (an 84.5% increase), with a mean yield of 7.91 Mg ha<sup>-1</sup> in 1972 and a mean yield of 14.6 Mg ha<sup>-1</sup> in 2021.

Seedling emergence percentage and final plant stands showed a strong (64%) or very strong (82%) correlation with yield across the 50 years of the trials. Improvements higher per ha<sup>-1</sup> for OCPT in yield were observed, compared to the average for the state of Ohio. By quantifying agronomic differences between commercially available corn hybrids, annual OCPT results have contributed to the selection of adapted and high-yielding hybrids across the state. This study indicates that yield improvements in the OCPT test have been achieved through synergistic responses to new genotypes, technologies seeds, and management practices.

## 1. INTRODUCTION

Corn (*Zea mays* L.) is a world's widely grown cereal crop, due to its high production potential and wide adaptability (Mohammed et al., 2023). The United States (US) represents about 32% of worldwide production, being the largest corn producing country with 383.5 M Mg. Historically, Ohio has been one of the eastern Corn Belt corn-producing states due to its edaphoclimatic conditions, currently, Ohio is the 10th highest corn producer state and accounts for about 4% of production of the United States (USDA FAS, 2023). Corn uses are diverse, including for fuel, feed, fiber, food, and industrial purposes. With such diverse uses, corn is a critical commodity in the United States and global economy, and the second most produced crop (after soybean) grown on Ohio's farmed land.

Significant production changes have occurred over the last half-century. In the last 60 years, the most significant corn production improvements have been attributed to better hybrids, environmental changes, and improvements in agronomic management (Duvick, 2005; Hall et al., 2013; Cooper et al., 2020; Rizzo et al., 2022; Ortez et al., 2023b). Crop breeding programs have developed greater corn vigor associated with the development of single-cross hybrids (Beckett et al., 2017), hybrids that incorporate traits for improved stalk quality, earlier maturity and dry-down, resistance to herbicides, insects, and diseases, and improved abiotic stress

tolerance, nutrient use efficiency, and nutritional quality (Texeira et al., 2021; Yassitepe et al., 2021). These traits have been accompanied by the adoption of new management techniques and technologies; selective pesticides, soil conservation practices, diversified production systems, higher seeding rates, and improved seed treatments (Lahm et al., 2009; Fromme, et al., 2019; Medeiros et al., 2023; Ortez et al., 2022a; Peixoto et al., 2020).

The study of hybrid adaptation to varying conditions has played a pivotal role in recent yield gains (Lana et al., 2017). As observed by Zhou et al. (2022) tested hybrids in different environments allow decision-makers to select the most adapted and productive genotypes that drive crop productivity and profitability across all regions. Duvick et al. (2004) explored a historical series of improvements from 1991 to 2001 also documented an increase of 0.077 Mg year<sup>-1</sup> in corn productivity in Iowa, United States.

The Ohio Corn Performance Test (OCPT) started in 1972 to test commercially available hybrids across multiple, regionally representative in terms of environmental and soil characteristics, in Ohio locations (Minyo et al., 2022). The main goal of this annual project has been to provide regionally specific hybrid agronomic performance information to farmers, educators, agronomists, and seed companies. Results of the OCPT program aid stakeholders in selecting hybrids or genotypes adapted to Ohio's variable environments. However, an historical narrative built upon a summary of these emerging and developing characteristics has yet to be leveraged to assess how corn production practices, agronomic characteristics, and grain yield metrics have changed since the project began. The objective of this study was to highlight overall trends as they relate to agronomic performance and crop management and how these factors have contributed to the gains obtained in grain yields in the state of Ohio over the last 50 years.

## 2. MATERIALS AND METHODS

### 2.1 Experimental sites and design

This work has been achieved through public and private partnerships between Ohio State University, seed companies, and cooperating farmers. Each company submitting hybrids for the test has been responsible for providing seeds and their recommended seeding rates. The OCPT program has gathered corn hybrid performance data from 1972 to 2021. Altogether, this dataset accounts for a wide range of genetics, spatial, and temporal conditions on the 50 years of test in Ohio.

The test is normally conducted at nine to eleven locations per year. The sites depend on the year and the conditions. Fertilizers, herbicides, insecticides, and fungicides are applied according to the recommended cultural practices for obtaining optimum grain yields at each location. Each plot consisted of four 0.76 m rows and each row was 7.6 m long, adding to about 23.1 m<sup>2</sup> per plot. Each hybrid was replicated three times at each site in a randomized complete block design.

**Table 1.** Regions and counties in the state of Ohio where the Ohio Corn Performance Test was conducted from 1972 to 2021.

Region*	County
Central	Licking
Northcentral	Marion Morrow Huron
Northeast	Portage Mahoning Columbiana Coshocton Wayne
Northwest	Wood Wyandot Van Wert Hancock
Southcentral	Scioto
Southwest	Darke Clark Fayette

## 2.2 Data Collection

The number of *hybrid entries*, *companies*, and genetically modified, *transgenic traits* (when applicable) tested within the OCPT program from 1972 to 2021 were collected.

The recommended *seeding rates* of hybrids was provided by seeds companies.

*Plant emergence*: the percentage of plant emergence was calculated as a ratio of the number of seeds planted and number of plants that emerged, and the results are reported as a percentage.

*Final plant stands*: determined by counting the number of viable plants at the V3 to V7 stages, with 3 to 7 developed-collared leaves (Abendroth et al., 2011). Final plant stands are reported as plants per hectare.

*Grain yield*: determined from the two center rows in each plot (about 11.5 m<sup>2</sup> area) using a small plot research combine. Grain yield was reported in megagrams per hectare at a standard grain moisture content of 155 g kg<sup>-1</sup>.

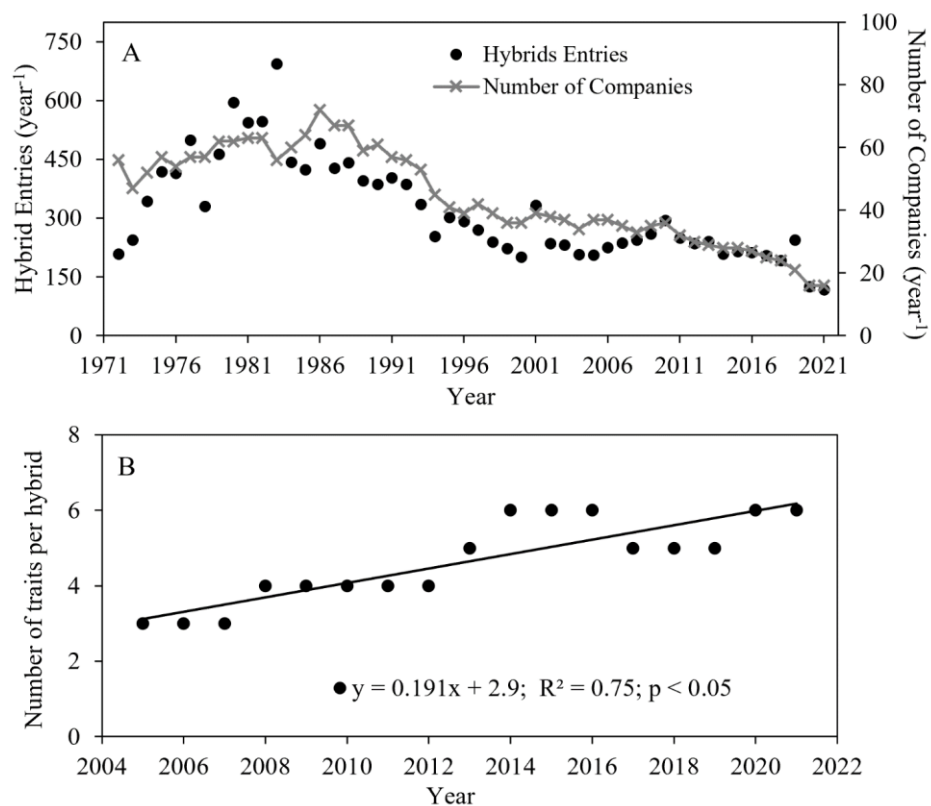
## 2.3 Data Analyses

Data for agronomic characteristics was based on the averages of the hybrids at all locations tested in the year. The historical parameters were analyzed using year as the fixed effect in a linear regression model to determine significance (alpha = .05).  $R^2$  and significance levels were included in the plot series. The annual averages of the agronomic variables also were subjected to Pearson's linear correlation matrix employing the correlogram method ( $p < 0.05$ ), in the R package "Hmisc" (Harrell, 2023). The analyses were performed using R 4.0 software (RStudio Team, 2020).

### 3. RESULTS AND DISCUSSION

#### 3.1 Hybrid Entries, Companies, and Traits

Given changes in partnerships, mergers, company acquisitions, and developing seed industry trends, the number of companies and hybrids participating in the OCPT has varied over the years. The late 1980s saw the beginning of a trending decrease in number of companies and hybrids entered in the trials (Figure 1A), fact that may be related to mergers and acquisitions in the sector, which intensified after 2010.



**Figure 1.** The left axis of the upper graph (A) represents the number of hybrid entries per year, and the right axis represents the number of companies submitting hybrid entries in the Ohio Corn Performance Test (OCPT) from 1972 to 2021. The lower graph (B) shows maximum number of transgenic traits tested in a single hybrid in the OCPT from 2005 to 2021.

From 1972-2021, an average of 319 hybrids, representing 44 companies, were tested each year. This averages to approximately seven hybrids per company per year. Since the program's inception, 9,119 unique hybrid names were tested (many of which were tested across multiple years), and a total of 234 companies participated in the trials. In total, more than 52,000 hybrid

combinations (hybrids × environments × years) were conducted across all sites and years. The greatest number of entries for one year was recorded in 1983, with 694 hybrid entries. The greatest seed company participation occurred in 1986, with 72 companies represented. By 2021, the number of entries had reduced to 118 hybrids representing only 16 seed companies. According to Clapp (2018), the corporate concentration among agribusiness results has resulted in a reduction in, and market-share dominance by a smaller number of companies. OCPT submission data represents these dynamics, with fewer hybrids and companies participating in more recent trials as compared to prior decades. The reduction in the number of seed companies and hybrids participating in crop performance tests has posed a leading challenge for the OCPT and similar crop performance programs across other United States universities. As a result, and over the past two decades, several comparable university-led performance programs have ceased activities, and third-party site-specific information is no longer available for agronomist and producer consideration in those regions. This can make hybrid selection, especially regionally-specific performance decisions, more difficult.

According to OCPT records, before 1995, none of the tested hybrids contained transgenic materials (data not shown). By 2018, 95% of the corn planted in the OCPT was transgenic. The first transgenic or Genetically Modified (GM) hybrids, both Bt hybrids with European Corn Borer (ECB) resistance, were entered in the OCPT in 1996.

Though the extent of trait “stacking” has fluctuated, the total number of transgenic traits per hybrid as increased over time (Figure 1B). Beginning in 2014 through 2016, up to 6 traits were available in the same hybrid. From 2017 to 2019, the maximum number of stacked traits per hybrid decreased slightly to five, but returned again to six traits in years 2020 and 2021. In recent years (2013 and later), most hybrids in the test had four to six transgenic traits (Figure 1B). Crop production challenges, developing technologies, and market-force drivers will determine future trends in cultivated hybrids. Given the continuing evolution of novel

technologies and hybrids, there is an ongoing need for agronomic performance testing services, like those offered by the OCPT, to test these traits in hybrids for their regional adaptability and site-specific selection.

Traits represented by OCPT-trialed hybrids (Table 2) have primarily included insecticidal proteins against corn borers (Cry1Ab or Cry1A.105 and Cry2Ab2) and corn rootworms (Cry3Bb1 from *Bacillus thuringiensis*) (Siebert et al., 2012; DiFonzo & Porter, 2023), tolerance to glyphosate (5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp.) (Achary et al., 2020) and glufosinate herbicides (*Streptomyces* spp originating enzymes that detoxify l-phosphinothricin) (Takano and Dayan, 2020; DiFonzo and Porter, 2023), and season-long drought tolerance derived from multiple genes (Messina et al., 2022a) (Table 2).

**Table 2.** Summary of transgenic traits identified across all test combinations (hybrids × sites × years) from 2005 to 2021 in the Ohio Corn Performance Test (OCPT).

Transgenic Trait	Frequency	Percentage
Drought tolerant	451	0.9%
Glufosinate tolerant <sup>1</sup>	7,253	14.2%
Rootworm <sup>2</sup>	9,232	18.1%
Glyphosate tolerant <sup>3</sup>	15,579	30.6%
Corn borer <sup>4</sup>	18,415	36.2%
<b>Total</b>	<b>50,930</b>	

<sup>1</sup>Bacterium *Agrobacterium tumefaciens* encodes the ammonium glufosinate acetyltransferase enzyme (GAT).

<sup>2</sup>Bacterium *Bacillus thuringiensis* encodes Cry (Cry3Bb1 and/or Cry34/35Ab1) proteins.

<sup>3</sup>Bacterium *Agrobacterium tumefaciens* encodes the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSPS).

<sup>4</sup>Bacterium *Bacillus thuringiensis* encodes Cry (Cry1Ab, Cry2Ab2, Cry3Bb1, Cry1A.105, Cry1F) and/or Vip3Aa20 proteins.

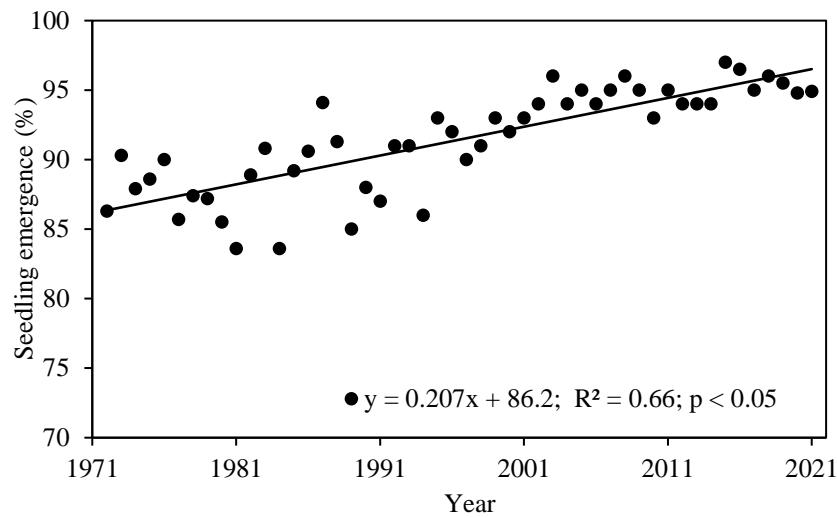
A revolutionary milestone in North American corn production was the introduction of genes derived from *Bacillus thuringiensis* spp. (Bt) that improved crop yield mainly in areas of high incidence of European corn borer (ECB) (Lauer & Wedberg, 1999). This development was quickly followed by the release of Roundup Ready corn in the 1990's. The development ushered in an era of hybrids resistant to the herbicide glyphosate (Padgett et al., 1996; Franz et al., 1997). The proliferation of these technologies, as well as ongoing advancements in corn

breeding likely played pivotal roles in overall yield increases (Figure 1B and Table 2). Research has shown that advances in corn breeding and hybridization, including advances relating to drought tolerance, standability, above and below-ear leaf angle ratios, increased leaf area duration, photosynthetic capacity at maturity, biomass accumulation, and harvest index have all greatly contributed to higher corn yields (Duvick, 2005; Edgerton et al., 2012; Liu et al., 2021).

The development and use of transgenic hybrids and the introduction of novel genetic traits in corn have conferred resistance to major lepidopteran and coleopteran insect pests and provided tolerance to herbicides and drought, which, when combined, have contributed to the mitigation of yield-limiting factors and increased overall yield and stability (Achary et al., 2020; Cooper et al., 2014; Siebert et al., 2012; Takano & Dayan, 2020). There is an ongoing demand for new transgenic traits, as negligent use, the evolution and developed resistance of targeted factors (i.e., weeds, insects), leading to loss of crop resistance and selection pressure added to weather variability (Mason et al., 2018).

### **3.2 Seedling Emergence and Seed Treatment**

Seedling emergence increased by 0.2 percentage points per year, from 86% in 1972 to 97% in 2021 (Figure 2). Research has shown the vital role that high physiological quality, genetic potential, phytosanitary and physical quality, and improvements in pre- and post-harvest seed handling play in obtaining productive and healthy crops (Marcos-Filho et al., 2015). Productivity components such as plant emergence directly correlate with the achievement of optimum plant stands under unfavorable environmental conditions (Ebone et al., 2020). These findings have been corroborated by results from the OCPT.



**Figure 2.** Percent changes in seedling emergence for the Ohio Corn Performance Test (OCPT) from 1972 to 2021.

The use of seeds with better emergence and vigor is important as it provides fast and uniform emergence (Egli et al., 2012). Plant emergence can also affect plant height, leaf area index, dry matter accumulation, and grain yield by 4% to 8%, as plants adjacent to a late emerging corn plant do not exhibit compensatory growth in corn (Liu et al., 2004). The importance of high and uniform emergence is also demonstrated by Lawles et al. (2012), who found that variable and delayed corn emergence resulted in yield decrease ranging from 0.225 to 1.379 Mg ha<sup>-1</sup>. Thus, the percentage of seedling emergence is an important yield component.

Besides improved genetics, new technologies in seed treatment (Table 3), such as fungicides, insecticides, biologicals, and nematicides, have likely contributed to increased seedling emergence results, such as the products used in 2021 (Figure 2). Seed treatment is an important tool for protection against pests and pathogens during the vulnerable emergence phase (Pes et al., 2020; Medeiros et al., 2023). Elbert et al. (2008) found that imidacloprid, thiamethoxam, and clothianidin are the most important broad-spectrum chemical insecticides to control pests in corn seed. Similar results were observed for the active ingredient chemical group of insecticides currently used in the OCPT (Table 3). Another trend also observed in our

study, is the use of Insecticide/Nematicide for the seeds treatment of biological origin, with reduced toxicity and improved biodegradability (Vojvodić & Bažok, 2021).

Additionally, fungicides with the active ingredient metalaxyl, pyraclostrobin, fluxapyroxad, and erthaboxam are important tools for managing seedlings diseases caused by *Phytophthora*, *Pythium*, and *Phytophthora* in corn and soybeans in Ohio (Broders et al., 2007; Scott et al., 2020).

**Table 3.** Seed treatment used in 2021 at the Ohio Corn Performance Test (OCPT).

Seed Treatment	Commercial name	Active ingredient
Fungicide	Acceleron	Metalaxyl, fluxapyroxad and pyraclostrobin
	Acceleron Elite	Metalaxyl, fluxapyroxad, pyraclostrobin, and imidacloprid
	Apron	Metalaxyl and fluxapyroxad
	Avicta Complete	Abamectin, thiamethoxam, mefenoxam, and fludioxonil
	Encase	Cyclohexane
	Vibrance	Sedaxane
	LumiGEN	Ethaboxam, inpyrfluxam and metalaxyl
Insecticide /Nematicide	Vibrance Extreme	Sedaxane, difenoconazole and mefenoxam
	Avicta	Abamectin
	Cruiser 250 and 500	Thiamethoxam
	Poncho 500	Clothianidin
	Varnimo	<i>Bacillus amyloliquefaciens</i> strain PTA-4838
	Votivo	<i>Bacillus firmus</i> cepa I-1582
	Lumisure	Clothianidin
Lumialza	<i>Bacillus amyloliquefaciens</i> strain PTA-4838	

\*Source, OCPT 2021 (Minyo et al., 2021).

Combined with the improvement in seed quality over the years at the OCPT, new planting technologies, crop management, application of agrochemicals, fertilizers and harvesting for field trials were observed (Figure 3). As in the early years of the OCPT, planting was conducted using a commercial-type planter adapted for small plots. Since 2014, planting has been conducted using an Almaco Seed Pro 360 plot planter with high-precision count-down seeding and in row spacing, guided by a SkyTrip GPS (Figure 3, first row).

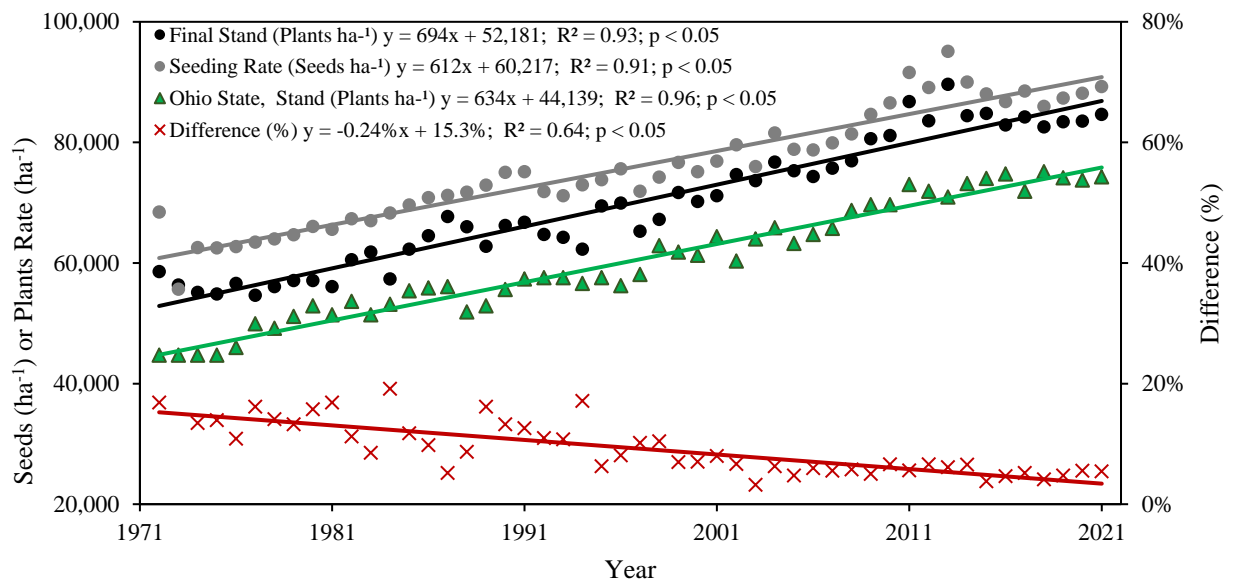


**Figure 3.** Planting (first row), crop management, agrochemical and fertilizer application technology, crop development (second and third row), pre-harvest, equipment/harvest for field trials (fourth row) for the Ohio Corn Performance Test (OCPT) in 2023. Source: Jhonata Cantuaria Medeiros, 2023

### 3.3 Seeding Rates and Plant Stands

In 1972, when the OCPT program was initiated, seeding rates and final stands were approximately 60,000 seeds per ha<sup>-1</sup> and 52,000 plants per ha<sup>-1</sup>, respectively. Over the years, the seeding rate the OCPT program increased at an annual rate of about 612 seeds and 694 plants per ha<sup>-1</sup>, reaching a maximum average of 90,826 seeds and 86,871 plants per hectare in the 2021. This represents 51% increase in seeding rate and 66% increase in plant stands, respectively (Figure 4). In addition to the increases in seeding rates and final plant stands, there has been a decline in the difference between the seeding rate and the final plant population,

decreasing from an average of 15.3% in 1972 to an average of only 3.4% in 2021 (Figure 4). The well crop development and establishment in the OCPT 2023 can be observed in Figure 3 (second and third row). These results corroborate the ongoing evolution of seed quality, and hybrid and seed treatment technologies, reflecting the ever-increasing emergence ratings (Figure 2).



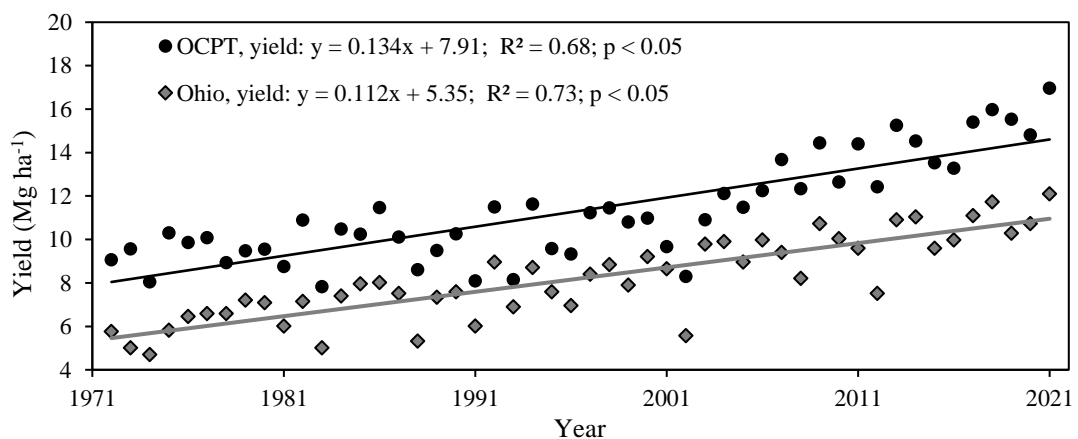
**Figure 4.** The left axis represents the seeding rate and final stand counts. The right axis represents the difference between the seeding rate and final stand expressed as a percentage for the Ohio Corn Performance Test (OCPT) and the state of Ohio (as reported by the United States Department of Agriculture, USDA) from 1972-2021.

Optimal plant stands are an important component contributing to overall productivity (Ortez et al., 2022b). Studies with plant densities ranging from 38,400 to 107,900 plants  $\text{ha}^{-1}$  saw maximal corn grain yields at  $\geq 81,700$  plants  $\text{ha}^{-1}$  for southern Minnesota (Van Roekel & Coulter, 2011). An adequate establishment of plants per unit of area is one of the primary and earliest-observable components that determines corn grain yield (Ortez et al., 2022b). Optimum plant populations, adequate growth and development, and favorable weather conditions are all necessary for successful crop establishment and to achieve high-yielding results (Abendroth et al., 2011). Given the nature of the trial, and given that the program follows the seed companies' recommendations for seeding rates of their new and high-performing hybrids, the higher

seeding rates reported in the OCPT were to be expected. A unique feature of the OCPT program is that companies can select (recommend) seeding rates for their hybrids entered in the test, and this capability has allowed them to explore hybrids phenotypic potentials at higher (desired) plant populations.

### 3.4 Grain Yields

Other notable changes were seen in improvements mean corn grain yields, in 1972 the OCPT were 9 Mg ha<sup>-1</sup>, whereas in 2021, mean corn yields were 17 Mg ha<sup>-1</sup>, an almost a 2-fold increase, and representing an annual increase of roughly 0.134 Mg ha<sup>-1</sup> according the regression model (Figure 5). Average corn yields for farmers in Ohio also increased from 5.35 to 10.95 Mg ha<sup>-1</sup>, or a 0.112 Mg ha<sup>-1</sup> year<sup>-1</sup> increase per year in 1972 to 2021, according to statistics reported by USDA NAS (2021, 2023). The difference between OCPT yield results and Ohio's reported results show the potential attainable yields for farmers in the state when utilizing similar hybrid, management, and environmental conditions.



**Figure 5.** Average grain yield in the Ohio Corn Performance Test (OCPT) from 1972 to 2021 (black dots). The average grain yield for Ohio from 1972 to 2021, as reported by the United States Department of Agriculture (USDA), is also included (gray diamond).

Historical US corn data (1956 to 2015) has shown significant nationwide increases in yield (0.117 Mg ha<sup>-1</sup> year<sup>-1</sup>), with seed derived from high heterosis in single-cross hybrids serving as the largest source of corn seed planted for commercial production (Beckett et al., 2017). Other

Midwestern studies have also shown increases in corn yields of around 0.15% to 0.30% year<sup>-1</sup> for 7 of the 12 states represented in the Corn Belt region (Bundy & Gensini, 2022). The investigation of genes contributing to agronomic performance in elite corn breeding has also been determined to be a critical factor in increasing corn grain yields in the United State (Beckett et al., 2017; White et al., 2020). Some of those advancements have included the introduction of transgenics and its overexpression, specific genes produce more traits of agronomic interest than their normal level, which have improved the growth of plant organs and the resilience of genotypes under stress conditions, providing better yields (Guo et al., 2014).

In our study, the direct relationship between aspects of seed quality and crop establishment was evidenced by the correlation test, seedling emergence, seeding rates, final plant stand showed a positive correlation of  $r = .64$ ,  $r = .81$  and  $r = .82$ , respectively with grain yield (Figure 6).

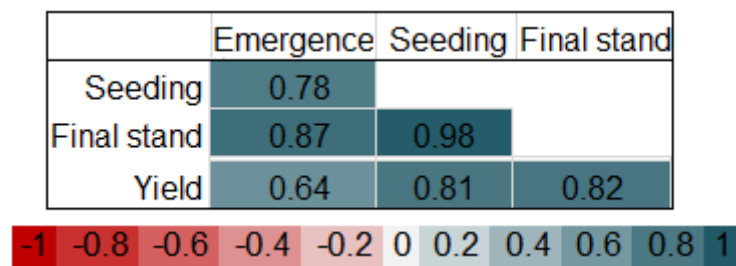


Figure 6. Pearson Correlation matrix (correlogram) between seedling emergence percentages, seeding rates, final plant stands and grain yield in Ohio Corn Performance Test, blue  $r$  values indicate positive correlations and red  $r$  values indicate negative correlations,  $p < 0.05$ . From 1972 to 2021 in the Ohio Corn Performance Test (OCPT).

When filtering the dataset, it was observed that the 50 highest-yield hybrids were obtained mostly in the last two years of trials, 2020 and 2021, with an average yield of 19.9 Mg ha<sup>-1</sup> or 316.9 bu ac<sup>-1</sup>, being 94% of the top productivity hybrids with an emergence rate  $\geq 95\%$  and average of 97%, and final stand average of 86.5 thousand plants per hectare (data not shown). Which emphasizes the relationship between seed quality to obtain ideal stand and high performance. TeKrony and Egli (1991) also found seed quality can influence aspects of

performance as total emergence and rate of emergence had a positive relationship with yield. According to TeKrony and Egli, the use of high-vigor planting seed can be justified to ensure adequate plant populations across the wide range of field conditions that occur during emergence. Like our results, Beruski et al. (2020) reported a relationship between plant population above 80,000 plants ha<sup>-1</sup> and best crop yields. As well as, research show non-uniform germination, emergence, and slower plant growth has often been associated with lower grain yields mainly in low precipitation and/or high temperatures environments (Boomsma et al., 2010). Reis et al. (2022) found that high-vigor corn seeds favor the initial development of the crop and promote greater soil cover, positively influencing the final stand population and increasing the number of grains per ear to promote greater grain yields. For each increase of 1.0 percentage point in seed vigor, there is an estimated increase in grain production translating to up to 0.0435 Mg ha<sup>-1</sup>.

Overall, as new genetics become available, management practices improve, and climate patterns shift, continued third-party testing of hybrids across regions remains an important task that serves farmers, practitioners, and seed companies when selecting hybrids well-suited to specific crop growing conditions across the state (Ortez et al., 2023b).

The main goal of the OCPT has been to provide agronomic performance information for hybrids to farmers, educators, agronomists, and seed companies. Annual results of the OCPT program assist in selecting hybrids or genotypes adapted to Ohio's varying environments. The OCPT has served as an invaluable tool that has contributed to increased productivity while helping growers and their advisors to minimize production losses across the state.

#### **4. CONCLUSION**

Over the past 50 years, the OCPT has tested more than 52,000 hybrid-environment combinations across the state, critical agronomic traits associated with seed quality,

technologies and newer genetics have important improvements. An increase of 10.3% (or 0.66% year<sup>-1</sup>) in seedling emergence, an increase of 66% or 34 thousand plants ha<sup>-1</sup> (equivalent to 694 plants year<sup>-1</sup>) in final stand population. And correlations  $r = .64$  and  $r = .82$ , respectively with yield.

The evolution of new hybrids, vigor and technologies related to corn seeds, contributed to an 84.7% increase in grain yield, an annual increase of 0.134 Mg ha<sup>-1</sup> or 6.69 Mg ha<sup>-1</sup> from 1972-2021.

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## CONSIDERAÇÕES FINAIS

Através das investigações conduzidas, importantes informações voltadas para a manutenção da qualidade fisiológica e combate a fitotoxidez em sementes de milho tratadas, bem como a relação do emprego de sementes de alta qualidade fisiológica, genética, sanitária e física para obtenção de altas produtividades foram obtidos/evidenciadas como:

Os processos de tratamento e disposição dos ingredientes ativos junto as sementes afetaram a manutenção da qualidade das sementes de milho tratadas. O uso de fungicida mais polímero (FP) em primeiro estágio cria uma camada de proteção que evita ou diminui o contato direto da molécula inseticida com a semente, o que auxilia na preservação do vigor e mitiga fitotoxidez junto as sementes e plântulas principalmente no período de 12 meses de armazenamento. Nessa linha, o procedimento operacional de tratamento estratificado FP/Inseticida/Pó demonstrou ser uma boa alternativa para promover menor intensidade de deterioração por fitotoxidez de sementes de milho tratadas com produtos fitossanitários e armazenadas.

Há redução do vigor das sementes tratadas ao longo do armazenamento principalmente aos 21 meses de armazenamento, caracterizado pela deterioração acentuada devido a combinação do período avançado de armazenamento em condições não controladas e uso do inseticida neonicotinóide, indicando que esse período de armazenamento não é seguro para sementes de milho com essas moléculas.

Os princípios ativos imidaclopride + tiodicarbe e tiametoxam apresentaram maior efeito fitotóxico, maior impacto na germinação e no vigor. Além disso, lotes de baixo vigor foram mais suscetíveis à fitotoxicidade com tratamento com inseticida neonicotinóide. Entretanto, os híbridos tolerantes a inseticidas e de alto vigor apresentaram desempenho e desenvolvimento morfoanatômico superiores.

Lotes de alto vigor apresentaram maiores diâmetros de amido, o que pode ser sinônimo de desenvolvimento superior que contribui para vigor e potencial de armazenamento. Além da maior espessura do pericarpo, deve ser levado em consideração, pois acreditamos que essa característica pode servir como mecanismo de proteção física contra o contato de tecidos e órgãos internos com moléculas inseticidas auxiliando na manutenção da qualidade fisiológica.

Melhorias importantes nos últimos 50 anos de características agronômicas associadas à qualidade (emergência e vigor), tecnologia (aumento do número de produtos fitossanitários e tecnologia de plantio) e genética (novos híbridos e traits transgênicos) de sementes foram observados, como demonstrado nos mais de 52.000 testes, combinações de ambientes e híbridos

nos ensaios do Ohio Corn Performance Test, evidenciaram a importância de sementes híbridas de milho de alta qualidade para alto rendimento. Como aumento de 10,3% (ou 0,66 ano<sup>-1</sup>) na emergência de plântulas, aumento de 66% ou 34 mil plantas ha<sup>-1</sup> (equivalente a 694 plantas ano<sup>-1</sup>) no estande final. Com correlação de  $r = 0,64$  e  $r = 0,82$ , respectivamente com rendimento. Contribuíram para o aumento de 84,7% ou 6,69 Mg ha<sup>-1</sup> na produtividade de grãos entre 1972-2021.