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Regina Tomiozzo

**ECOFISIOLOGIA, MODELAGEM E DESEMPENHO AGRONÔMICO DE
Helianthus annuus L. E *Limonium sinuatum* Mill. COMO FLORES DE CORTE**

Santa Maria, RS
2024

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Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutora em Agronomia.**

Orientador: Prof. PhD. Nereu Augusto Streck

Santa Maria, RS
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Santa Maria, RS
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“Que a tua vida não seja uma vida estéril. - Sê útil. - Deixa rasto.”

São Josemaria Escrivá

RESUMO

ECOFISIOLOGIA, MODELAGEM E DESEMPENHO AGRONÔMICO DE *Helianthus annuus* L. E *Limonium sinuatum* Mill. COMO FLORES DE CORTE

AUTORA: Regina Tomiozzo
ORIENTADOR: Nereu Augusto Streck

A floricultura brasileira é uma promissora e típica atividade da agricultura familiar, que entre 2012 e 2023 cresceu a uma taxa média de 12% ao ano. É uma atividade que está alinhada com as necessidades da produção agrícola global, que enfrenta o desafio de aumentar a produtividade sem expandir a área cultivada. A pandemia do Covid-19 trouxe inúmeras perdas para este setor, ocasionando um intenso período de vulnerabilidade. Uma questão chave é como fortalecer toda a cadeia produtiva brasileira de flores e plantas ornamentais em escala nacional para enfrentar futuros imprevistos. Seria realizando melhorias que acompanhem as tendências do mercado internacional, desde a produção até a comercialização? Neste sentido, existem dois caminhos possíveis. Um, é através da produção e comércio local, que permitem a redução de custos e fomentam o consumo interno de flores. Este moderno modelo de floricultura é popular em países da Europa e da América do Norte, e no Brasil é um caminho que já vem sendo seguido e tem se consolidado com o Projeto Flores para Todos, o maior projeto inclusivo de extensão em Floricultura do Brasil. O outro caminho é desenvolver inovação e gerar tecnologias de ponta para a floricultura, através da criação de modelos que simulem o desenvolvimento de espécies de flores. Juntos, estes caminhos têm potencial para consolidar um crescimento sustentável e de sucesso para a floricultura brasileira e aumentar a participação do Brasil no mercado internacional de flores e plantas ornamentais. Unindo esses caminhos estratégicos, foram conduzidos ensaios de campo em diversas regiões do Brasil e na região da Toscana, na Itália, com o objetivo de aprofundar o entendimento dos processos ecofisiológicos que regem o desenvolvimento de duas espécies cultivadas como flores de corte: o girassol e a statice, visando assim, a sua aplicação em modelos de simulação do desenvolvimento vegetal para área da floricultura. Desta forma, esta tese está dividida em três capítulos, com os seguintes objetivos: i) avaliar o crescimento, desenvolvimento e produção de girassol de corte em ambientes tropicais, subtropicais e temperados; ii) estimar o filocrono em genótipos de girassol de corte cultivado a campo considerando diversos locais e épocas de semeadura e, iii) descrever o padrão de florescimento da cultura da statice em ambiente subtropical, investigando fatores que influenciam seu ciclo reprodutivo. Os genótipos de girassol de corte utilizados para este estudo são bem adaptados nos ambientes tropical, subtropical e temperado, apesar das variações proporcionadas pelas diferentes condições ambientais. O número de folhas e o tempo térmico em girassol de corte tem relação bi-linear, resultando em dois filocronos. O período de colheita de statice pode variar de 5 a 18 semanas no rendimento e componentes de produção de flores variam em cada semana de colheita. Os resultados fornecem informações importantes aos produtores de flores sobre ambiente e seus efeitos nos processos ecofisiológicos no cultivo de girassol de corte e statice e compõe uma base sólida para o desenvolvimento futuro de modelos de simulação do desenvolvimento baseados em processos destas espécies.

Palavras-chave: Floricultura. Girassol de Corte. Statice. Ambiente. Cultivo a campo.

ABSTRACT

ECOPHYSIOLOGY, MODELLING AND AGRONOMIC PERFORMANCE OF HELIANTHUS ANNUUS L. AND LIMONIUM SINUATUM MILL. AS CUT FLOWERS

AUTHOR: Regina Tomiozzo
ADVISOR: Nereu Augusto Streck

Brazilian floriculture is a promising and typical activity of family farming, which grew at an average rate of 12% per year between 2012 and 2023. It is an activity aligned with the needs of global agricultural production, which faces the challenge of increasing productivity without expanding cultivated areas. The Covid-19 pandemic brought numerous losses to this sector, causing an intense period of vulnerability. A key issue is how to strengthen the entire Brazilian production chain of flowers and ornamental plants on a national scale to face future uncertainties. Could this be achieved by making improvements that align with international market trends, from production to marketing? In this regard, there are two possible paths. One is through local production and trade, which reduce costs and promote domestic consumption of flowers. This modern floriculture model is popular in European and North American countries, and in Brazil, it is a path that has already been pursued and has been consolidated with the "Flowers for All" Project, the largest inclusive floriculture extension project in Brazil. The other path is to develop innovation and generate cutting-edge technologies for floriculture, creating models that simulate the development of flower species. Together, these paths have the potential to consolidate sustainable and successful growth for Brazilian floriculture and increase Brazil's participation in the international market for flowers and ornamental plants. Combining these strategic approaches, field trials were conducted in various regions of Brazil and in the Tuscany region of Italy to deepen understanding of the ecophysiological processes governing the development of two cultivated species as cut flowers: sunflower and statice. Thus, this thesis is divided into three chapters with the following objectives: i) evaluate the growth, development, and production of cut sunflowers in tropical, subtropical, and temperate environments; ii) estimate the phyllochron in field-grown cut sunflower genotypes considering various locations and sowing times to estimate the phyllochron using a single and a bilinear model in field-grown cut sunflower genotypes considering several sowing dates in tropical, subtropical, temperate locations, and; and iii) describe the flowering pattern of the statice crop in the subtropics of Brazil, investigating factors that influence its reproductive phase. The cut sunflower genotypes used in this study are well adapted to tropical, subtropical, and temperate environments, despite variations due to different environmental conditions. The number of leaves and thermal time in cut sunflowers show a bi-linear relationship, resulting in two phyllochrons. The harvesting period of statice can vary from 5 to 18 weeks, and flower yield and production components vary each harvesting week. The results provide important information to flower producers about the environment and its effects on ecophysiological processes in cut sunflower and statice cultivation, forming a solid foundation for the future development of developmental simulation models based on these species.

Keywords: Floriculture. Cut sunflower. Statice. Environment. Field grown.

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1 INTRODUÇÃO

Em 2023, o mercado brasileiro de flores e plantas ornamentais movimentou o montante de U\$3,798 bilhões e teve um crescimento de 8% em relação a 2022, sendo a floricultura responsável por 17% do PIB interno do setor (IBRAFLOR, 2024), comprovando a importância e o alto potencial de crescimento deste setor no país como uma atividade do agronegócio atrativa, estratégica e economicamente rentável (SCHWAB et al., 2014; JUNQUEIRA; PEETZ, 2018). Além disso, é um mercado ávido por produtos de qualidade por um preço acessível, e que possuam maior durabilidade e baixa manutenção, demandas que se acentuaram durante a pandemia do Covid-19.

De acordo com Junqueira e Peetz (2018), a Cadeia Produtiva de Flores e Plantas Ornamentais brasileira se encaminha para a implantação de um modelo de qualidade internacional de gestão, governança e intensificação dos processos de introdução e adaptação de novas espécies, cultivares e híbridos no Brasil. Esse avanço promove a atualização do setor ao acompanhar as tendências do mercado mundial de flores, podendo alcançar o reconhecimento e a excelência internacional de maneira competitiva, eficiente e sustentável.

A priorização da pesquisa na área da floricultura em escala nacional e global permite aumentar essa capacidade, com alto grau de sofisticação de manejo, aplicando práticas agronômicas e utilizando variedades com boa relação custo-benefício, visando a máxima eficiência do uso dos recursos disponíveis. Segundo Cavalcante (2017) ao olhar para as perspectivas de expansão deste setor, é necessário estar em sintonia com os avanços na produção vegetal, garantir melhorias na qualidade das flores e plantas ornamentais cultivadas e atender os consumidores cada vez mais exigentes. A autora ainda salienta que realizar mudanças nas práticas de cultivo visando tornar o setor mais viável, especialmente para pequenos produtores, implica na adaptação das habilidades e conhecimentos. Visando melhorar o cultivo e manejo de espécies globalmente, cada vez mais a pesquisa está voltada para desenvolver tecnologias que tragam inovação, otimização dos recursos e sustentabilidade para o produtor. Dentre as tecnologias disponíveis, os modelos de simulação do desenvolvimento vegetal são importantes ferramentas de gestão que integradas ao dia a dia e tornam-se essenciais para os diversos setores do agronegócio brasileiro, inclusive para a floricultura.

A capacidade de expansão e aumento do volume de produção por área de maneira sustentável torna a floricultura uma excelente oportunidade de gerar emprego e renda para a agricultura familiar. Por isso, além de valorizar o espaço da propriedade rural e maximizar os lucros, inserir espécies da floricultura para diversificação da produção é essencial para o enfrentamento de períodos de vulnerabilidade, por proporcionar a redução dos riscos econômicos e incertezas do produtor rural. Uma das alternativas da floricultura para atender a esta demanda é a produção de flores de corte como o *Helianthus annuus* L. e o *Limonium sinuatum* (L.) Mill, popularmente conhecidas como girassol e statice, respectivamente (LORENZI; SOUZA, 2001). Tanto o girassol de corte como a statice são consideradas “flores de corte especiais”, pois apresentam as seguintes características conforme Darras (2021): são espécies anuais que podem ser produzidas sazonalmente em pequenas quantidades, favorecendo a venda em mercados locais de flores. Além disso, são espécies de flores rústicas, apropriadas para o cultivo a campo e que não requerem grandes investimentos financeiros e de infraestrutura. Por demanda de produtores e extensionistas, de acordo com Streck e Uhlmann (2021), estas espécies de flores de corte foram introduzidas no projeto “Flores para Todos”, um projeto de extensão com abrangência nacional que visa levar a floricultura como uma alternativa de renda para agricultores familiares e manter o jovem no campo.

Dada a extensão territorial brasileira, com latitudes desde 5°N até 34°S, com climas que variam desde clima úmido e quente o ano todo (Região Norte) até subtropical com invernos frios e úmidos (Região Sul) passando pelo clima tropical monsonico nas Regiões Centro-Oeste e Sudeste e pelo clima de savana em transição para um regime mediterrâneo em precipitação (Região Nordeste), o primeiro passo antes de desenvolver um modelo robusto e com alta taxa de acerto, é compreender o desenvolvimento e crescimento destas espécies nos distintos ambientes de cultivo. Considerando as diferenças de temperatura, fotoperíodo e radiação solar, que são variáveis meteorológicas que exercem uma influência significativa nos processos ecofisiológicos fundamentais de desenvolvimento do girassol e da statice (BAHUGUNA; JAGADISH, 2015; SHILLO; ZAMSKI, 1985), a sua adaptabilidade pode variar em diferentes localizações brasileiras. Nesse sentido, estudos que descrevam detalhadamente os processos ecofisiológicos envolvidos no desenvolvimento e produção de girassol de corte e statice necessitam ser realizados.

1.1 HIPÓTESES

O crescimento, desenvolvimento e produção de girassol de corte são influenciados por variações climáticas, com o potencial de apresentar características distintas em cada região.

Há um ponto de inflexão entre a sexta e a sétima folha em genótipos de girassol de corte que alterará a velocidade de emissão de folhas ao longo do desenvolvimento foliar.

O padrão de florescimento da cultura da *statice* em ambiente subtropical seguirá uma determinada sequência temporal, influenciada por fatores como temperatura e fotoperíodo, com potenciais variações em seu ciclo reprodutivo em outras regiões e épocas do ano.

1.2 OBJETIVO GERAL

Aprimorar o conhecimento dos processos ecofisiológicos que governam o desenvolvimento de duas espécies cultivadas como flores de corte, o girassol e a *statice*, através de ensaios de campo multi-anos e multi-locais, visando a sua aplicação em modelos futuros de simulação do desenvolvimento vegetal para área da floricultura.

1.2.1 Objetivos específicos

1. Avaliar o crescimento, desenvolvimento e produção de girassol de corte em ambientes tropicais, subtropicais e temperados.
2. Estimar o filocrono em genótipos de girassol de corte cultivado em campo considerando diversos locais e épocas de semeadura.
3. Descrever o padrão de florescimento da cultura da *statice* em ambiente subtropical, investigando fatores que influenciam seu ciclo reprodutivo.

2 REFERENCIAL TEÓRICO

2.1 MERCADO BRASILEIRO DE FLORES E PLANTAS ORNAMENTAIS

No Brasil, o setor de flores e plantas ornamentais registrou um montante de US\$ 1,157 milhões em 2021, representando um crescimento de 15% em relação a 2020. Nos últimos 10 anos, esse setor tem mantido uma taxa média de crescimento de 12% ao ano (IBRAFLOR, 2022). Em 2022, observou-se um aumento de 17% em relação a 2021 e em 2023, o setor cresceu 8% em relação a 2022, movimentando US\$ 3,798 bilhões (IBRAFLOR, 2024). Países como Itália e Estados Unidos alcançaram respectivamente, em 2019, US\$ 1,439 milhões e US\$ 3,715 milhões, e a Inglaterra registrou valores de US\$ 534 milhões em 2020, respectivamente (AIPH, 2021). No entanto, o consumo *per capita* de flores no Brasil ainda é baixo, cerca de US\$ 19,57/ano (IBRAFLOR, 2024), em comparação com países como a Itália (US\$ 82,33/ano), Estados Unidos (US\$ 129,26/ano) e Inglaterra (US\$ 152,07/ano) em 2021 (AIPH, 2022). Se metade dos brasileiros consumissem esse valor (US\$ 19,57/ano) por mês, o faturamento anual do setor da floricultura no Brasil subiria para US\$ 25.2 bilhões/ano, ou seja, um crescimento de 631.7%.

A atividade de flores e plantas ornamentais tem atraído muitos investidores, pela sua performance no mercado comercial, uma vez que esta é uma atividade familiar, e por ser um mercado comercial em expansão, comprovando a importância e o alto potencial de crescimento da floricultura no país (JUNQUEIRA; PEETZ, 2018; REIS et al., 2020). O Brasil possui mais de 8.000 produtores de flores e plantas ornamentais que cultivam mais de 350 espécies e 3.000 cultivares. No entanto, a maior parte da produção de flores e plantas ornamentais brasileira está concentrada na região Sudeste do país, no estado de São Paulo (JUNQUEIRA; PEETZ, 2017; REIS et al., 2020; IBRAFLOR, 2024). A concentração da produção nesta região do Brasil dá-se pela imigração holandesa iniciada na década de 50, onde estabeleceu-se um formato de floricultura empresarial e comercial sob a gestão da Cooperativa Veiling Holambra e a partir disso, polos de consumo foram criados em outras regiões brasileiras (por exemplo Minas Gerais, Rio de Janeiro, Rio Grande do Sul, Santa Catarina e Ceará) para o escoamento da produção a curtas, médias e longas distâncias (JUNQUEIRA; PEETZ, 2008).

A principal demanda de flores no Brasil ocorre em datas específicas, concentrando as vendas em datas como Dia das Mães e Dia dos Namorados (JUNQUEIRA; PEETZ, 2017).

Da produção brasileira, 97,5% são absorvidos pelo mercado nacional e ainda são importados produtos da Colômbia, Equador, China, Chile e Holanda (AIPH, 2022; IBRAFLOR, 2024). Por isso, segundo Castro et al. (2022), a oferta regular de produtos padronizados e de melhor qualidade pode abastecer muitos mercados, causando influência e homogeneização dos hábitos de consumo. Além disso, a variedade de produtos encontrados no varejo brasileiro é limitada e praticamente indistinguível de Norte a Sul do país. Em virtude disso, os produtos oriundos deste setor apresentam alto custo para o consumidor final em outras regiões brasileiras, intensificado em parte pelo custo do transporte dos produtos que está embutido no preço final estabelecido ao consumidor e, em parte pelo baixo poder aquisitivo dos brasileiros em relação aos demais países, que impedem o aumento do consumo de flores e plantas ornamentais no país.

Este cenário se acentuou durante a pandemia do Covid-19, que trouxe para a floricultura brasileira um forte e intenso período de vulnerabilidade econômica (CAVALCANTE, 2021). O cancelamento em massa dos eventos e o fechamento do comércio, especialmente no início da pandemia em 2020, impediu o escoamento da produção, levando a perda massiva de produtos perecíveis não comestíveis (flores de corte) e elevando o preço dos poucos produtos disponíveis (ANACLETO et al., 2021). Isto instalou uma forte crise em toda a cadeia produtiva, acarretando a falência de inúmeros produtores, especialmente aqueles que investiram na especialização e não na diversificação da produção. No entanto, com os atuais conflitos geopolíticos que o mundo está vivendo em decorrência de Guerras entre Rússia e Ucrânia e entre Israel e Hamas, um cenário de aumento dos preços dos produtos deve persistir em 2024 e, o consumo de flores e plantas ornamentais fica em segundo plano na lista de prioridades dos brasileiros.

Apesar das dificuldades, o mercado de flores e plantas ornamentais brasileiro está expandindo e gerando empregos, com 60% da mão-de-obra feminina das 272.000 pessoas envolvidas no setor (IBRAFLOR, 2024). Com os resultados positivos de crescimento dos últimos anos, a regularização e reconhecimento do setor no âmbito nacional como parte integrante da horticultura, tem sido incentivada também através da criação de uma nova Política Nacional de Incentivo à Cultura de Flores e de Plantas Ornamentais de Qualidade que tem o objetivo de fomentar a produção de flores e plantas ornamentais no Brasil, bem como a sua comercialização nos mercados interno e externo, pela Lei n. 14.637, de 25 de julho de 2023 (DIÁRIO OFICIAL DA UNIÃO, 2023).

2.2 PROJETO FLORES PARA TODOS

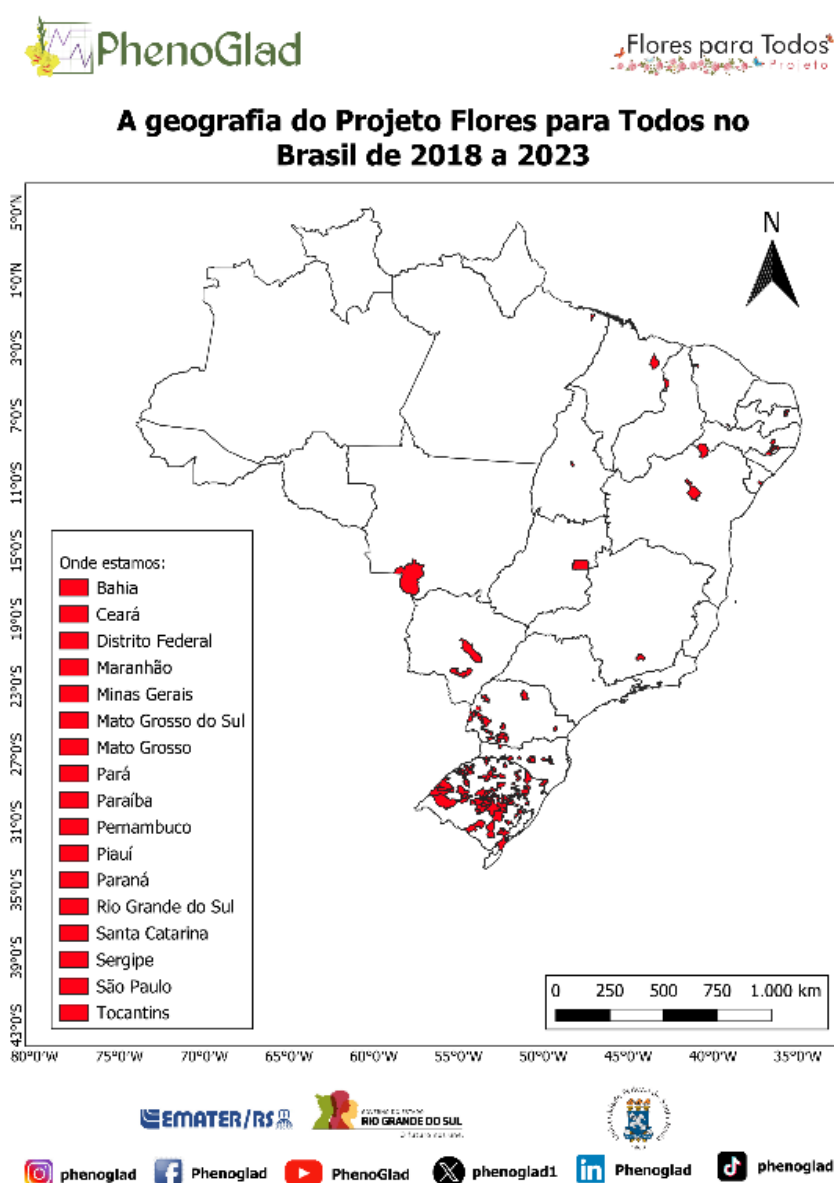
Investir no fortalecimento e crescimento do setor de flores e plantas ornamentais no Brasil é fundamental e, uma forma de fazer isso, é através da introdução da floricultura como alternativa de diversificação da produção em pequenas propriedades rurais. Além de valorizar o espaço da propriedade rural e maximizar os lucros, a diversificação da produção é essencial para o enfrentamento de períodos de vulnerabilidades como esse, por proporcionar a redução dos riscos econômicos e incertezas. Esta atividade permite ao agricultor familiar empreender, criar e inovar dentro da sua propriedade, agregando valor e crescendo economicamente. Além disso, permite a descentralização da produção, criando outros canais de comercialização, produzindo espécies mais adaptadas ao clima e cultura local e, ainda, encurtar cadeia, reduzindo o custo ao consumidor, alavancando o consumo per capita, tornando-a uma cadeia sustentável economicamente, socialmente e ambientalmente.

O modelo moderno de floricultura, com produção e comercialização local tem dado certo em outros países do mundo, como nos Estados Unidos com o projeto chamado “Local Flowers, Local Farmers: a Growing Movement” (<https://localflowers.org/>; <https://www.youtube.com/watch?v=PEXs9UUgqqg>), uma iniciativa da Association of Specialty Cut Flowers Growers, que visa o resgate no cultivo de flores de corte e as floriculturas e feiras locais comercializam apenas as flores produzidas no seu município. No Brasil, um projeto inclusivo de extensão que utiliza este modelo moderno em todo o território nacional gerando renda para agricultores familiares, mulheres rurais e jovens do campo é o Projeto “Flores para Todos” (STRECK; UHLMANN, 2021) (https://www.youtube.com/watch?v=-sR6LW_P8dU). Desde seu início em 2018 até 2023, este projeto já alcançou, em nível de Brasil, 324 famílias e 59 escolas do campo de 151 municípios em 17 estados de regiões brasileiras (Figura 1). Em 2024, o projeto continua em andamento e estes números seguem aumentando.

As espécies de flores são escolhidas visando atender as seguintes premissas básicas agronômicas do Projeto Flores para Todos: i) rusticidade; ii) cultivo a céu aberto; iii) fácil propagação e manejo; iv) baixo custo de implantação e produção e, v) ótima aceitação pelos consumidores. A cultura pioneira do projeto é o gladiolo, uma bulbosa com forte importância como flor de corte no país para o dia das Mães e o dia de Finados (UHLMANN et al., 2019). Com a expansão do projeto por todo o Brasil e dada a sua extensão territorial, houve a

necessidade de introduzir novas espécies de flores para aumentar as opções de diversificação ao longo do ano. Por isso, novas culturas já foram introduzidas no projeto: statice (*Limonium sinuatum* Mill.) https://youtu.be/YK_J73zMQvk), girassol de corte (*Helianthus annuus* L.) (<https://youtu.be/cQqUg3zrAkI>), dália (*Dahlia pinnata*) (https://youtu.be/Jtrl_I20QEc) e ornitogalum (*Ornithogalum saundersiae*) (<https://www.youtube.com/watch?v=v5yXkGxoVIA>).

Figura 1 - Geografia do Projeto Flores Para Todos no Brasil de 2018 a 2023.



2.3 GIRASSOL DE CORTE

O girassol (*Helianthus annuus*) é uma planta dicotiledônea, herbácea de ciclo anual. Pertencente à família Asteraceae e nativa da região temperada da América do Norte (KAYA; JOCIC; MILADINOVIC, 2011; SHATOORI et al, 2021). Como planta ornamental, o girassol tem grande representatividade como flor de corte por sua beleza exuberante e imponente, sendo uma das culturas de flores mais conhecidas e apreciadas em todo o mundo (MLADENOVIC et al. 2020; PUTTHA et al., 2024).

Morfologicamente, possui um caule ereto e robusto, que pode atingir alturas significativas, variando de acordo com a cultivar e as condições de crescimento. Suas folhas são grandes, de formato oval ou em formato de coração, com uma textura áspera e uma cor verde vibrante, que contribui para sua estética visualmente marcante. A inflorescência do girassol é do tipo capítulo, que se caracteriza pela dilatação do pedúnculo na parte superior, formando um receptáculo, sobre o qual se inserem as flores sésseis (CURTI et al., 2012). Envolvendo o receptáculo, está o involúcro, um conjunto de brácteas que protege e impede a queda dos frutos (aquênios). Apresenta dois tipos de flores sésseis: as liguladas e as tubulares. As liguladas (corola amarela) são incompletas e inférteis e servem como atrativo para insetos, como abelhas. As tubulares são as flores do disco, completas e férteis, que formarão os aquênios. No entanto, para o girassol ornamental, as flores do disco também são estéreis, pois para ornamentação o pólen é indesejável (CURTI et al., 2012; NEVES et al., 2005).

É uma planta propagada por sementes e sua emergência ocorre rapidamente, cerca de 8 dias após a sementeira. Responsiva a temperatura do ar, a duração do ciclo é calculada em °C dia, que geralmente é curto (50 a 70 dias) dependendo da cultivar (ARMITAGE; LAUSHMAN, 2003; SHATOORI et al., 2021). Segundo Alberio et al. (2015), a temperatura base do girassol pode variar de 4,0 °C a 8,0 °C. Em condições de baixa umidade relativa do ar e temperatura elevada, o ciclo é acelerado antecipando o florescimento (CURTI et al., 2012). O fotoperíodo também apresenta influência no desenvolvimento do girassol, sendo classificado como uma planta neutra ao fotoperíodo (DN), uma planta facultativa de dias longos ou como planta facultativa de dias curtos, resultando na antecipação do florescimento (GOUNE; HAMMER, 1982; YAÑEZ et al., 2012). Não há um consenso entre autores sobre qual seria essa resposta e entende-se que é pode ser variável com a cultivar.

O estresse hídrico é a principal limitante para produção de hastes florais de qualidade e em condições sem limitação de água, é uma planta que apresenta alta taxa de transpiração (ALBERIO et al., 2015). Um mecanismo de defesa da planta, em situações de deficiência hídrica é o murchamento das folhas (redução da transpiração), que fará com que a água fique conservada no solo por mais tempo, protegendo-as do estresse térmico. Nutricionalmente, o girassol é exigente em macro e micronutrientes. Na implantação da cultura exige-se uma adubação de base com NPK e posterior adubação de cobertura, durante a fase vegetativa, quando ocorre maior absorção dos nutrientes (ALBERIO et al., 2015; CURTI et al., 2012).

A produtividade do girassol ornamental pode ser medida através do comprimento da haste floral e da qualidade da sua inflorescência, pelo diâmetro do capítulo e aspectos visuais (NEVES et al., 2005) e isto pode variar com a genética da planta e o ambiente em que estão expostas. A colheita é realizada conforme o estágio de abertura floral, geralmente quando as pétalas das flores liguladas apresentam abertura em um ângulo de 90°. O ponto de colheita pode diferir conforme a demanda do mercado. Colhe-se mais fechada, quando o objetivo é a comercialização em mercados distantes e mais abertas para mercados próximos. Após o corte, o girassol continua a abertura das flores tubulares, por isso devem ser acondicionadas em recipiente com água. A vida de vaso pode variar de acordo com a região, época do ano, genética, sistema de cultivo e distância do consumidor (CURTI et al., 2012). De modo geral, a durabilidade pós-colheita é em torno de 7 a 10 dias. Por essas características, é uma espécie que tem grande potencial para conquistar o mercado regional de flores de corte, pela crescente demanda local por flores de qualidade a um baixo custo.

2.4 A CULTURA DA STATICE

O gênero *Limonium* pertence à família Plumbagianaceae e possui mais de 300 espécies, dentre as quais está o *Limonium sinuatum* L. Mill., natural da região do Mediterrâneo e popularmente conhecida como statice, estátice, lavanda do mar ou sempre viva (LORENZI; SOUZA, 2001; CIOTTA; NUNES, 2011). É uma planta herbácea de ciclo anual, multiplicada por sementes, de caule com entrenós muito curtos durante a fase vegetativa e por isso as folhas basais crescem em forma de roseta, inflorescências eretas ramificadas e duráveis que podem ser ora do tipo cacho ou racimo (flores situadas em pedicelos saindo de diversos níveis no eixo primário e atingindo diferentes alturas) e ora do tipo corimbo (flores situadas em pedicelos saindo de diversos níveis no eixo primário e

atingindo todas a mesma altura), numerosas flores de cálice azulado e corola branca, amarela, roxa e rósea (BARROSO et al., 2004). De acordo com Blas (1992), classifica-se como um rácemo-corimbiformes.

A produção de *statice* é de especial interesse, pois é amplamente utilizada por floristas e decoradores na composição arranjos e vasos para ornamentar interiores e ocasiões especiais, como datas comemorativas. A preferência pela utilização desta flor ocorre pela sua longevidade quando secas, podendo também ser usada fresca, além da variedade de cores (WHIPKER; HAMMER, 1994; NATARAJ et al., 2009). Tanto no Brasil como em outras regiões do mundo, como é o caso de Taiwan, o cultivo de grande parte das cultivares de *statice* atualmente disponíveis inicia no outono, passando por um período de vernalização natural durante o inverno, florescendo na primavera e verão em regiões subtropicais (CHANG; YEH; YANG, 2010). Em função destas exigências bioclimáticas, o período de colheita das flores de *statice* em cultivo de campo no Sul do Brasil é de setembro a dezembro.

O cultivo da *statice* é realizado, preferencialmente, a campo e pleno sol (BARROSO et al., 2004) e em pequenas áreas. A planta de *statice* adapta-se bem a diferentes tipos de solos desde que tenha boa aeração e drenagem (GONZÁLES; RE, 2003). O solo deve ser previamente preparado antes do plantio, a fim de proporcionar uma boa estrutura para o desenvolvimento da planta. Solos menos férteis são preferíveis para controlar a floração e evitar a emissão demasiada de folhas (crescimento vegetativo intenso e pouco crescimento reprodutivo). Adapta-se bem em solos com pH de 6,0 a 6,5 e durante seu cultivo é necessário realizar adubação suplementar de potássio e magnésio (GONZÁLES; RE, 2003).

O plantio pode ser realizado em canteiros com fileiras duplas espaçadas 50 cm e com 30 cm entre plantas, para proporcionar o bom crescimento e desenvolvimento das plantas. Densidades maiores diminuem a qualidade das hastes, além favorecer o aparecimento de doenças fúngicas devido à falta de ventilação dentro do dossel. Nataraj et al. (2009) estudaram o crescimento e o desenvolvimento de cinco cultivares de *statice* e verificaram que seu ciclo pode durar de 70 a 90 dias até a floração. Dependendo da cultivar e da época de cultivo, uma planta de *statice* pode emitir cerca de 180 a 200 folhas durante seu ciclo (BLAS, 1992; NATARAJ et al., 2009).

A desenvolvimento da *statice* é influenciado principalmente pela temperatura do ar e pelo fotoperíodo (SHILLO, 1976; SHILLO; ZAMSKI, 1985). Altas temperaturas promovem o crescimento das folhas, mas inibem a indução floral (SHILLO; ZAMSKI, 1985). A *statice* é considerada uma planta de dia longo, com fotoperíodo crítico (a partir do qual o

florescimento é induzido) de 13 horas (SEMENIUK; KRIZEK, 1972; SHILLO; ZAMSKI, 1985; CHEN et al., 2010). Além disso, seu crescimento é maior quando exposta a alta intensidade luminosa (SHILLO; ZAMSKI, 1985).

Shillo e Zamski (1985) descrevem como os fatores ambientais afetam o desenvolvimento da *stative*. Segundo os autores, o requerimento de temperatura está interrelacionado com a cultivar. Por exemplo, as cultivares de cores amarela e branca podem florescer em altas temperaturas em relação as cultivares de cor rósea e roxa. Caso não ocorra a indução floral, a planta de *stative* permanecerá emitindo folhas, aumentando a fase vegetativa e atrasando a floração. Shillo (1976) descreve que a faixa ideal de temperatura para o ciclo de desenvolvimento da *stative* é de 12 °C a 16 °C durante a noite e de 22 °C a 27 °C durante o dia.

A vernalização das sementes de *stative* não é efetiva para o desenvolvimento da cultura (SHILLO; ZAMSKI, 1985). No entanto, a exposição das plantas a baixas temperaturas durante a fase plântula (a partir da quinta folha) é essencial para desencadear o florescimento. As necessidades de vernalização são alcançadas expondo plântulas de *stative* a temperaturas de 11 a 13 °C durante três semanas, sendo que, o fotoperíodo durante a vernalização não influencia o florescimento (SHILLO, 1976; SHILLO; ZAMSKI, 1985) e esta necessidade de frio é alcançada nos cultivos de campo nos estados do Sul do Brasil quando o transplântio é realizado no outono e as plantas ficam expostas no inverno.

Segundo Nataraj et al. (2009) apesar da ampla utilização desta flor, alguns gargalos estão associados à sua produção, como: indisponibilidade de material propagativo, falta de variedades melhoradas e alta flutuação de mercado. Quanto à indisponibilidade de material propagativo, atualmente no Brasil, este é encontrado no mercado de duas maneiras: por sementes e por *plugs*. As sementes disponíveis no mercado brasileiro são *mixes* de cultivares, com custo mais acessível, porém com alguns obstáculos para o cultivo pela diferença de duração de ciclo, cor da flor e em número e tamanho de folhas, pois cada cor da flor é uma cultivar de *stative* no mesmo lote de sementes. Sementes importadas e *plugs* (produzidos a partir da cultura de tecidos) proporcionam uma produção uniforme com cultivares específicas, porém com alto custo decorrente da legislação e burocracia para importação deste material de outros países.

3 RESULTADOS E DISCUSSÕES

3.1 CAPÍTULO 1 – DEVELOPMENT, GROWTH, AND FLOWER PRODUCTION OF CUT SUNFLOWER IN TROPICAL, SUBTROPICAL, AND TEMPERATE ENVIRONMENTS

(Submetido à *Horticulture, Environment, and Biotechnology*, portanto as normas estão de acordo com as diretrizes exigidas)

1 **Development, growth, and flower production of cut sunflower under tropical,**
 2 **subtropical, and temperate environments**

3
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Abstract

32

33 Sunflower (*Helianthus annuus L.*) is globally recognized as a popular cut flower,
34 boasting an annual developmental cycle influenced primarily by temperature and
35 photoperiod. To assess the response of field grown cut sunflower in tropical, subtropical,
36 and temperate environments, 28 genotypes of cut sunflower were tested in field trials
37 conducted for 4 years (2020 – 2023) in different locations from Northern to Southern Brazil
38 and in the Tuscany region, Italy. These trials provided a large and robust dataset containing
39 results of developmental cycle (in days and °C day), vegetative and reproductive phase (°C
40 day), final leaf number (FLN), leaf number at R1 stage (LN at R1), number of leaves still to
41 appear after R1 stage [FLN – (LN at R1)], phyllochron (°C day leaf), leaf area index (LAI),
42 plant height (cm), capitulum diameter (cm) and stem diameter (cm). The dataset was divided
43 into three environments (tropical, subtropical, and temperate) and relationships were applied
44 by linear regressions. As a result, cut sunflower genotypes used for this study are well
45 adapted to tropical, subtropical, and temperate environments, despite the variations provided
46 by different climatic regimes. In general, it is possible to establish the following relationship:
47 the longer the developmental cycle, the greater the LN at R1, the higher the FLN, and bigger
48 the plant height. The novelty of this study is the response of several cut sunflower in three
49 major climates worldwide (tropical, subtropical and temperate) that provides a basis for
50 futures studies by identifying significant patterns that influence their development, growth,
51 and flower production.

52

53 **Key words:** *Helianthus annuus*, floriculture, cut flower, field grown, multilocation trial.

54 3.1.1 Introduction

55

56 Ornamental crops with short developmental cycle, ease of cultivation, low
57 production cost, wide adaptability to different environmental conditions, and profitability
58 are more and more preferred among farmers (Curti et al., 2012; Junqueira and Peetz, 2018).
59 Sunflower (*Helianthus annuus*), native of North America (Seiler and Gulya, 2016), as a cut
60 flower fulfills these requirements with impressive beauty and aesthetic features of its vibrant
61 capitulum-type inflorescence.

62 With the advancement of genetic improvement, new genotypes of ornamental crops
63 including cut sunflower with selected characteristics for quality that fits well in floral
64 compositions and gardens are released every year (Ahn et al., 2020). Breeders around the
65 world have been dedicated to generating ornamental sunflower genotypes that meet market
66 needs, such as garden plants, potted plants, or cut flowers, that differ in visual characteristics
67 such as size of the flower head, plant height, flower color, branching, longer vase life, and
68 sterile and pollen-free genotypes (Atlagić et al., 2005; Sloan and Harkness, 2006).

69 As a cut flower, sunflower has high potential and increasingly conquering the
70 Brazilian flower market. Additionally, its popularity has been boosted for diversifying
71 production and profit increase through the Flowers for All Project, a nationwide initiative in
72 Brazil led by PhenoGlad Teams (Uhlmann, 2019, Streck and Uhlmann, 2021). However, one
73 of the challenges is the introduction of new genotypes to keep up with international market
74 trends, given that Brazil lacks tradition in genetic improvement of floriculture species and
75 has large territorial extension and diversity of climates and soils.

76 Genotypes can respond differently in each location because of differences in
77 temperature, photoperiod, and solar radiation that drive sunflower development and growth
78 processes (Aiken, 2005; Ungaro et al., 2009, Bahuguna and Jagadish, 2015). Despite its
79 ability to tolerate different climatic conditions, temperature and photoperiod have been
80 shown to significantly affect the timing of sunflower flowering according to the genotype
81 (Villalobos et al., 1996; Yañez et al., 2005). The duration of the vegetative phase, leaf area
82 index (LAI), and leaf appearance rate (or its inverse, the phyllochron), primarily depend on
83 temperature, whereas the final leaf number (FLN), which is genetically a fixed trait (Alberio
84 et al., 2015), may be affected by the photoperiod. Some authors classify the sunflower as a
85 day-neutral plant (DN) or facultative long-day plant, while others classify it as a facultative
86 short-day plant (Goune and Hammer, 1982; Yañez et al., 2012). Thus, temperature and

87 photoperiod are major factors that drive sunflower developmental rate in field conditions
88 (Connor and Sadras, 1992).

89 Studies on the adaptability and stability have been conducted for sunflower
90 genotypes for grain and oil production purposes (Grunvald et al., 2013; Porto et al., 2008,
91 2009; Matta et al., 2020) but basic studies on development and growth in ornamental
92 sunflower as cut flower are scarce. Improving knowledge about cut sunflower development
93 and growth in different growing environments is essential for the fine tuning of management
94 practices and therefore to improve farmers profit and may help breeders to define priorities
95 and shorten their breeding programs. Therefore, the objective in this study was to evaluate
96 the development, growth, and flower production of field grown cut sunflower genotypes in
97 tropical, subtropical, and temperate environments. Development variables such as final leaf
98 number, phyllochron, duration of development cycle, vegetative and reproductive phases, as
99 well as growth variables such as leaf area index and quantitative parameters of the floral
100 stem (plant height, capitulum and stem diameter) and their relationships were performed.

101

102 **3.1.2 Material and Methods**

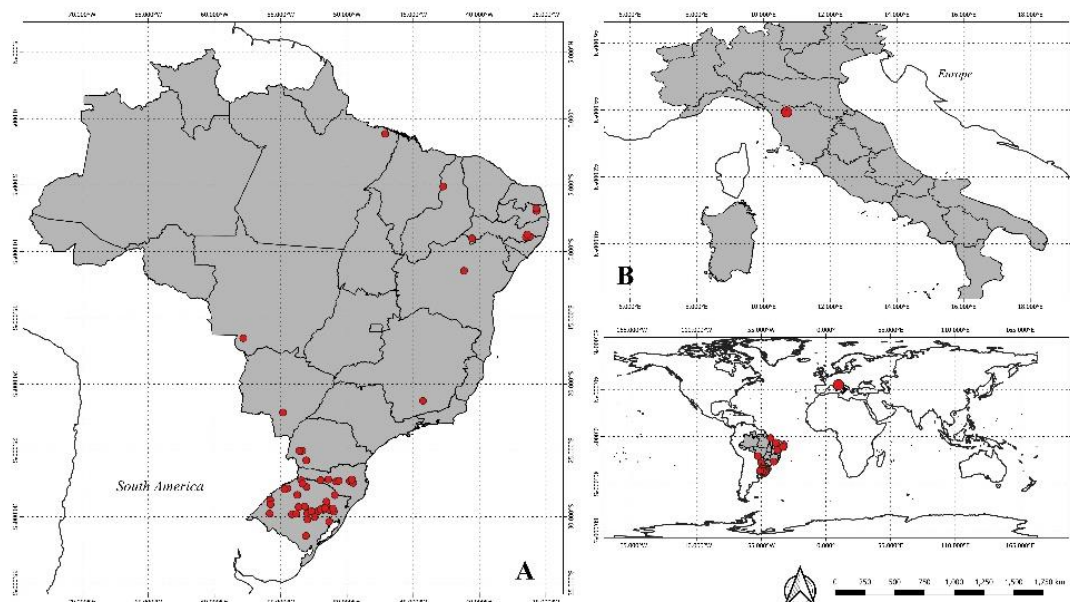
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104 3.1.2.1 Field studies

105

106 Five field trials were performed for four years, from 2020 to 2023, in several
107 locations, sowing dates, and regions of Brazil, South America (Fig. 1A), and in
108 Pescia/Tuscany, Italy, Europe (Fig. 1B). Details of each location are in Table 1. The locations
109 and sowing dates represent a wide variety of soil types and climate conditions.

110



111

112

113 **Figure 1.** Geographic location of the field trials with cut sunflower genotypes conducted in
 114 Brazil (A) and in Italy (B).

115

116 All trials followed the same protocol. The sunflower seeds were sown in polystyrene
 117 trays containing a commercial substrate, with one seed per hole at a depth of one centimeter.
 118 The trays were kept in a protected location until seedlings had the first pair of true leaves
 119 with a blade length of 2 cm and a well-developed root system forming a clod in the substrate,
 120 when they were then transplanted to 1m in width and 25-30 cm in height beds in the field.
 121 Plant spacing was 0.20 m among rows and 0.125 m within rows, with 4 rows per bed,
 122 resulting in a plant density of 32 plants/m². The length of the beds varied according to the
 123 location and each genotype or sowing date was separated in the bed by a 50 cm empty area.
 124 In the two central rows, ten plants (five plants per row) were tagged for data collection.

125 **Table 1.** Locations in Brazil and in Italy and their characteristics of climate, biome, latitude, longitude, altitude, sowing date (mm/dd/yyyy),
 126 transplanting date (mm/dd/yyyy), and institution used in field trials with cut sunflower genotypes.
 127

| Trial | Location | Climate | Biome | Latitude | Longitude | Altitude | Sowing date | Transplant date | Institution |
|-------|-----------------------------------|---------|-----------------------------|----------|-----------|-----------|--------------------------|--------------------------|-------------|
| 1 | Santa Maria, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°43'S | 53°43'W | 95 m | 08/06/2020 | 08/17/2020 | UFSM |
| 2 | Santa Maria, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°43'S | 53°43'W | 95 m | 01/22/2021 02/26/2021 | 01/30/2021 03/06/2021 | On farm |
| | Júlio de Castilhos, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°23'S | 53°68'W | 5 29 m | 2/25/2021 | 03/06/2021 | On farm |
| 3 | Herval D'Oeste, SC, Brazil | Cfa | Atlantic Forest | 27°19'S | 51°49'W | 520 m | 1/29/2021 2/26/2021 | 02/13/2021 03/06/2021 | On farm |
| | Cáceres, MT, Brazil | Awa | Cerrado/ Amazon/Pantanal | 16°07'S | 57°41'W | 143 m | 09/02/2022 | 09/12/2022 | UNEMAT |
| 3 | Capanema, PA, Brazil | Ami | Amazon | 01°11'S | 47°10'W | 32 m | 09/10/2022 | 09/17/2022 | UFRA |
| | Curitibanos, SC, Brazil | Cfb | Atlantic Forest | 27°16'S | 50°30'W | 992 m | 09/16/2022 | 10/17/2022 | UFSC |
| | Dois Vizinhos, PR, Brazil | Cfa | Atlantic Forest | 24°44'S | 53°04'W | 565 m | 01/27/2023 | 02/04/2023 | UTFPR |
| | Dourados, MS, Brazil | Am | Cerrado/Pantanal | 22°13'S | 54°48'W | 430 m | 01/19/2023 | 01/27/2023 | UFGD |
| | Petrolina, PE, Brazil | Bsh | Caatinga | 09°23'S | 40°30'W | 380 m | 08/08/2022 | 08/16/2022 | UNIVASF |
| | Santa Maria, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°43'S | 53°43'W | 95 m | 08/08/2022 | 08/25/2022 ^a | UFSM |
| 4 | São João Del Rei, MG, Brazil | Cwa | Atlantic Forest | 21°08'S | 44°15'W | 904 m | 09/13/2022 | 09/23/2022 | EPAMIG |
| | Pescia, PT, Italy | Csa | Appenine ^b | 43°54'N | 10°41'E | 42 m | 02/28/2023 | 03/17/2023 | CREA-OF |
| 5 | Morro do Chapéu, BA, Brazil | BSh | Caatinga | 11°33'S | 41°9'W | 1017 m | 04/17/2023 ^c | 04/25/2023 | On farm |

Cont. **Table 1**

| | | | | | | | | |
|--------------------------------------|-----|------------------------------|---------|----------|-------|-------------------------|------------|---------|
| Dourados, MS, Brazil | Am | Cerrado/Pantanal | 22°13'S | 54°48'W | 430 m | 10/15/2021 ^c | 10/15/2021 | UFGD |
| | | | | | | 16/08/2021 | 08/25/2021 | |
| Teresina, PI, Brazil | Aw | Cerrado | 5°5'S | 42°48'W | 87 m | 09/30/2021 ^c | 10/07/2021 | On farm |
| | | | | | | 03/09/2022 ^c | 03/22/2022 | |
| Areia, PB, Brazil | As | Caatinga | 6°57'S | 35°41'W | 573 m | 09/11/2022 ^c | 09/22/2022 | On farm |
| Solânea, PB, Brazil | As | Caatinga | 6°45'S | 35°43'W | 589 m | 09/11/2022 ^c | 09/22/2022 | On farm |
| Canhotinho, PE, Brazil | As | Atlantic Forest /Caatinga | 8°52'S | 36°11'W | 552 m | 11/03/2022 ^c | 11/16/2022 | On farm |
| Garanhuns, PE, Brazil | As | Atlantic Forest /Caatinga | 8°53'S | 36°29'W | 841 m | 04/03/2023 ^c | 04/12/2023 | On farm |
| Jupí, PE, Brazil | As | Caatinga | 8°42'S | 36°25'W | 788 m | 04/05/2023 ^c | 04/15/2023 | On farm |
| Petrolina, PE, Brazil | Bsh | Caatinga | 09°23'S | 40°30'W | 380 m | 02/03/2022 ^c | 02/11/2022 | UNIVASF |
| | | | | | | 05/26/2022 ^c | 06/03/2022 | |
| Cascavel, PR, Brazil | Cfa | Atlantic Forest | 24°57'S | 53°27'W | 782 m | 09/29/2021 ^c | 10/12/2021 | FAG |
| Santa Tereza do Oeste, PR, Brazil | Cfa | Atlantic Forest | 29°7' S | 51°42'W | 749 m | 03/17/2022 ^c | 03/28/2022 | On farm |
| Aurora, SC, Brazil | Cfa | Atlantic Forest | 27°18'S | 49° 38'W | 259 m | 10/05/2021 ^c | 10/15/2021 | On farm |
| Brunópolis, SC, Brazil | Cfb | Atlantic Forest | 27°18'S | 50°52'W | 843 m | 03/17/2021 ^d | 03/31/2021 | On farm |
| | | | | | | 03/31/2021 ^d | 04/14/2021 | |
| Concórdia, SC, Brazil | Cfa | Atlantic Forest | 27°14'S | 52°1'W | 578 m | 10/06/2021 ^c | 10/25/2021 | On farm |
| Curitibanos, SC, Brazil | Cfb | Atlantic Forest | 27°16'S | 50°30'W | 992 m | 09/08/2022 ^c | 10/05/2022 | On farm |
| Ituporanga, SC, Brazil | Cfa | Atlantic Forest | 27°24'S | 49°36'W | 347 m | 10/18/2021 ^c | 10/27/2021 | On farm |
| Herval D'Oeste, SC, Brazil | Cfa | Atlantic Forest | 27°19'S | 51°49'W | 520 m | 03/22/2021 ^d | 03/29/2021 | On farm |
| Rio do Sul, SC, Brazil | Cfa | Atlantic Forest | 27°12'S | 49°38'W | 332 m | 03/29/2021 ^d | 04/09/2021 | On farm |
| | | | | | | 10/05/2021 ^d | 10/15/2021 | |

Cont. Table 1

| | | | | | | | | |
|----------------------------------|------------|------------------------|---------|---------|-------|-------------------------|------------|---------|
| Seara, SC, Brazil | Cfa | Atlantic Forest | 27°9'S | 52°18'W | 517 m | 09/26/2022 ^c | 10/13/2022 | On farm |
| Trombudo Central, SC, Brazil | Cfa | Atlantic Forest | 27°17'S | 49°47'W | 323 m | 10/18/2021 ^c | 10/26/2021 | On farm |
| Cachoeira do Sul, RS, Brazil | Cfa | Pampa | 30°0'S | 52°55'W | 73 m | 03/20/2021 ^d | 03/29/2021 | On farm |
| | | | | | | 04/13/2021 ^d | 04/27/2021 | |
| Júlio de Castilhos, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°23'S | 53°68'W | 529 m | 04/15/2021 ^d | 04/25/2021 | On farm |
| | | | | | | 10/13/2021 ^c | 10/22/2021 | |
| | | | | | | 11/04/2021 ^c | 11/16/2021 | |
| | | | | | | 01/08/2022 ^c | 01/20/2022 | |
| | | | | | | 04/12/2022 ^c | 04/26/2022 | |
| Dilermando de Aguiar, RS, Brazil | Cfa | Pampa | 29°42'S | 54°12'W | 133 m | 03/15/2021 ^d | 03/24/2021 | On farm |
| | | | | | | 10/07/2022 ^c | 10/14/2022 | |
| Santa Maria, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°43'S | 53°43'W | 95 m | 10/24/2022 ^c | 11/04/2022 | On farm |
| | | | | | | 03/05/2021 ^e | 03/13/2021 | |
| | | | | | | 03/05/2021 ^f | 03/16/2021 | |
| | | | | | | 03/16/2021 ^d | 03/23/2021 | |
| | | | | | | 05/11/2021 ^d | 06/01/2021 | |
| | | | | | | 06/08/2021 ^d | 07/02/2021 | |
| | | | | | | 07/02/2021 ^d | 07/13/2021 | |
| | | | | | | 07/13/2021 | 08/10/2021 | |
| | | | | | | 09/07/2021 | 09/24/2021 | |
| | | | | | | 10/15/2021 ^c | 10/26/2021 | |
| 01/27/2022 ^c | 02/04/2022 | | | | | | | |
| 04/20/2022 ^c | 04/29/2022 | | | | | | | |
| | | | | | | 09/16/2022 ^c | 09/29/2022 | On farm |
| | Cfa | Atlantic Forest | 29°36'S | 52°11'W | 29 m | 03/15/2021 ^d | 03/23/2021 | On farm |

Cont. Table 1

| | | | | | | | | |
|-------------------------------------|-----|---------------------------|---------|----------|-------|-------------------------|------------|---------|
| Venâncio Aires, RS, Brazil | | | | | | 04/05/2021 ^d | 04/13/2021 | |
| Caiçara, RS, Brazil | Cfa | Atlantic Forest | 27°16'S | 53°25'W | 580 m | 09/27/2021 ^c | 10/08/2021 | On farm |
| Novo Xingú, RS, Brazil | Cfa | Atlantic Forest | 27°43'S | 53°3'W | 451 m | 09/23/2021 ^c | 10/05/2021 | On farm |
| | | | | | | 10/09/2021 ^c | 10/21/2021 | |
| | | | | | | 10/25/2021 ^c | 11/10/2021 | |
| | | | | | | 11/16/2021 ^c | 11/30/2021 | |
| Seberi, RS, Brazil | Cfa | Atlantic Forest | 27°28'S | 53°24'W | 526 m | 09/27/2021 ^c | 10/08/2021 | On farm |
| | | | | | | 10/22/2021 ^c | 11/04/2021 | On farm |
| | | | | | | 10/21/2021 ^c | 11/04/2021 | On farm |
| Vale do Sol, RS, Brazil | Cfa | Atlantic Forest | 29°36'S | 52°40'W | 213 m | 10/20/2021 ^c | 11/12/2021 | On farm |
| Boa Vista do Sul, RS, Brazil | Cfa | Atlantic Forest | 29°21'S | 51°40'W | 526 m | 03/12/2022 ^c | 03/22/2022 | On farm |
| Novo Cabrais, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°44'S | 52°57'W | 60 m | 02/22/2022 ^c | 03/02/2022 | On farm |
| | | | | | | 03/11/2022 ^c | 03/19/2022 | |
| Picada Café, RS, Brazil | Cfa | Atlantic Forest | 29°26'S | 51°8'W | 121 m | 03/12/2022 ^c | 03/28/2022 | On farm |
| | | | | | | 09/29/2022 ^c | 10/14/2022 | On farm |
| Santa Bárbara do Sul, RS, Brazil | Cfa | Atlantic Forest /Pampa | 28°21'S | 53°14'W | 511 m | 03/14/2022 ^c | 03/26/2022 | On farm |
| Teutônia, RS, Brazil | Cfa | Atlantic Forest | 26°56'S | 51°48'W | 47 m | 03/14/2022 ^c | 03/23/2022 | On farm |
| Piratini, Brazil | Cfa | Pampa | 31°26'S | 53°6'W | 345 m | 03/11/2022 ^c | 03/24/2022 | On farm |
| Rio Pardo, RS, Brazil | Cfa | Atlantic Forest /Pampa | 29°59'S | 52°22'W | 41 m | 02/04/2022 ^c | 02/14/2022 | On farm |
| | | | | | | 02/14/2022 ^c | 02/24/2022 | |
| Bom Princípio, RS, Brazil | Cfa | Atlantic Forest | 29°29'S | 51°21'W | 29 m | 02/22/2022 ^c | 03/02/2022 | On farm |
| Cândido Godói, RS, Brazil | Cfa | Atlantic Forest/Pampa | 27° 7'S | 54° 45'W | 308 m | 03/11/2022 ^c | 03/21/2022 | On farm |

Cont. **Table 1**

| | | | | | | | | |
|------------------------------|-----|-----------------------|---------|---------|-------|-------------------------|------------|---------|
| Maçambará, RS, Brazil | Cfa | Pampa | 29°8'S | 56° 4'W | 88 m | 03/15/2022 ^c | 03/22/2022 | On farm |
| Estrela Velha, RS, Brazil | Cfa | Atlantic Forest/Pampa | 29°10'S | 53°9'W | 388 m | 03/10/2022 ^c | 03/21/2022 | On farm |
| São Marcos, RS, Brazil | Cfa | Atlantic Forest | 28°58'S | 51°4'W | 724 m | 09/15/2022 ^c | 10/07/2022 | On farm |
| Lajeado, RS, Brazil | Cfa | Atlantic Forest | 29°28'S | 51°57'W | 21 m | 09/10/2022 ^c | 10/01/2022 | On farm |
| Faxinalzinho, RS, Brazil | Cfa | Atlantic Forest | 27°24'S | 52°39'W | 694 m | 09/29/2022 ^c | 10/17/2022 | On farm |
| Barra do Ribeiro, RS, Brazil | Cfa | Pampa | 30°17'S | 51°18'W | 14 m | 10/17/2022 ^c | 10/28/2022 | On farm |
| Colinas, RS, Brazil | Cfa | Atlantic Forest | 23°19'S | 51°52'W | 36 m | 09/20/2022 ^c | 10/03/2022 | On farm |
| Santa Rosa, RS, Brazil | Cfa | Atlantic Forest/Pampa | 27°52'S | 54°28'W | 268 m | 09/17/2022 ^c | 09/27/2022 | On farm |
| Alegrete, RS, Brazil | Cfa | Pampa | 29°47'S | 55°46'W | 76 m | 02/23/2023 ^c | 03/02/2023 | On farm |
| | | | | | | 03/02/2023 ^c | 03/09/2023 | |
| | | | | | | 03/09/2023 ^c | 03/16/2023 | |
| | | | | | | 03/16/2023 ^c | 03/23/2023 | |
| Bozano, RS, Brazil | Cfa | Atlantic Forest | 28°22'S | 53°46'W | 429 m | 01/27/2023 ^c | 02/03/2023 | On farm |
| | | | | | | 02/03/2023 ^c | 02/10/2023 | |
| | | | | | | 02/10/2023 ^c | 02/17/2023 | |
| | | | | | | 02/17/2023 ^c | 02/24/2023 | |
| Vila Flores, RS, Brazil | Cfb | Atlantic Forest | 28°52'S | 51°33'W | 702 m | 04/03/2023 ^c | 04/18/2023 | On farm |
| Vacaria, RS, Brazil | Cfb | Atlantic Forest | 28°30'S | 50°55'W | 960 m | 04/03/2023 ^c | 04/13/2023 | On farm |
| Nova Petrópolis, RS, Brazil | Cfa | Atlantic Forest | 22°57'S | 51°70'W | 581 m | 04/03/2023 ^c | 04/14/2023 | On farm |
| Bento Gonçalves, RS, Brazil | Cfa | Atlantic Forest | 29°10'S | 51°31'W | 671 m | 04/04/2023 ^c | 04/22/2023 | On farm |
| Sapiranga, RS, Brazil | Cfa | Atlantic Forest | 29°38'S | 51°00'W | 47 m | 04/05/2023 ^c | 04/21/2023 | On farm |

Cont. **Table 1**

| | São Borja, RS, Brazil | Cfa | Pampa | 28°40'S | 55°58'W | 74 m | 04/01/2023 ^c | 04/14/2023 | On farm |
|-----|--|-----|-------|---------|---------|------|-------------------------|------------|---------|
| 128 | a. Genotype VO-09 transplanted on 08/29/2022, due to low germination and late emergence. | | | | | | | | |
| 129 | b. According to Blasi et al. (2014). | | | | | | | | |
| 130 | c. Only genotype VC-12. | | | | | | | | |
| 131 | d. Only genotypes SO-06 and AG-01. | | | | | | | | |
| 132 | e. Only genotype DD-03. | | | | | | | | |
| 133 | f. Only genotype DD-04. | | | | | | | | |

For each location, a soil sampling was taken for physical and chemical test. Soil acidity was corrected along with nutrient supplementation based on the soil analysis and technical recommendations for sunflowers. Before transplanting, base fertilization was applied with 50 g per square meter of NPK Formula 05-20-20, spread and incorporated into the soil. Approximately 10 to 15 days after transplanting, when the plants had about 10 leaves, topdressing fertilization was applied using 25 g/m² of Potassium Chloride and 25 g/m² of Urea, also incorporated into the soil. Pest and disease control were performed as needed with chemicals. Weed control was carried out through manual hoeing. Drip irrigation was applied as needed in order to avoid soil water deficit. Each plot received individual staking with bamboo stakes or wooden slats at the four external corners and the use of raffia string at 2 to 3 heights as the plants increased in height throughout the developmental cycle. Twenty-eight sunflower genotypes for cutting purposes were used (Table 2) from different private international breeding companies.

Table 2. Cut sunflower genotypes names and codes used in each field trial in Brazil and in Italy.

| Trial | Code | Genotype name |
|--------------|-------------|----------------------|
| Trial 1 | AG-01 | Amalfi Golden |
| | AO-02 | Amalfi Orange |
| | SO-06 | Stromboli Orange |
| | VO-09 | Vesuvio Orange |
| | VT-11 | Vesuvio Tangy |
| | MO-03 | Magic Orange |
| Trial 2 | AG-01 | Amalfi Golden |
| | AO-02 | Amalfi Orange |
| | SO-06 | Stromboli Orange |
| | VO-09 | Vesuvio Orange |
| | VT-11 | Vesuvio Tangy |
| | MO-03 | Magic Orange |
| | OBS-11 | OBS-11 |
| | OBS-29A | OBS-29A |
| | OBS-36 | OBS-36 |
| | OBS37 | OBS37 |
| | FV - 01 | FV - 01 |
| | FV-07 | FV-07 |
| FV-13 | FV-13 | |
| FV-33 | FV-33 | |

Cont. **Table 2**

| | | |
|---------|-------|--------------------------------|
| | AG-01 | Amalfi Golden |
| | AO-02 | Amalfi Orange |
| | VT-11 | Vesuvio Tangy |
| | LT-05 | Luxor Tangy |
| | DD-03 | Double Delight Black Center |
| Trial 3 | MP-06 | Magic Orange Pro |
| | VO-09 | Vesuvio Orange |
| | FA-04 | Favola |
| | ST-08 | Stromboli Tangy |
| | SP-07 | Stromboli Orange Pro |
| | VP-10 | Vesuvio Orange Pro |
| | VC-12 | Vincent's Choice |
| | MP-06 | Magic Orange Pro |
| | VP-10 | Vesuvio Orange Pro |
| | VT-11 | Vesuvio Tangy |
| | MP-01 | Marco Polo Orange |
| | MP-02 | Marco Polo Tangy |
| | MP-03 | Marco Polo Deep Orange Pro |
| Trial 4 | MP-04 | Marco Polo Deep Tangy |
| | MP-05 | Marco Polo Sun Orange |
| | ST-08 | Stromboli Tangy |
| | SO-06 | Stromboli Orange |
| | SP-07 | Stromboli Orange Pro |
| | AG-01 | Amalfi Golden |
| | FA-04 | Favola |
| | AG-01 | Amalfi Golden |
| | SO-06 | Stromboli Orange |
| Trial 5 | DD-03 | Double Delight Black Center |
| | DD-04 | Double Delight Green Center |
| | VC-12 | Vincent's Choice |

Specific details of each trial were as follows:

Trial 1 – The first field trial was conducted from August to November 2020, in Santa Maria - RS, Brazil. The experimental design was a randomized complete block with four replications and 6 treatments (6 cut sunflower genotypes) (Table 2). In each replication, 6 plants per genotype were selected and tagged for data collecting.

Trial 2 – Data of the second field trial was collected from January to April 2021, in three locations of Southern Brazil (Santa Maria - RS, Júlio de Castilhos – RS, and Herval D’Oeste – SC). The experimental design was completely randomized with 6 replications, where each plant evaluated was considered one replication. The treatments were formed by the combination of locations (Santa Maria - RS, Júlio de Castilhos – RS, and Herval D’Oeste – SC), sowing dates, and genotypes. Two sowing dates were carried out in Santa Maria - RS and Herval D’Oeste – SC and one sowing date was carried out in Júlio de Castilhos – RS. Six cut sunflower genotypes were tested in the three locations, and on the second sowing date in Santa Maria - RS eight additional sunflower genotypes were tested (Table 2). In each replication, 6 plants per genotype were selected and tagged for data collecting.

Trial 3 - A multilocation field trial named Brazilian Cut Sunflower Trial was conducted from August 2022 to May 2023 in eight states (Table 1), covering all regions and biomes of Brazil. The experiment was conducted using a randomized block design with two replicates in a factorial arrangement. The treatments were formed by the combination of 8 locations x 12 cultivars x 7 sowing dates. Each block consisted of a bed measuring 25 meters in length. In each block, 10 plants per genotype were selected and tagged for data collecting.

Trial 4 – From February to June 2023, a trial was conducted at the Research Centre for Vegetable and Ornamental Crops (CREA-OF), located in Pescia, Tuscany, Italy. The methodology followed the same protocol of the trials in Brazil. Sunflower seeds were sown in polystyrene trays containing substrate prepared with vermiculite + perlite + sphagnum peat. Thirteen genotypes were used (Table 2). The experimental design was completely randomized, with eight replications. Each replication constituted a plant in the plot. Each plot consisted of one genotype with an area of 0.80 m², and 30 plants distributed in three rows spaced 20 cm apart, with 0.125 m between plants in the row. In the plots, 50 g per plot of 16-9-12 NPK fertilizer was used at transplanting. Nitrogen and phosphorus were supplied with 50 g per plot of 18-46 NP fertilizer as side dressing at 28 days after transplanting when plants had 6-8 leaves. It was not necessary to correct the soil pH.

Trial 5 – On farm trials were conducted from 2020 to 2023, across various regions of Brazil, from North to the South, as part of the “Flowers for All” Project (Streck and Uhlmann, 2021). Following the project methodology, the activities were led by rural extensionists from Emater/RS-Ascar and PhenoGlad Teams from research centers and universities in Brazil. The activities comprised the following steps: a) selection of the family farmers or rural schools with an affinity for flower production; b) an initial meeting with the

family farmers and rural extensionists for training and taking their doubts; c) send seeds and installing; d) cultivation and data collection with support from the PhenoGlad teams; e) tabulation and data analysis; f) final meeting for delivering results to the farmers and feedback.

A detailed protocol for implementation, management and collecting data was provided during the training, including spreadsheets for recording the data. The farmers received 100 seeds, which were sown in polystyrene trays and seedlings were transplanted to the beds. Five cut sunflowers genotypes were cultivated in this approach (Table 2). In each genotype, 10 plants were selected and tagged for data collecting.

3.1.2.2 Data collecting and statistical analysis

One week after transplanting to the beds, some selected plants (according to the Trials described in 3.1.2.1) were tagged with colored labels. In the tagged plants, the flowing variables were measured or observed: the accumulated number of leaves (ANL) once or twice a week, depending on the location, until reaching the final leaf number (FLN) using the criterion to consider a leaf with blade length of greater than 2 cm, and the date of the phenological stages R1 (visible bud) and R5 (corolla at 90° with the capitulum disk, harvest point) according to the phenological scale for sunflower by Schneiter and Miller (1981). At the R1 stage, the accumulated leaf number in the plant (LN at R1) was counted and then the number of leaves still to appear until the FLN [$FLN - (LN \text{ at } R1)$] was calculated.

On the day of harvest, the total height of the plant (from the soil surface to the insertion of the inflorescence), stem diameter at 70 cm from the top of insertion of the inflorescence, and capitulum diameter (excluding petals) were measured. Additionally, at flower harvest the greatest length (L) and width (W) of each green leaf was measured from the base towards the apex and individual leaf area (LA) was calculated by (Maldaner et al., 2009): $LA = 0.7330 \cdot (L \times W)$. Total leaf area was calculated by summing the individual leaf area and then the leaf area index (LAI) was calculated as $LAI = \text{leaf area} / \text{area occupied by each plant in the plant spacing}$.

In Trial 4 a destructive approach for calculating specific leaf area was used, which consisted of selecting sub-samples of leaves (20% of the total weight of green leaves) and measuring through a scanner (WinDIAS Image Analysis System, Delta-T Devices, Cambridge, UK). The fresh material was dried at 65 °C and weighed until the weight was

constant to determine the dry matter. The leaf area, in cm^2 , was calculated by $LA = (DM_{\text{leaves}} * LA_{20\%})/DM_{20\%}$, where DM_{leaves} is the dry matter of all leaves, $LA_{20\%}$ is the leaf area of the sub-sample measured by scanner and $DM_{20\%}$ is the dry matter of the sub-sample measured by scanner. Subsequently, the LAI was calculated.

Daily minimum and maximum air temperature were collected from the meteorological stations located as close as possible to each site during the experimental period. The daily mean air temperature (T_{mean}) was calculated by arithmetic average between the daily minimum and maximum temperature. The daily thermal time (DTT, $^{\circ}\text{C}$ day) was calculated by (Gilmore and Rogers, 1985; Arnold, 1960):

$$DTT = \{(T_{\text{opt}} - T_b) * [(T_b - T_{\text{mean}}) / (T_b - T_{\text{opt}})]\}$$

$$\text{If } T_b > T_{\text{mean}} > T_b, DTT = 0$$

$$\text{If } T_{\text{mean}} < T_{\text{opt}}, DTT = T_{\text{m}} - T_b$$

$$\text{If } T_{\text{mean}} > T_{\text{opt}}, DTT = T_o - T_b * \left(\frac{T_{\text{mean}} - T_b}{T_o - T_b} \right)$$

where T_b , T_{opt} and T_b are the lower base, the optimum, and the upper base temperatures for sunflower development defined by Villalobos et al. (1996) as 4°C , 28°C and 40°C , respectively. Accumulated thermal time (ATT, $^{\circ}\text{C}$ day) from the sowing (SO) to FLN, to R1, and to R5 was calculated by accumulating the DTT values.

The phyllochron (PHY, $^{\circ}\text{C}$ day leaf⁻¹) was calculated by the inverse of the slope of linear regression between ANL and ATT (Streck et al., 2009, Ferreira et al., 2019). Also, we calculated the time in $^{\circ}\text{C}$ day for the vegetative phase (from sowing to R1), for the reproductive phase (from R1 to R5) and the total developmental cycle (from sowing to R5).

The five trials conducted for four years provided a large and robust dataset on cut sunflower development and growth. We divided the data into three environments: tropical (Northeast, Central and South-eastern Brazil), subtropical (Southern Brazil), and temperate (Italy). Relationships that influence the development, growth, and flower production components were tested using linear or quadratic regressions as follows. Relationships between accumulated thermal time ($^{\circ}\text{C}$ day) for the total developmental cycle (SO-R5), the vegetative (SO-R1) and the reproductive (R1-R5) phases as well as between the vegetative phase and LN at R1, reproductive phase and FLN-(LN at R1). Reproductive phase, vegetative phase, LN at R1 and FLN – (LN at R1) as a function of the final leaf number

(FLN), and Phyllochron ($^{\circ}\text{C day leaf}^{-1}$) against FLN, LN at R1, vegetative and reproductive phase were also regressed.

The relationship between leaf area index (LAI) against FLN and phyllochron was also tested. To understand how LAI influences the flower production components, we applied linear regression between plant height, stem diameter and capitulum diameter and LAI. Some relationships among the production components were also applied such as plant height and developmental cycle ($^{\circ}\text{C day}$), plant height and FLN, capitulum diameter and plant height and finally, stem diameter and plant height. Furthermore, descriptive statistics such as minimum and maximum values, median, mean, 25% percentile and 75% percentile were calculated for developmental cycle, phyllochron, final leaf number, plant height, stem diameter and capitulum diameter (Supplementary File 1). We also calculated dispersion statistics as standard deviation and coefficient of variation, and distribution statistics as Skewness and Kurtosis. The relationships with probability (p) lower than 5% by the Test F were assumed to be significant. Statistics analyses were performed with the GraphPad 8.0.2 version software.

3.1.3 Results

3.1.3.1 Meteorological data

The five trials provided a wide variation in edaphoclimatic conditions where the plants were exposed (Supplementary File 2). In Southern Brazil, considered as subtropical environment, temperature varied from -2°C to 39°C . In this region, the temperature amplitude was the highest, mostly because of the large dataset with sowing dates along the twelve months of the year for four years. The other Brazilian regions, considered as tropical environment presented temperatures varying from 8°C to 41.5°C . The temperate environment is represented by the trial conducted in Italy and the temperatures varied from 0.5°C to 32.7°C .

3.1.3.2 Sowing date

The sowing date had a strong effect on the duration in days of the total developmental cycle in cut sunflower genotypes grown in the subtropical locations throughout the year whereas in the tropical locations the duration was the shortest and did not vary throughout

the year and in the temperate location the duration was among the longest (Fig. 2A). When the duration of the total developmental cycle is described in thermal time, with unit of °C day, there is no variation throughout the year in the subtropics and the developmental cycle in all locations and regions fall into around the mean duration, represented by the line in Fig. 2B, confirming that thermal time is a better time descriptor for plants than calendar days (Streck et al., 2011).

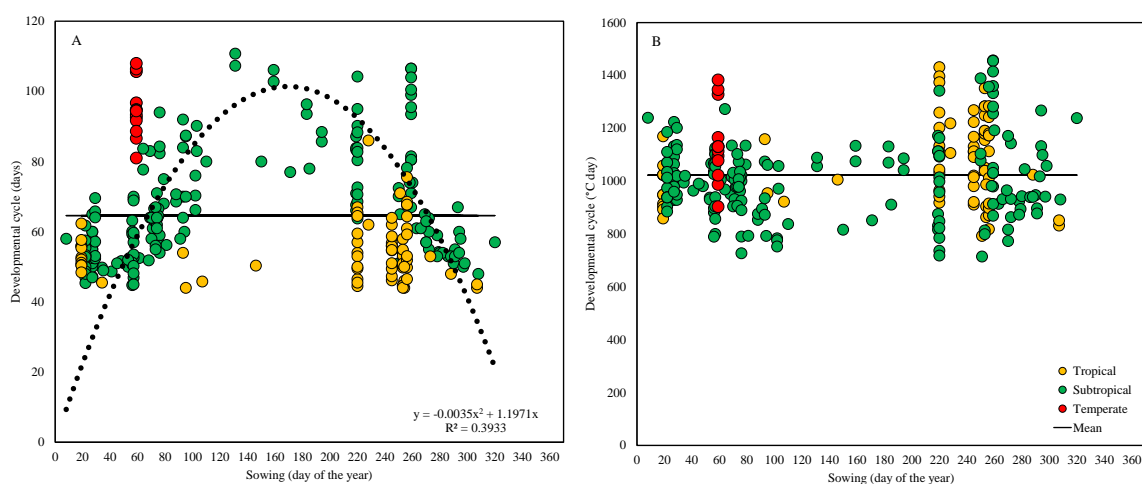


Figure 2. Developmental cycle of field grown cut sunflower genotypes in days (A) and in °C day (B) as a function of sowing date. Data from 28 field grown cut sunflower genotypes in cultivated in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype in a trial. Dashed line in panel A indicates the quadratic equation for the subtropical dataset. The solid line in panel B indicates the mean value of the dataset.

Sowing date and climate type did not affect LN at R1 (Fig. 3A). FLN and phyllochron demonstrated to be dependent of sowing date when analyzed all the dataset, without separating the environments (Fig. 3B and 3C, respectively). LAI was the variable most influenced by sowing date and the only that showed influence in the tropical and subtropical locations separately (Fig. 3D). As only one growing season was conducted in Italy, it was not possible to identify any relationship between the sowing date and the variables analyzed in temperate environment.

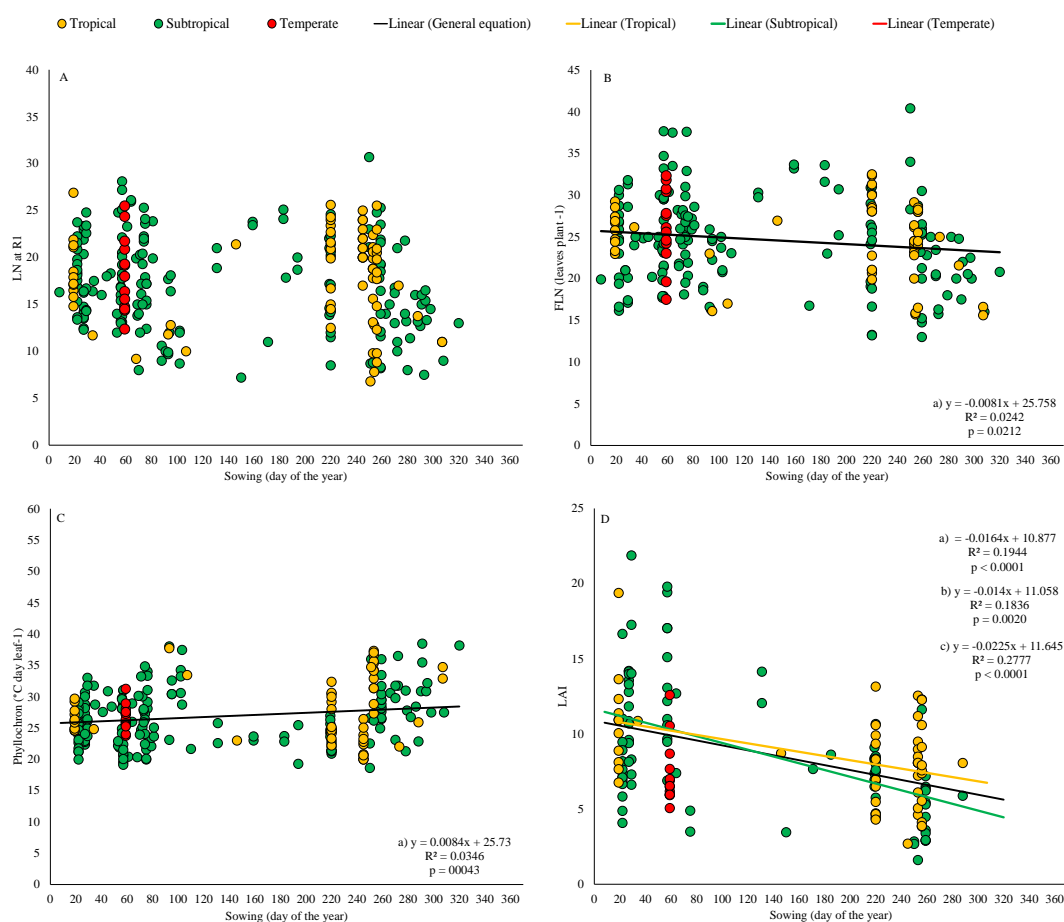


Figure 3. Relationship between (A) LN at R1, (B) final leaf number (FLN), (C) phyllochron and (D) leaf area index (LAI) and sowing date (day of the year). Data from 28 field grown cut sunflower genotypes in cultivated in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype in a trial. Each equation corresponds to a) General equation, b) Tropical, c) Subtropical and d) Temperate.

3.1.3.3 Developmental variables and their relationships

The relationships between developmental phases and total developmental cycle are considered only for thermal time used as time descriptor. Pooling all data, the duration of the total developmental cycle ($^{\circ}\text{C day}$), from sowing to R5 stage, is influenced primarily by the vegetative phase ($R^2 = 0.6954$, $p < 0.001$, Fig. 4A) but also by the reproductive phase ($R^2 = 0.3125$, $p < 0.001$, Fig. 4B), with a trend for an increase in the developmental cycle as the duration of the vegetative and reproductive phases prolong. Analyzing separately the

environments, tropical, subtropical, and temperate locations also presented significant relationships between the duration of total developmental cycle and the duration of vegetative and reproductive phases. The duration of the vegetative phase depends on the leaf number at R1 stage (LN at R1) in all three environments (Figure 3C). Conversely, the duration of reproductive phase does not depend on the number of leaves still to appear after the R1 stage [FLN-(LN at R1)] until FLN (Figure 4D).

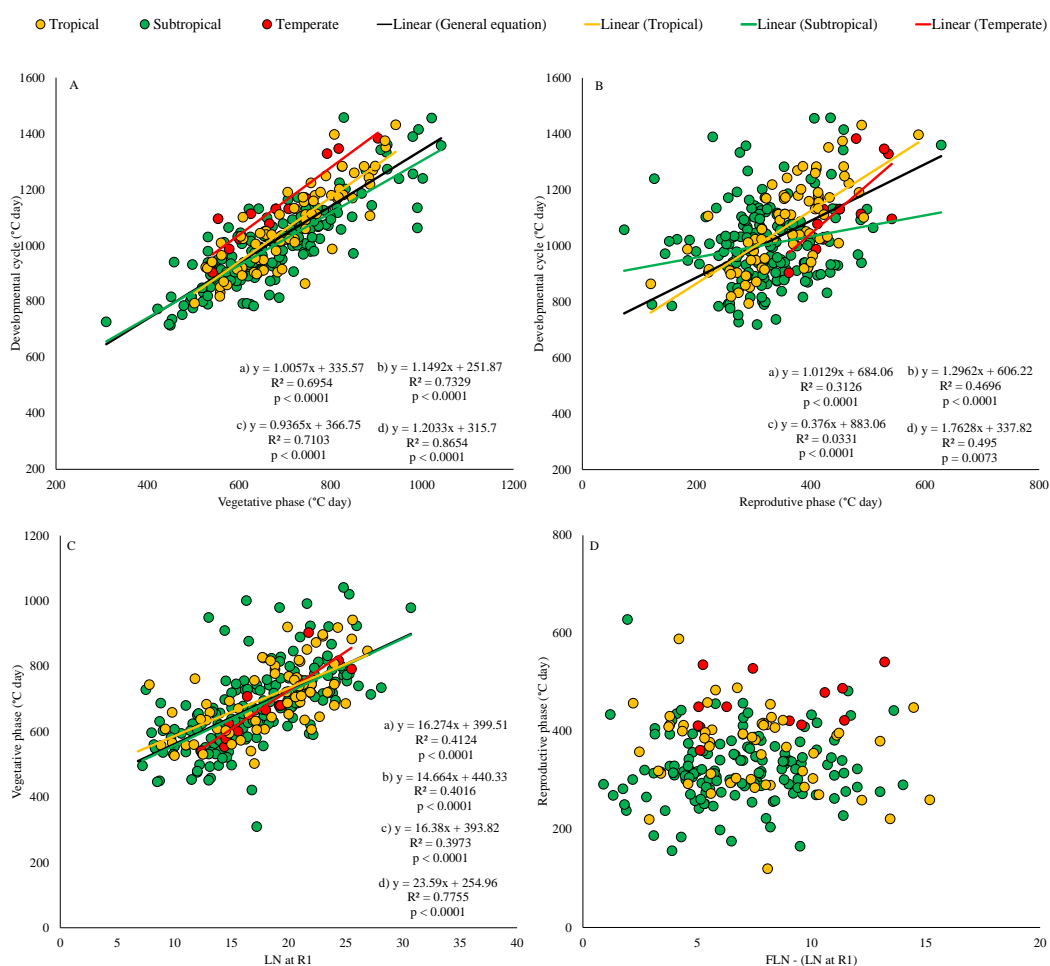


Figure 4. Relationship between the duration of total developmental cycle and (A) duration of the vegetative phase and (B) duration of the reproductive phase, (C) between the duration of the vegetative phase and the accumulated leaf number (LN) at the R1 Stage, and (D) duration of the reproductive phase and the leaf number after R1 [FLN – (LN at R1)]. Data from 28 field grown cut sunflower genotypes in cultivated in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype in a trial. Each equation corresponds to a) General equation, b)

Tropical, c) Subtropical and d) Temperate. Test F with p-value 5% of probability, when appear is significant.

3.1.3.4 Final leaf number

Overall, final leaf number (FLN) presented a significant relationship with all four variables analyzed (LN at R1, FLN – (LN at R1), duration of vegetative and duration of reproductive phases – Fig. 5). Here, we describe the results before and after the R1 stage. Before the R1 stage, the variable LN at R1 was dependent on the FLN (Fig. 5A), i.e., the higher the FLN, the greater the accumulated leaf number at the R1 stage, and the longer the vegetative phase (Fig. 5C). After R1 stage, FLN - (LN at R1) showed to be dependent on FLN in subtropical locations (Fig. 5B) and the reproductive phase was dependent on FLN only in tropical and temperate locations (Fig. 5D).

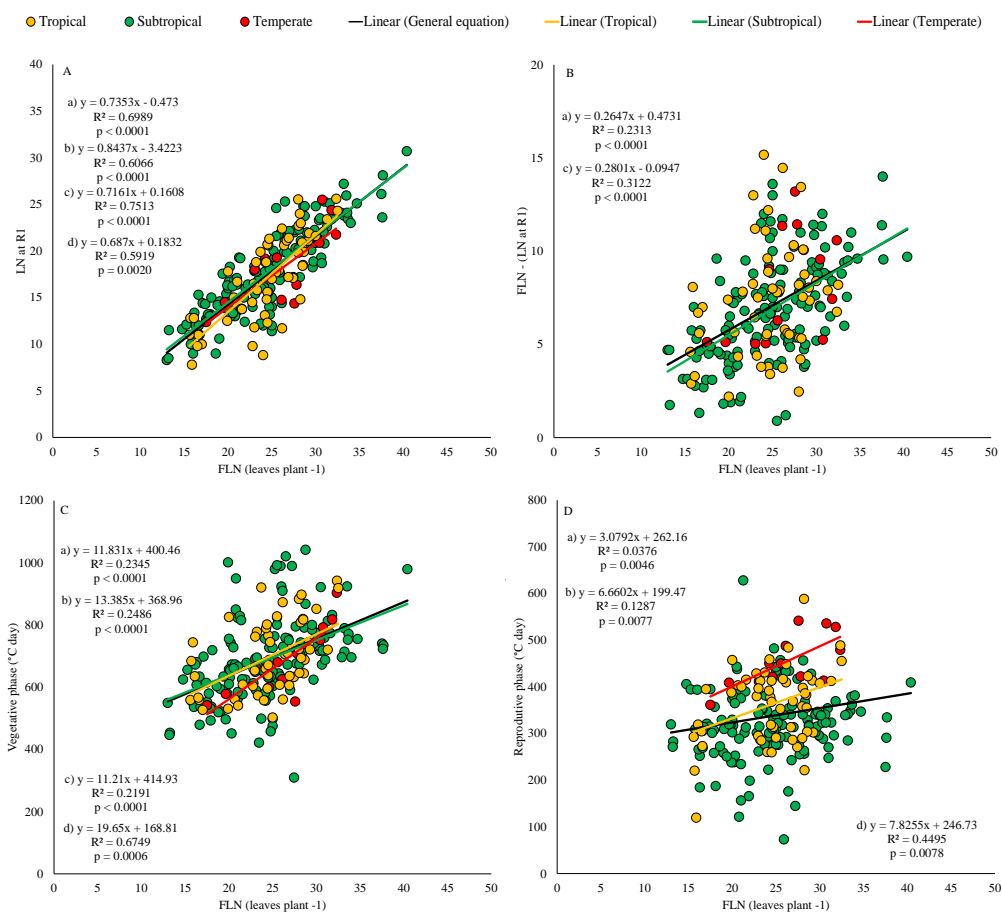


Figure 5. Relationship between (A) LN at R1 and final leaf number (FLN), (B) FLN – (LN at R1) and FLN, (C) vegetative phase and FLN and (D) reproductive phase and FLN. Data from 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype in a trial. Each equation corresponds to a) General equation, b) Tropical, c) Subtropical and d) Temperate.

The significant relationships found in Figures 2-5 are hypothesized to be due to genotypes. Aiming to test this hypothesis, we fit linear regressions separately for each genotype. Table 3 and 4 show the equations, *p* value, and R^2 for each genotype. Genotypes AG-01, VC-12, SP-07, MO-03 and DD-03 were the genotypes that had significance (Table 3) between FLN and FLN – (LN at R1). The “Other genotypes” was a group of genotypes that had only one point. We also analyzed each genotype for the FLN and LN at R1 relationship (Table 4). Five genotypes (VO-09, LT-05, SP-07, ST-08 and VP-10) did not show significant relationship.

Table 3. Equation, probability (*p*) value, and coefficient of determination (R^2) of linear regression of leaf number at R1 stage ([FLN – (LN at R1)] = *y*) against final leaf number (FLN = *x*) in 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial).

| Genotype | Linear regression | | |
|----------|--------------------------|-----------------------|--------|
| | Equation | <i>p</i> | R^2 |
| AG-01 | $y = 0.3213x - 1.098$ | 0.0055 ^s | 0.2796 |
| AO-02 | $y = 0.01495x + 7.213$ | 0.9536 ^{ns} | 0.0004 |
| SO-06 | $y = 0.1247x + 5.179$ | 0.2815 ^{ns} | 0.0607 |
| VC-12 | $y = 0.3551x - 0.6953$ | < 0.0001 ^s | 0.3087 |
| VT-11 | $y = 0.4440x - 4.156$ | 0.0880 ^{ns} | 0.2633 |
| VO-09 | $y = 0.5606x - 3.847$ | 0.0530 ^{ns} | 0.3915 |
| LT-05 | $y = 0.7059x - 15.39$ | 0.1196 ^{ns} | 0.6085 |
| SP-07 | $y = 0.8550x - 17.00$ | 0.0138 ^s | 0.7341 |
| MP-06 | $y = 0.2531x - 0.006715$ | 0.1122 ^{ns} | 0.4256 |
| ST-08 | $y = 0.6193x - 8.926$ | 0.1093 ^{ns} | 0.4308 |

| | | | |
|-----------------|-------------------------|----------------------|--------|
| FA-04 | $y = 0.1744x + 0.09671$ | 0.0716 ^{ns} | 0.5619 |
| MO-03 | $y = 0.5146x - 4.849$ | 0.0246 ^s | 0.8549 |
| DD-03 | $y = 0.2865x - 1.907$ | 0.4945 ^{ns} | 0.1235 |
| VP-10 | $y = 0.5553x - 5.207$ | 0.0450 ^s | 0.5854 |
| Other genotypes | $y = 0.3816x - 3.078$ | 0.0345 ^s | 0.3460 |

s. Significant 5% of probability ns. Not significant

Table 4. Equation, probability (p) value, and coefficient of determination (R^2) of linear regression of leaf number at R1 stage (LN at R1 = y) against final leaf number (FLN = x) in 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial).

| Genotype | Linear regression | | |
|----------|-------------------------|-----------------------|----------------|
| | Equation | p | R ² |
| AG-01 | $y = 0.6767x + 1.179$ | <0.0001 ^s | 0.6343 |
| AO-02 | $y = 1.035x - 8.759$ | 0.0014 ^s | 0.6940 |
| SO-06 | $y = 0.8802x - 5.236$ | <0.0001 ^s | 0.7578 |
| VC-12 | $y = 0.6445x + 0.7072$ | < 0.0001 ^s | 0.5947 |
| VT-11 | $y = 0.5072x + 4.778$ | 0.1514 ^s | 0.1777 |
| VO-09 | $y = 0.4401x + 3.964$ | 0.0945 ^{ns} | 0.2795 |
| LT-05 | $y = 0.2941x + 15.39$ | 0.4345 ^{ns} | 0.2125 |
| SP-07 | $y = 0.1547x + 16.75$ | 0.5314 ^{ns} | 0.0828 |
| MP-06 | $y = 0.7505x - 0.04923$ | 0.0024 ^s | 0.8648 |
| ST-08 | $y = 0.3807x + 8.926$ | 0.2854 ^{ns} | 0.2224 |
| FA-04 | $y = 0.8256x - 0.09671$ | 0.0323 ^s | 0.6335 |
| MO-03 | $y = 0.4758x + 5.144$ | 0.0098 ^s | 0.8427 |

Cont. **Table 3**

| | | | |
|-----------------|-----------------------|----------------------|--------|
| DD-03 | $y = 0.7152x + 1.876$ | 0.0063 ^s | 0.7379 |
| VP-10 | $y = 0.4447x + 5.207$ | 0.0866 ^{ns} | 0.4752 |
| Other genotypes | $y = 0.6156x + 3.154$ | 0.0003 ^s | 0.6856 |

s. Significant 5% of probability **ns.** Not significant

3.1.3.5 Phyllochron

Phyllochron was dependent on FLN ($R^2 = 0.1815$, $p < 0.0001$, Fig. 6A), decreasing as FLN increases. This decreasing relationship was also observed between phyllochron and LN at R1 ($R^2 = 0.1264$, $p < 0.0001$, Fig. 6B), where genotypes with higher phyllochron exhibited lower LN at R1. Duration of the vegetative and reproductive phases were not related to the phyllochron (Fig. 6C and 6D). In temperate climate, no relationships were identified. Tropical and subtropical presented significant relationship between phyllochron and FLN ($R^2 = 0.1417$ and $R^2 = 0.2323$, respectively) and between phyllochron and LN at R1 ($R^2 = 0.1481$ and $R^2 = 0.2393$). The slope of the linear regressions indicates a decrease in both cases.

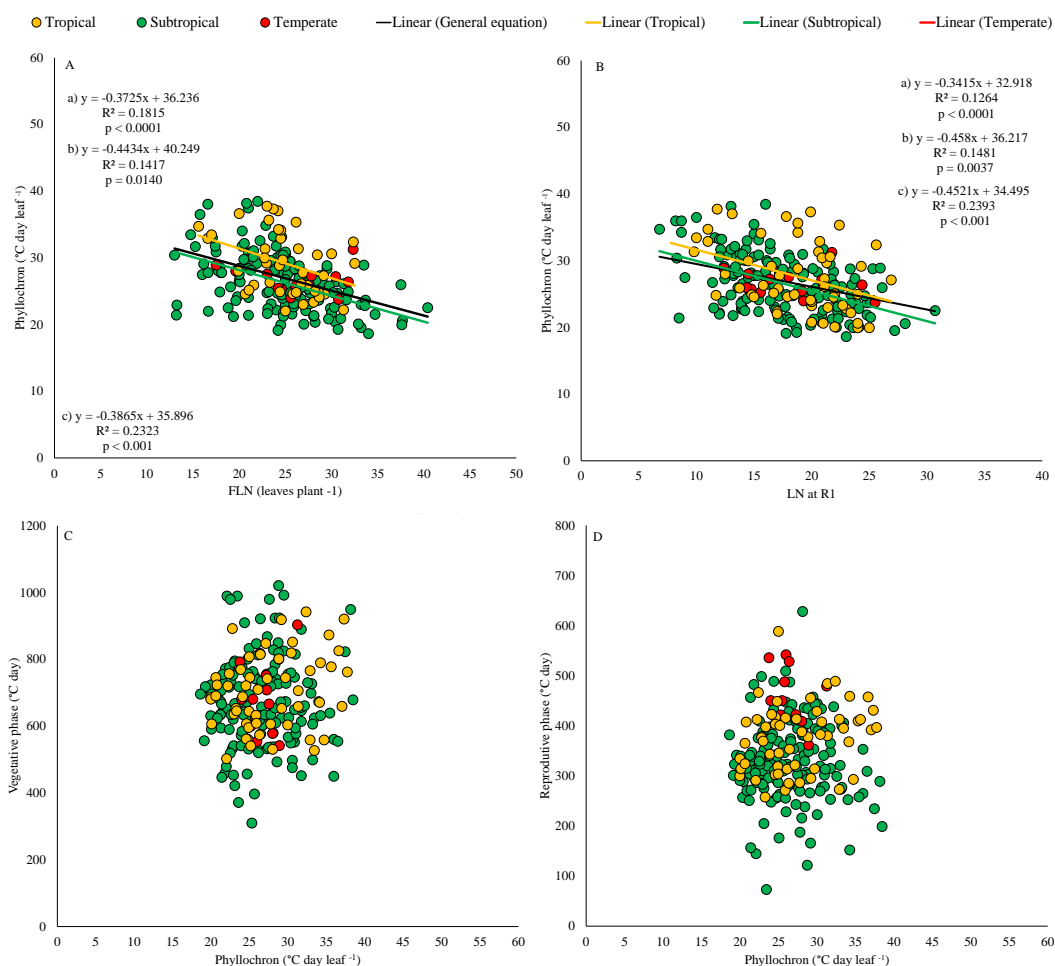


Figure 6. Relationship between (A) phyllochron (°C day leaf⁻¹) and final leaf number (FLN), (B) phyllochron and LN at R1, (C) vegetative phase (°C day) and phyllochron and, (D) reproductive phase (°C day) and phyllochron. 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype in a trial. Each equation corresponds to a) General equation, b) Tropical, c) Subtropical and d) Temperate. Test F with p-value 5% of probability, when appear is significant.

3.1.3.6 Leaf area index

Leaf area index (LAI) was shown to be dependent on FLN and phyllochron (Fig. 7). Thus, cut sunflower plants with higher FLN tend to have a higher LAI. This increasing trend was identified in tropical and subtropical locations (Fig. 7A). On the other hand, plants with a higher phyllochron tend to exhibit lower LAI values (Fig. 7B). This characteristic was more evident in the subtropical environment.

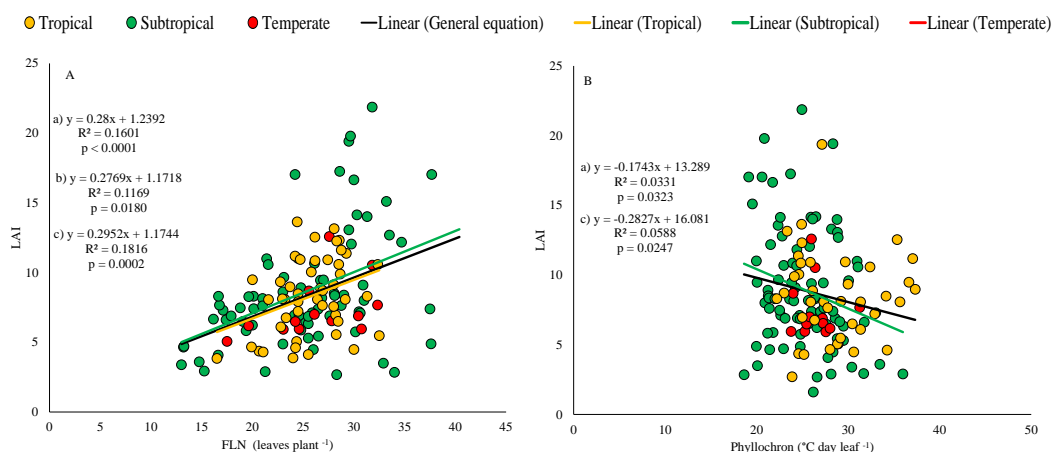


Figure 7. Relationship between leaf area index (LAI) and (A) final leaf number (FLN) and between (B) LAI and phyllochron ($^{\circ}\text{C day leaf}^{-1}$). Data from 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype inside the trial. Each equation corresponds to a) General equation, b) Tropical, c) Subtropical and d) Temperate. Test F with p-value 5% of probability, when appear is significant.

3.1.3.7 Flower production components

Relationships among flower production components were also analyzed by linear regressions (Fig. 8). It was observed that plants with a higher accumulation of thermal time from sowing to R5 stage, represented by developmental cycle ($^{\circ}\text{C days}$), had the tallest plants (Fig. 8A). Similarly, plant height was also related to FLN (Fig. 8B). This trend was observed in all three climates. As for head diameter, it is dependent on plant height, so plants with greater height have larger inflorescences (Fig. 8C). No relationship between stem diameter and plant height was observed (Fig. 8D).

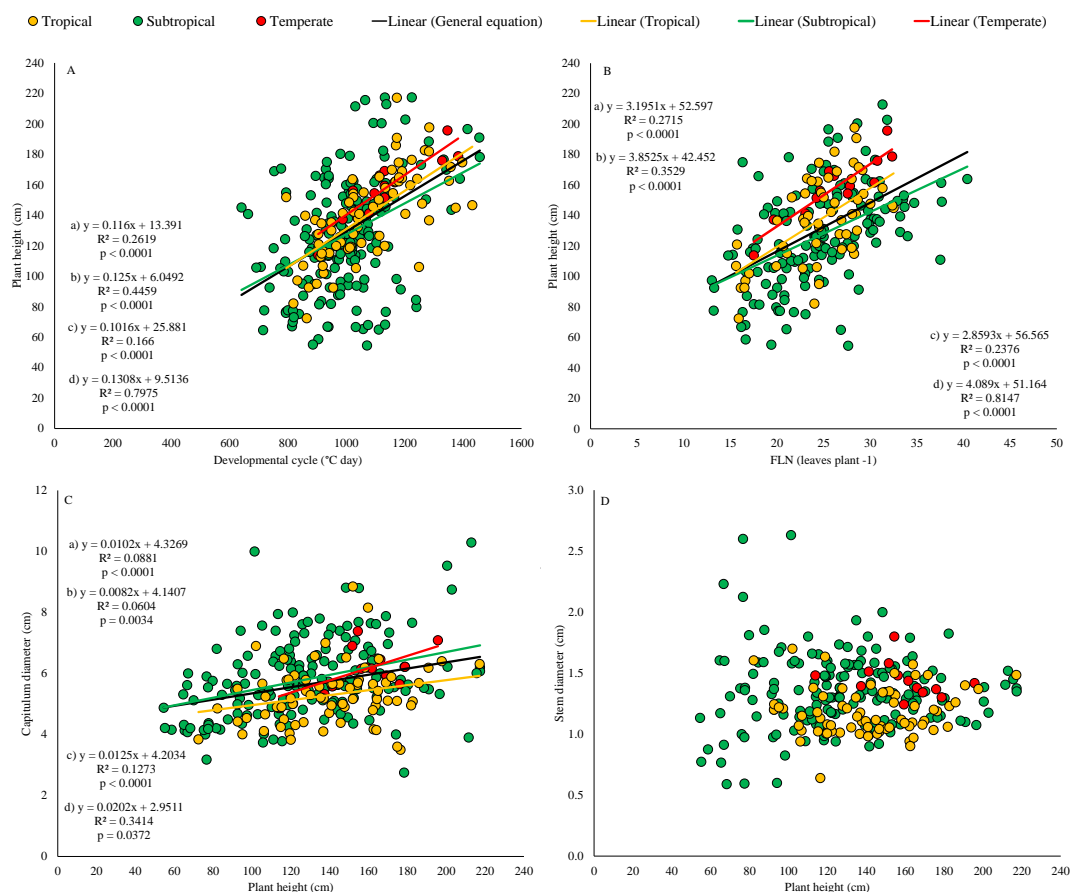


Figure 8. Relationship between (A) plant height (cm) and developmental cycle (°C day), (B) plant height (cm) and FLN (leaves plant⁻¹), (C) capitulum diameter (cm) and plant height (cm) and (D) stem diameter (cm) and plant height (cm). Data from 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype inside the trial. Each equation corresponds to a) General equation, b) Tropical, c) Subtropical and d) Temperate. Test F with p-value 5% of probability, when appear is significant.

The investigation of the relationship between leaf area index and plant height ($R^2 = 0.1947$, $p < 0.0001$), capitulum diameter ($R^2 = 0.3127$, $p < 0.0001$) and stem diameter ($R^2 = 0.1357$, $p < 0.0001$) revealed a positive and significant association through linear regression analysis (Fig. 9). In all tropical, subtropical, and temperate locations, positive relationships were found between LAI and flower production components so that plants with higher LAI

exhibit larger plant height, capitulum diameter, and stem diameter. Only for temperate environment there was no significant relationship between LAI and stem diameter (Fig. 9C).

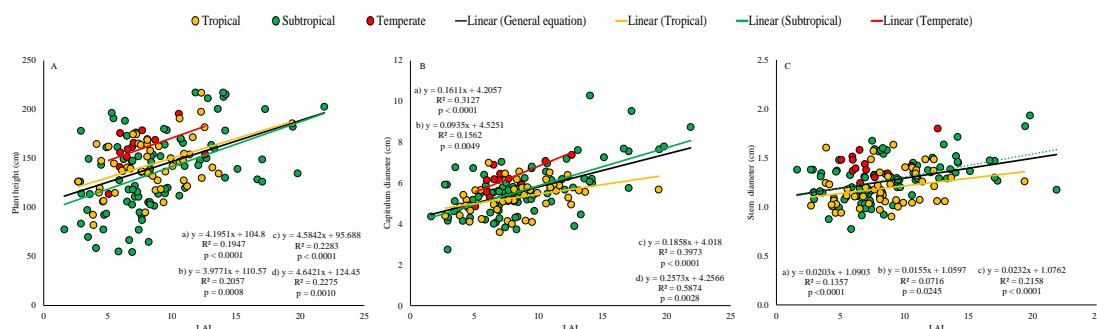


Figure 9. Relationship between leaf area index (LAI) and (A) plant height, (B) capitulum diameter and (C) stem diameter. Data from 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial) are pooled. Each point represents one genotype inside the trial. Each equation corresponds to a) General equation, b) Tropical, c) Subtropical and d) Temperate. Test F with p-value 5% of probability, when appear is significant.

3.1.3.8 Pooled data analysis

A pooled analysis of the three environments provided an overall summary about each single variable. The result from this analysis is presented in Supplementary File 1 and in box plots in Figure 10. The subtropical environment exhibits the greatest variation, especially in the FLN (Fig. 10D), LN at R1 (Fig. 10E), FLN – (LN at R1) (Fig. 10F), plant height (Fig. 10G), capitulum and stem diameter (Fig. 10H and 10I, respectively) variables. For example, FLN varied from 15.6 to 32.5 leaves plant⁻¹ in the tropics, from 13.0 to 40.4 leaves plant⁻¹ in the subtropics and from 17.5 to 32.3 leaves plant⁻¹ in temperate environment (Supplementary File 1). Variable FLN – (LN at R1) had the highest coefficient of variation in tropical (41.53%), subtropical (40.93%) and temperate (36.84%) environments.

Under subtropical environment, the coefficient of variation for plant height (CV = 28.85 %), capitulum diameter (CV = 21.88 %) and stem diameter (28.14 %) was higher than in tropical environment, with CV = 20.54 %, CV = 18.44 %, CV = 16.90 % for plant height, capitulum diameter and stem diameter, respectively. The smallest variation was in the

temperate environment for all three production components (Plant height, CV = 12.99%; Capitulum diameter, CV = 11.49%; Stem diameter, CV = 10.44%).

Values of the duration of total development cycle varied from 792.8 to 1431.0 °C day, 714.7 to 1457.0 °C day and 903.5 to 1383.0 °C day for tropical, subtropical, and temperate environments with median 1042.0, 999.0 and 1131.0 °C day, respectively. For the duration of total developmental cycle in days, only descriptive analyses were applied (Supplementary Table S1). The shortest cycle was 43, 40 and 81 days and the longest cycle 78.2, 110.8, 108 days for tropical, subtropical, and temperate environments, respectively.

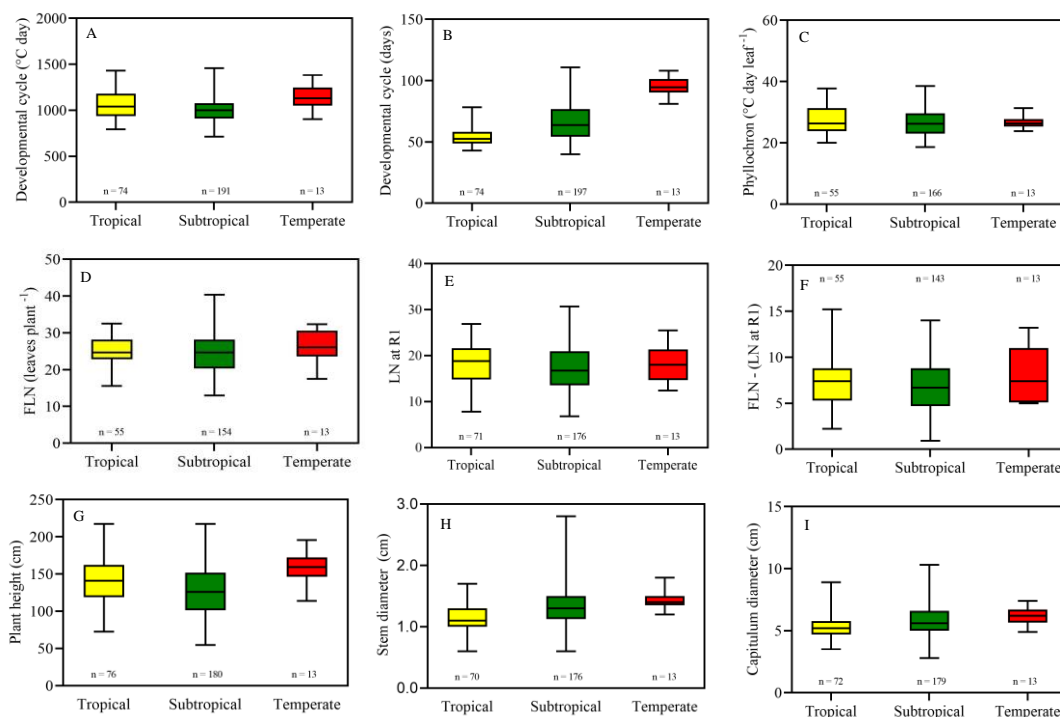


Figure 10. Box plots for (A) duration of total developmental cycle (°C day), (B) duration of total developmental cycle (days), (C) phyllochron, (D) final leaf number, (E) LN at R1, (F) FLN – (LN at R1), (G) plant height, (H) stem diameter and (I) capitulum diameter of 28 field grown cut sunflower genotypes in tropical, subtropical, and temperate environments in Brazil (four trials) and Italy (one trial). Solid line in the boxes is the median. n = number of samples.

3.1.4 Discussion

The network established among different Brazilian regions and Italy provided a robust dataset for understanding the genotype x environment x management x farmer (G x E x M x F) interaction for cut sunflower, providing robust information about adaptability and stability, as suggested by Matta et al. (2020). As a species of tropical and subtropical environments, that grow at an extensive range of latitudes in both hemispheres (northern and southern), sunflowers are affected by different environmental conditions (Schneiter, 1997, Hussain et al. 2018, Silva et al., 2022). According to Curti et al. (2012) sowing date is a key factor to obtain success in sunflower cultivation, prioritizing seasons that can satisfy the edaphoclimatic exigences on temperature, photoperiod, solar radiation, and water availability. Our trials provided sowings all year round, exposing the cut sunflower plants to several environmental conditions.

The time required for development depends on the genetic and growing season environment (Proietti et al., 2022). Also, different sunflower genotypes require different accumulated thermal time for development (Schneiter, 1997). Figures 2A and 2B graphically demonstrate the differences in total cycle time in days and in degree days, respectively. In the temperate environment, the cultivation of cut sunflowers occurred in late Winter in the Northern Hemisphere, extending into Spring. This condition exposed the genotypes to lower temperatures for a longer period, resulting in cycle (days) above average, and varying among genotypes. In the Southern Hemisphere, cut sunflower genotypes were grown in the tropics and subtropics of Brazil. The climate is warm in a tropical environment leading to the total development cycle being shorter than the average. The values above the mean value, represented by the black line, occurred only in the cultivation carried out in São João Del Rei/MG for some genotypes. This location is indicated as a subhumid tropical environment and the temperatures during the growing season were lower than the other locations in the same environment, influencing the duration of the developmental cycle in days (Supplementary File 2).

However, in °C day, there is less variation between environments than in days, and the duration of the developmental cycle is maintained within the same range (Fig. 3B and Fig. 10A). Essentially, these results reassure that the biological clock of cut sunflowers is regulated by air temperature and that thermal time is a better descriptor of time than calendar days (Streck et al., 2008, Streck et al., 2009a, Streck et al., 2011, Silva et al., 2020). The duration of total developmental cycle in °C day is driven by the duration of the vegetative and the reproductive phases, i.e., longer vegetative, and reproductive phases result a longer

development cycle. These results agree with those for *Gladiolus x grandiflorus* (Streck et al., 2011), a cut flower that also strongly responds to temperature.

During the developmental cycle of cut sunflower, there is an overlap between the vegetative and reproductive phases, so that leaf appearance continues after the R1 stage. The duration of the vegetative phase tends to be longer as the leaf number at the R1 stage (LN at R1) increases, while the reproductive phase does not depend on the number of leaves still to appear after this stage [FLN - (LN at R1)]. Our hypothesis is that when the cut sunflower plant initiates the reproductive phase at the R1 stage, the final leaf number is already defined, as reported by Anderson et al. (1978). These two main phases occur concurrently with the elongation sub-phase; thus, after the R1 stage, the leaves that have not yet emerged visually begin to become apparent due to the elongation of the internodes, which is more evident after the R1 stage. Additionally, the variables LN at R1 and FLN - (LN at R1) were found to be dependent on FLN, as well as the vegetative and reproductive phases (Fig. 5). Meanwhile, when genotypes were analyzed separately (Tables 3 and 4), just some genotypes demonstrate a relationship between these variables. Streck et al. (2009b) found similar results in *Oryza sativa* L., with differences among genotypes, among sowing dates and among years.

The phyllochron also showed a linear relationship with FLN and LN at R1. However, there is no relationship between the phyllochron and the duration of the phases in °C day. Villalobos and Ritchie (1992) found phyllochron for sunflower ranging from 20 to 25 °C day leaf⁻¹ and reported that low temperatures at the beginning of development are associated with low phyllochron, and there may be an inverse relationship between the phyllochron and the duration of the developmental cycle. Similarly, Aiken (2005) found a mean phyllochron of 25.3 °C day leaf⁻¹ for grain sunflower. Our results indicate a variation from 18.6 to 38.5 °C day leaf⁻¹, with greater variation in subtropical environments. These differences can be attributed to the wide range of genotypes evaluated and how each one responds to the conditions of each environment, as already demonstrated for *Dahlia* spp. (Fernandes et al., 2023), especially in countries like Brazil, which has a vast territorial extension and temperature conditions can vary within each environment, depending on latitude. The lack of relationship between the phyllochron and the developmental phases is not a characteristic found only in cut sunflowers. For instance, Walter et al. (2009) found the same lack of relationship for *Triticum aestivum* L., indicating that the trait responsible for the duration of the vegetative phase in cut sunflower is the final number of leaves and not the rate at which these leaves appear on the stem.

The sowing date plays a significant role in influencing the leaf area index, which decreased as the sowing date progressed throughout the year (Fig. 3D). But it also is influenced by the FLN and phyllochron (Fig. 7). This is an ecophysiological factor that has a significant influence on defining the flower production components of distinct species, especially in Southern Brazil where there is a wide variation in temperature and solar radiation throughout the year (Tagliapietra et al., 2018, Höhn et al., 2023). Due to its robustness and ease of field cultivation, cut sunflower producers typically sow from January to December, aiming to supply the flower market periodically and increasing the number of produced plants for specific celebrating dates. As shown in Figure 9, LAI has a major influence on the production components studied and considerable variation among genotypes. Moreover, cut sunflower genotypes with a longer developmental cycle and greater FLN produce plants with a bigger plant height and consequently, inflorescences with a large capitulum, in tropical and subtropical environments. Recent studies about ornamental sunflower conducted in the tropical semi-arid climate in Brazil by Silva et al. (2018) found interesting results among the cut sunflower growth, validating our results on the relationship between plant height and FLN. Contradictory to our results, a strong relationship between plant height and stem diameter was verified by the authors.

For cut sunflower, long stem are a desirable feature due to the stem management usually practiced until the flower reaches the final consumer (Mladenovic et al., 2020). The association of plant height, stem diameter, and inflorescence diameter are the main flower production components for commercial purposes (Silva et al., 2018). Furthermore, the results underscore the importance of considering temperature and other climatic variables in positioning the genotypes at sowing dates that provide suitable growing conditions and consequently improve aesthetic aspects. This is essential to ensure the supply of products that meet the standards required by the flower market.

The results from our study indicate that cut sunflower genotypes are well adapted to tropical, subtropical, and temperate environments. Indeed, the performance of cut sunflowers across such a wide range of temperatures provided by this extensive exposure is outstanding. Originality, *Helianthus annuus* as species, is from temperate climate. This knowledge about species associated with the advances in biotechnology aiming improve genetic variability allow conditions for cultivation in different climatic zones, as highlighted by Paiva and Cavalcante (2023). From the standpoint of the production chain, our results support the premise used by the "Flowers for All" project that, despite variations among genotypes and

environments, cut sunflowers are versatile and well adapted for the different edaphoclimatic conditions across Brazil, becoming an excellent opportunity for small and medium-sized farmers who wish to diversify their production throughout the year, holding considerable economic significance and represent a promising avenue for agricultural development, as emphasized by Puttha et al. (2023).

3.1.5 Conclusion

The results presented in this study demonstrated a significant impact of the tropical, subtropical, and temperate environments on the development, growth, and flower production of cut sunflower. The duration of vegetative phase has positive linear relationship with developmental cycle, as so reproductive phase, and it is determinate by final leaf number. Leaf number at R1 stage are positively related to vegetative phase, but number of leaves still to appear after R1 stage are not related to reproductive phase. On the other hand, leaf area index has negative linear relationship with sowing dates, likewise phyllochron and final leaf number. Flowers' production components are defined by leaf area index and final leaf number. Briefly, cultivars with longer development cycle have high final leaf number, low phyllochron and greater plant height than cultivars with shorter cycle. These findings contribute to expanding the knowledge about sunflower cultivation as a cut flower and offer valuable insights for developing more effective and sustainable strategies for floriculture not only in Brazil but also in regions with similar climatic characteristics.

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Credit authorship contribution statement

R.T. Writing – review & editing, Writing - original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **L. O. U.** Methodology, Investigation, Data curation. **L. C. B.** Investigation, Resources. **M. Z. B.-C.** Investigation, Resources. **P. B. L.** Investigation, Resources. **J. C. S.** Investigation, Resources. **S. N. R.** Investigation, Resources. **L. M. C.** Investigation, Resources. **A. T. P.** Investigation, Resources. **L. M. L.** Investigation, Resources. **H. F. F.** Investigation, Data curation. **S. M. S. V.** Investigation, Data curation. **L. G. O. S.** Investigation, Data curation. **T. L. T.** Investigation. **T. P. R.** Investigation, Data curation. **G. V. N.** Investigation, Data curation. **V. B. S. O.** Investigation, Data curation. **J. B. T. J.** Investigation. **A. T. I. C.** Investigation **D. B.** Investigation, Resources. **C. P. O. F.** Investigation, Data curation. **B. N.** Investigation, Resources. **A. M.** Conceptualization, Resources. **C. M. T. M.** Conceptualization, Methodology, Resources. **L. B.** Investigation, Data curation. **J. S. F. C.** Investigation, Data curation. **A. M. F. S.** Investigation, Data curation. **A. J. Z.** Supervision. **N.A.S.** Supervision, Project Administration, Conceptualization, Methodology, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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3.2 CAPÍTULO 2 – IMPROVING THE KNOWLEDGE ABOUT PHYLLOCHRON IN CUT SUNFLOWER

(Submetido à *Annals of Botany*, portanto as normas estão de acordo com as diretrizes exigidas)

1 **Issue section: Original Article**

2

3 **Improving knowledge about the phyllochron in cut sunflower**

4

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23

1 Abstract

2 • **Background and Aims:** A breakpoint in leaf appearance rate of *Helianthus annuus*
3 L., suggest a bilinear relationship to estimate phyllochron, an important developmental
4 variable that can be used to predict leaf appearance in crop simulation models. We aimed
5 (i) to estimate the phyllochron using a single and a bilinear model in field-grown cut
6 sunflower genotypes considering several sowing dates in tropical, subtropical, temperate
7 locations, and (ii) to test the hypothesis of a breakpoint as the indicator of change in
8 phyllochron during the ontogeny of cut sunflower.

9 • **Methods:** Leaf number was evaluated in several trials carried out during from 2020
10 to 2023 in different locations in Brazil, South America, and one location in Italy, Europe.
11 Leaf number was counted in fourteen cut sunflower genotypes until the final leaf number
12 and the accumulated thermal time was calculated from transplanting date. The
13 phyllochron was estimated by linear and bilinear regression between the leaf number and
14 the accumulated thermal time, and expressed in °C day leaf⁻¹.

15 • **Key Results:** Field observations have identified a breakpoint in leaf appearance
16 between V6 and V7 stages in cut sunflower genotypes resulting in an early and a late
17 phyllochron. The early phyllochron is higher (34.87°C day leaf⁻¹) than the late (21.82°C
18 day leaf⁻¹) and single (23.83°C day leaf⁻¹) phyllochron phases. The division of the
19 phyllochron into two phases is hypothesized to be related to leaf phyllotaxy and stem
20 elongation.

21 • **Conclusions:** We confirmed the existence of a breakpoint between the V6 and V7
22 stages, as evidenced by the bilinear regression between leaf number and thermal time, in
23 cut sunflower genotypes. This led to identify two phyllochron phases, an early and a late.
24 These results enhance our knowledge about the phyllochron and also provide an

1 ecophysiological basis for developing a chronology response function in process-based
2 models for cut sunflower.

3
4 **Key words:** Floriculture, cut flower, development, phenology, leaf appearance,
5 temperature, thermal time, breakpoint, *Helianthus annuus* L.

6 7 **3.2.1 Introduction**

8
9 *Helianthus annuus* L., commonly known as sunflower, is an annual dicotyledonous
10 plant that belongs to the Asteraceae family, native to temperate North America (Azania et
11 al., 2003; Baldotto and Baldotto, 2015). Widely grown as an oilseed crop, the use of
12 sunflower as ornamental plant started more than 200 years ago (Kutschera and Briggs, 2015;
13 Cvejić et al., 2016). Over the decades, the importance of sunflower increased largely,
14 expanding the number of genotypes available for gardens, as a pot flower, and as a cut
15 flower, been considered one of the most valuable floriculture crops (Curti et al., 2012; Neves
16 et al., 2015; Shatoori et al., 2021; Puttha et al., 2023).

17 Basic studies on plant development and growth are important for several reasons as
18 they give information that have practical application such as for improving crop simulation
19 models and the fine tuning of management practices for farmers (Oteng-Darko et al., 2013;
20 Jones et al., 2016; Pasquel et al., 2022). Plant development and plant growth are related but
21 different processes: while plant development refers to cell differentiation, organ initiation
22 and appearance, and extends to plant senescence, plant growth is usually referred to an
23 irreversible increase in the physical dimension of an individual or organ with time (Anderson
24 et al., 1978; Wilhelm and McMaster, 1995; Dellai et al., 2005). In sunflowers, a few basic
25 studies on plant development and growth are available (Robison, 1971; Marc and Palmer,
26 1981; Sadras and Hall, 1988), and sunflower development was described by Schneiter and

1 Miller (1981) in developmental stages, dividing the developmental cycle into vegetative and
2 reproductive phases. Vegetative phase details the leaf appearance on the stem (Walter et al.,
3 2009; Schwab et al., 2015; Buffon et al., 2022). Leaf number depends upon the rate of
4 primordium formation at the stem apical meristem (apex) and the rate of leaf appearance
5 (Tenorio et al., 2017). In sunflower, the first leaves appear in opposite alternate pairs and
6 gradually develop a spiral phyllotaxy of alternate leaves (Schneiter and Miller, 1981). A
7 widely used variable to describe the rate that leaves appear in a plant is the phyllochron,
8 defined as time required for two successive leaves to appear on a stem, having as unit time
9 leaf⁻¹ (Wilhelm and McMaster, 1995; Xue et al., 2004).

10 Photoperiod plays an important role in sunflower development (Goyne and Hammer,
11 1982), but temperature is the major environmental factor driving leaf appearance in
12 sunflower (Doyle, 1975; Connor and Sadras, 1992) and many other floriculture crops as
13 *Chrysanthemum x morifolium* Ramat. (Streck, 2004), *Gladiolus x grandiflorus* (Streck et al.,
14 2012), *Eustoma grandiflorum* (Raf.) Shinnery (Höhn et al., 2023), and *Dahlia* sp. (Fernandes
15 et al., 2023). For sunflower, the base temperature for leaf appearance is 4°C (Villalobos and
16 Ritchie, 1992). One approach to consider the effect of temperature on plants from a
17 biological point of view is thermal time (TT), with units of °C day (Streck et al., 2009a;
18 Ongarato et al., 2020). Assuming a linear relationship between leaf appearance (leaves stem⁻¹
19 ¹) and thermal time (°C day), it is possible to estimate the phyllochron with unit of °C day
20 leaf⁻¹ (Wilhelm and McMaster, 1995; Xue et al., 2004).

21 In sunflower with grain purposes, Villalobos and Ritchie (1992) and Sadras and
22 Villalobos (1993) suggested an inflection point at six leaves in the relationship between
23 accumulated leaf number and accumulated thermal time from emergence, indicating that there
24 is a breakpoint in leaf appearance of sunflower between V6 and V7 stages (6 and 7 leaves,
25 respectively). The hypothesis to explain a change in phyllochron during the ontogeny of a

1 sunflower plant is that the inflection point occurs when sunflower leaf phyllotaxis changes
2 from opposite to alternate leaves (Schneiter and Miller, 1981; Sadras and Villalobos, 1993).
3 A breakpoint in leaf appearance and consequently two phyllochrons during the leaf
4 appearance phase was also reported for *Triticum aestivum* L. (Streck et al., 2003), *Hordeum*
5 *vulgare* L. (Abeledo et al. 2004), *Fragaria x ananassa* (Rosa et al., 2011), *Opuntia stricta*
6 Haw. (Silva et al., 2023), and *Oryza sativa* L. (Egle et al., 2015).

7 The fact that the phyllochron is not constant during the vegetative phase may have
8 direct impact on eco-physiological variables such as leaf area. The final leaf area is
9 determined by the emergence of new leaves during the ontogeny, which intercept solar
10 radiation and consequently influence several physiological processes, including crop
11 photosynthesis, growth, transpiration, photon interception, and defining yield components
12 (Rouphael et al., 2007; Tagliapietra et al., 2018). Moreover, practical applications of the
13 phyllochron concept extend to crop simulation models, which either utilize or are based on
14 the phyllochron to model the dynamics of leaf development such as for *Triticum aestivum*
15 L. (Streck et al., 2003; Xue et al., 2004), *Glycine Max* L. (Setiyono et al., 2007), and *Oryza*
16 *sativa* (Streck et al., 2008a). The hypothesis of a change in leaf appearance rate and
17 consequently a change in the phyllochron during the leaf appearance phase in ornamental
18 sunflower has not been tested, which constitutes a rationale for this study. The objectives in
19 this study were (i) to estimate the phyllochron using a single and a bilinear model in field-
20 grown cut sunflower genotypes considering several sowing dates in tropical, subtropical,
21 temperate locations, and (ii) to test the hypothesis of a breakpoint as the indicator of change
22 in phyllochron during the ontogeny of cut sunflower.

23

24 **3.2.2 Material and methods**

25

1 3.2.2.1 Field studies

2
3 Data used in this study are from four years (from 2020 to 2023) of field on farm trials
4 conducted at different locations and sowings to evaluate the leaf appearance of several cut
5 sunflowers (Table 1). The regions of the study were the Northeast, Midwest, and South
6 regions in Brazil, South America, and one location in Tuscany, Italy, Europe (Fig. 1). In
7 Brazil, the regions of study are divided in two climatic zones: tropical and subtropical. The
8 tropics in Brazil represents 81.4% of the territory and have high temperatures (above 18 °C),
9 with a wet and dry season. Southern Brazil is located below the Tropic of Capricorn and has
10 Cf climate (humid subtropical climate with no dry season), as defined in the Köppen climate
11 classification (Alvares et al. 2013; Roth, 2007). According to Blasi et al. (2014) the region
12 of study in Italy is in an Apennine province with temperate climate, where the minimum
13 temperature in the coldest month is below 3 °C and maximum temperatures can exceed 30
14 °C in the hottest month.

15 Twelve *Helianthus annuus* L. genotypes for cutting purposes were used from
16 different private international breeding companies: ‘Amalfi Gold’ (AG-01), ‘Amalfi Orange’
17 (AO-02), ‘Stromboli Orange’ (SO-06), ‘Vesuvio Orange’ (VO-09), ‘Vesuvio Tangy’ (VT-
18 11), ‘Magic Orange’ (MO-03), ‘Magic Orange Pro’ (MP-06), ‘Vesuvio Orange Pro’ (VP-
19 10), ‘Favola’ (FA-04), ‘Stromboli Orange Pro’ (SP-07), ‘Stromboli Tangy’ (ST-08), and
20 ‘Vincent’s Choice’ (VC-12). Information about experimental design and treatments of the
21 trials are described in Supplementary 1.

22 The trials in Brazil followed the same management practices. Sowing was carried out
23 in plastic trays with commercial substrate and kept protected until transplanting to beds,
24 which was performed when the first pair of true leaves had a blade length of 2 centimetres
25 and well-developed roots system. Beds were previously prepared with base fertilization of
26 500 g m⁻² of lime and 50 g m⁻² of NPK Formula 05-20-20, spread and incorporated into the

1 soil. Each bed was one meter in width and 25 – 30 cm in height. Plant density was 32 plants
2 m⁻² distributed in 4 rows spaced 0.20 m among rows and 0.125 m within rows. About 10 to
3 15 days after transplanting, a topdressing fertilization was applied using 25 g m⁻² of
4 potassium chloride and 25 g m⁻² of urea. Cultural practices, including pest and disease
5 control as required, manual hoeing for weed control, and drip irrigation applied as necessary
6 to prevent soil water deficit, were conducted. Each plot was individually staked with bamboo
7 stakes or wooden slats at the four corners, and raffia string was used at 2 to 3 heights as the
8 plants grew taller during their developmental cycle.

9 The field grown trial conducted in Pescia - Tuscany/Italy, at the Research Centre for
10 Vegetable and Ornamental Crops (CREA-OF), followed the same methodology of the trials
11 in Brazil adapting the cultural practices for the local conditions. Plants were cultivated in
12 plots standardized at 0.80 m² with 30 plants distributed in three rows spaced 20 cm apart,
13 with 0.125 m among plants in the row. Each plot received 50 g of 16-9-12 NPK fertilizer at
14 transplanting and 50 g of 18-46 NP fertilizer 28 days after transplanting, as side dressing.

15 In addition, a field trial was conducted in Santa Maria, located in the Central Region
16 of Rio Grande do Sul State, Southern Brazil, from August to November 2020. The objective
17 of this trial was to assess the influence of planting methods, namely direct sowing and
18 transplanting, on the phyllochron of cut sunflower. Six cut sunflower genotypes were
19 evaluated in a factorial randomized complete block experimental design with four
20 replications.

21

22 3.2.2.2 Data collecting and meteorological data

23

24 Plants in the central rows were tagged with coloured wires one week after
25 transplanting. The number of tagged plants varied from 10 to 24 according to the trial. The

1 accumulated leaf number (ALN) on the stem was counted once or twice a week in the tagged
 2 plants from V1 (one leaf) until the final leaf number (FLN), which occurred near the R4
 3 stage (Schneiter and Miller, 1981). A leaf was assumed and counted when it presented blade
 4 length of at least 2 centimetres. This approach rendered a thorough and detailed dataset on
 5 ALN. Weather data in Brazil were from the Brazilian National Institute of Meteorology
 6 meteorological stations or private meteorological stations located as close as possible to each
 7 site and weather data in Italy were from the meteorological station Research Centre for
 8 Vegetable and Ornamental Crops (CREA-OF). Mean daily air temperature (T_{mean}) was
 9 calculated as the average of daily minimum and maximum temperature.

10

11 3.2.2.3 Thermal time approach

12

13 Different methods can be used to calculate the thermal time (Streck et al., 2009). In
 14 this study, was used the same method to calculate thermal time as previously used by several
 15 authors such as Paula et al. (2005), Streck et al. (2008, 2009), Rosa et al. (2009), Delatorre
 16 et al. (2022). Daily thermal time (TT, °C day) was estimated according to the equation
 17 proposed by Gilmore and Rogers (1985) and Arnold (1960):

18

$$19 \quad TT = \{(T_{opt} - T_b) * [(T_B - T_{mean}) / (T_B - T_{opt})]\}$$

$$20 \quad \text{If } T_b > T_{mean} > T_B, TT = 0$$

$$21 \quad \text{If } T_{mean} < T_{opt}, TT = T_m - T_b$$

$$22 \quad \text{If } T_{mean} > T_{opt}, TT = T_{opt} - T_b * \left(\frac{T_{mean} - T_B}{T_{opt} - T_B} \right)$$

23

24 where T_b , T_{opt} and T_B are the cardinal (minimum, optimum, and maximum) temperatures
 25 for sunflower development defined by Villalobos et al. (1996) as 4 °C, 28 °C and 40 °C,

1 respectively. The accumulated thermal time (ATT, °C day) from transplanting was
2 calculated by accumulating the TT values, that is, $ATT = \sum TT$ (Streck et al., 2008b).

3 4 3.2.2.4 Phyllochron

5
6 The phyllochron (PHYL, °C day leaf⁻¹) was calculated by the inverse of the slope of
7 the linear regression (1/b) between ALN as dependent variable and ATT as independent
8 variable (Streck et al., 2009, Ferreira et al., 2019). Three phyllochron values were estimated
9 and named as Single, Early, and Late phyllochron phases. The Single phyllochron
10 (PHYL_{single}) was estimated considering the leaf appearance from V1 to FLN. The Early and
11 Late phyllochrons were estimated by dividing the leaf appearance into two phases, from V1
12 to V6 stage (PHYL_{early}), and from V7 to FNL (PHYL_{late}) as suggested by Villalobos and
13 Richie (1992).

14 15 3.2.2.5 Statistical analysis

16
17 Phyllochron data were analysed for planting methods and for sowing dates of multi-
18 location and multi-year dataset, that were grouped in seasons (Spring, Summer, Autumn,
19 and Winter), locations, and genotypes. Shapiro-Wilk and Levene tests were applied for test
20 the normality and homogeneity. The planting methods trial presented normal and
21 homogeneous data and was analysed using ANOVA. The significance of differences was
22 tested with Tukey's test at 5% probability, to compare the phyllochron phases in the different
23 planting methods. The dataset for seasons, locations and genotypes presented non-normality
24 and non-homogeneity and were analysed with Kruskal-Wallis non parametric test and a
25 post-hoc Dunn test, with p-value adjusted as Bonferroni's methodology. Location, season
26 and genotype were tested for each phyllochron phase. The statistical analysis was performed
27 using RStudio program (R Core Team).

1 3.2.3 Results

2

3 3.2.3.1 Meteorological data

4

5 There was variation in meteorological conditions during the leaf appearance phase in
6 the fourteen locations over the years (Table 2). Temperature varied from -2.0 °C in
7 Vacaria/RS (lower than the lower base temperature for sunflower) to 37.7 °C in Santa
8 Maria/RS (near to upper base cardinal temperature for sunflower). Photoperiod varied from
9 11.2 hours to 14.5 hours along the different growing seasons, especially in subtropical
10 locations, where photoperiods change as temperatures change, from Spring to Winter. This
11 variation in meteorological conditions provides a robust dataset of different environments
12 that sunflower genotypes were exposed during their leaf appearance phase.

13

14 3.2.3.2 ALN and ATT relationship

15

16 A linear highly correlated relationship between accumulated leaf number (ALN) and
17 accumulated thermal time (ATT), with an overall coefficient of determination (R^2) higher
18 than 0.90 was found for all genotypes, locations and sowing dates. These results indicate that
19 temperature was a major factor driving leaf appearance and therefore the estimate of the
20 phyllochron from the inverse of the slope of the relationship is a suitable approach (Streck
21 et al., 2009b; Rosa et al., 2011) Fig. 2 depicts an example when ALN was regressed against
22 ATT for a single phyllochron for the genotype AO-02 in Brazil (sowing in 06/08/2020, Fig.
23 2A) and for the genotype AG-01 in Italy (sowing in 28/02/2023, Fig. 2B), and for the two
24 phases of the phyllochronbreakpoint between six and seven leaves (Fig. 2C and D). It was
25 observed that in all locations and genotypes the breakpoint occurred between 200 and 300
26 °C day.

1 3.2.3.3 Planting methods

2
3 Shapiro-Wilk test indicates normality of the residuals (p -value = 0.1974) and
4 Levene's test indicates homogeneity (p -value = 0.4735). Among single-factor, ANOVA
5 indicates significant differences in planting methods, genotypes and phyllochron phases.
6 Between planting methods, transplanting has higher phyllochron ($29.15\text{ }^{\circ}\text{C day leaf}^{-1}$) than
7 direct sowing ($27.38\text{ }^{\circ}\text{C day leaf}^{-1}$). There are no significant interactions among three-
8 factor interactions planting methods x genotypes x phyllochron phase (p -value = 0.9179), at
9 5%. Among two-factor interactions, planting methods x genotypes (p -value = 0.7946), and
10 planting methods x phyllochron phases (p -value = 0.7129) were not significant. On other
11 hand, the interaction genotype x phyllochron phases was significant (p -value = 0.0005).
12 Differences are presented in Table 3. Phyllochron differed between phyllochron phases,
13 with the $\text{PHYL}_{\text{early}}$ having the highest values. $\text{PHYL}_{\text{single}}$ does not differ between genotypes,
14 varying from 23.78 to $25.93\text{ }^{\circ}\text{C day leaf}^{-1}$. On contrary, $\text{PHYL}_{\text{early}}$ and $\text{PHYL}_{\text{late}}$ differ
15 between genotypes, with 'AG-01' having the highest phyllochron in the early phase (41.75
16 $^{\circ}\text{C day leaf}^{-1}$) and 'SO-06' in the late phase ($23.91\text{ }^{\circ}\text{C day leaf}^{-1}$). Among genotypes, 'AG-
17 01', 'MO-03' and 'VO-09' differed between $\text{PHYL}_{\text{single}}$ and $\text{PHYL}_{\text{late}}$ and, for the other tree
18 genotypes single and late phyllochron are equals by Tukey test.

19

20 3.2.3.4 Phyllochron phase

21
22 Dataset present non-normality by Shapiro-Wilk test (p – value < 0.0000) and non-
23 homogeneity by Levene's test (p – value = 0.0002) and the phyllochron data were presented
24 as median with interquartile range. Among the phyllochron phases (Fig. 3), there was a
25 statistical difference by Kruskal-Wallis (p – value < 0.0000). Early phyllochron presented

1 the higher value ($34.87\text{ }^{\circ}\text{C day leaf}^{-1}$), followed by single phyllochron ($23.83\text{ }^{\circ}\text{C day leaf}^{-1}$) and late phyllochron ($21.82\text{ }^{\circ}\text{C day leaf}^{-1}$), i.e. $\text{PHYL}_{\text{early}} > \text{PHYL}_{\text{single}} > \text{PHYL}_{\text{late}}$.

3 The main effects on phyllochron (location, season, and genotype) were analysed for
4 each phyllochron phase. Among locations, Kruskal-Wallis and post hoc Dunn Test detected
5 differences (Fig. 4). Júlio de Castilhos/RS differed from Pescia/Italy, Santa Maria/RS and
6 Teresina/PI for the single phyllochron, while Santa Maria/RS differed from Rio do Sul/SC,
7 Novo Xingú/RS, Herval D'Oeste/SC and Bozano/RS (Fig. 4A). Median values of the single
8 phyllochron were $31.10\text{ }^{\circ}\text{C day leaf}^{-1}$ for Júlio de Castilhos/RS and $22.49\text{ }^{\circ}\text{C day leaf}^{-1}$ for
9 Santa Maria/RS. Differences in early phyllochron were also detected (Fig. 4B). Rio do
10 Sul/SC ($51.17\text{ }^{\circ}\text{C day leaf}^{-1}$) and Pescia/Italy ($45.30\text{ }^{\circ}\text{C day leaf}^{-1}$) obtained the highest
11 values of early phyllochron and differed from Boa Vista do Sul/RS, Herval D'Oeste/SC,
12 Santa Maria/RS, Dourados/MS and Teresina/PI. The lowest median value of the late
13 phyllochron was observed in Santa Maria/RS ($20.27\text{ }^{\circ}\text{C day leaf}^{-1}$) which differed from
14 other locations, such as Herval D'Oeste/SC, Júlio de Castilhos/RS, Novo Xingú/RS and
15 Bozano/RS, which presented higher values (Fig. 4C).

16 Among growing seasons, phyllochron differs between seasons for single phyllochron
17 (Fig. 5A) and late phyllochron (Fig. 5C). Dunn test indicates that the single and late
18 phyllochron of cut sunflowers sown in Autumn and Spring differed from those sown in
19 Winter. Single phyllochron varied from $23.22\text{ }^{\circ}\text{C day leaf}^{-1}$ in Winter to $26.09\text{ }^{\circ}\text{C day leaf}^{-1}$
20 in Spring and late phyllochron varied from $20.82\text{ }^{\circ}\text{C day leaf}^{-1}$ in Winter to $24.41\text{ }^{\circ}\text{C day}$
21 leaf^{-1} in Spring. The early phyllochron did not differ among seasons, ranging from $32.21\text{ }^{\circ}\text{C}$
22 day leaf^{-1} in Summer to $37.39\text{ }^{\circ}\text{C day leaf}^{-1}$ in Autumn (Fig. 5B).

23 Among genotypes (Fig. 6) no statistical differences were found between genotypes
24 in early phyllochron by Kruskal-Wallis (p – value = 0.2872). Early phyllochron varied from
25 the lower value of $27.2\text{ }^{\circ}\text{C day leaf}^{-1}$ for FA-04 to the greater value of $40.30\text{ }^{\circ}\text{C day leaf}^{-1}$

1 for MP-06. On other hand, Kruskal-Wallis indicated statistical differences in single
2 phyllochron (p – value = 0.0002) and late phyllochron (p – value = 0.0181). The genotype
3 VT-11 presented a single phyllochron of 21.40 °C day leaf⁻¹ and differed from SO-06 and
4 VC-12, with 25.15 °C day leaf⁻¹ and 24.30 °C day leaf⁻¹, respectively (Fig. 6A). However,
5 the post-hoc Dunn test with p -value adjusted as Bonferroni's did not show differences among
6 genotypes in late phyllochron (Fig. 6C).

7

8 **3.2.4 Discussion**

9 The multi-location, multi-year, and multi-environment field on farm trials conducted
10 in Brazil, along with the trial conducted in Italy, allowed for the assessment of leaf
11 appearance in different cut sunflower genotypes exposed to various edaphoclimatic
12 conditions, management practices, and variations in temperature and photoperiod. Our
13 approach advances by overcoming the limitations of previous studies about phyllochron in
14 sunflower (Vilallobos and Ritchie, 1992), by employing a comprehensive robust dataset
15 regarding leaf appearance in cut sunflower under field grown conditions, covering a wide
16 range of cut sunflower genotypes for analyzing patterns in the phyllochron. Exposing cut
17 sunflowers plants to a wide range of growing conditions provide robustness in studies
18 assessing basic plant growth and development processes, as well as in using thermal time as
19 a measure of biological time in plants (Streck et al., 2005; Streck et al., 2008a,b).

20 The relationship between accumulated leaf number and accumulated thermal time
21 showed a bilinear relationship with a high coefficient of determination (Fig. 2). Previous
22 studies on phyllochron in sunflower crops have indicated differences in leaf appearance
23 rates, emphasizing the necessity of measuring leaf emergence in two phyllochron phases
24 compared to a single phyllochron (Aiken, 2005). Similar studies have described the
25 occurrence of more than one phyllochron phase in other species. In strawberry, breaking the

1 phyllochron into vegetative and reproductive phases better represented leaf development
2 dynamic compared to a single phyllochron (Rosa et al., 2011). In long-cycle crops, such as
3 sugarcane and forage cactus, the phyllochron was also broken into early and late phases
4 (Streck et al., 2010; Silva et al., 2023).

5 The hypothesis that the planting method (direct sowing or transplanting) could
6 contribute to changes in the phyllochron was tested, given that transplanting seedlings could
7 accelerated maturity by 16 days compared to sunflowers grown by direct sowing (Ahmad et
8 al., 2020). However, the results of the planting methods trial (Table 3) demonstrated that the
9 planting method does not influence the phases of the phyllochron, thereby rejecting this
10 hypothesis. Furthermore, genotypes exhibited variation within both the early and late
11 phyllochron phases, contrary to the results obtained for the single phyllochron.

12 As mentioned, the results showed no difference among genotypes within the early
13 and late phyllochron phases. The greatest differences within the phases occurred mainly due
14 to location. Differences among mainly occurred for the late phyllochron. The results can be
15 explained by the environment, as they differ in temperature and photoperiod. From
16 emergence to the R1 stage, sunflower is more sensitive to long days, and after the R1 stage,
17 to short days (Aiken, 2005). According to MacDonough et al. (2004) the phyllochron in
18 Spring-sown (photoperiod 12-14 hours) ranged from 30 to 45 °C day leaf⁻¹ as a single
19 phyllochron, and this range was reduced to 30 to 35 °C day leaf⁻¹ when the photoperiod was
20 extended by 2 hours, reducing the final leaf number, in the same season.

21 Among the multilocation trials, the results demonstrate that in cut sunflower
22 genotypes the interquartile range of phyllochron varies from 21 to 27 °C day leaf⁻¹ in single
23 phyllochron, 30 to 42 °C day leaf⁻¹ in early phyllochron and 19 to 25 °C day leaf⁻¹ in late
24 phyllochron. These ranges are similar to the findings of Villalobos and Ritchie (1992), where
25 the single phyllochron in sunflower crop typically ranges from 20 to 25 °C day leaf⁻¹, the

1 early phyllochron (until six leaves) ranges from 35 to 43 °C day leaf⁻¹ and late phyllochron
2 (seven leaves upwards) ranges from 18 to 29 °C day leaf⁻¹. These results confirm that
3 phyllochron in cut sunflower is slower for the first six leaves (early phyllochron) and faster
4 from seven leaves upwards (late phyllochron). On the contrary, in some grass species such
5 as rice (Streck et al., 2008a) and sorghum (Clerget et al., 2008), the early phyllochron is
6 faster and the late phyllochron is slower. For wheat, ontogeny was identified as the cause of
7 these changes in leaf appearance (Streck et al., 2003; Baumont et al., 2019). The seed
8 reserves increases the leaf appearance rate for the first two leaves, and the subsequent leaves
9 take more time to appear due to the distance that each leaf tip needs to traverse until its
10 exposure, as a result of the elongation of the whorl (Streck et al. 2003).

11 In cut sunflower ontogeny, the opposite occurs. Seeds reserves are responsible for
12 seed germination and early seedlings development, until the development of cotyledons and
13 first leaves, when the photosynthetic activity starts to increase (Erbaş et al., 2016). Until the
14 V6 stage, leaves emerge in opposite pairs, and internode elongation is slow in an acropetal
15 direction, beginning only when the first pair of true leaves is about two to three centimeters
16 long (Garrison, 1973). As leaf expansion takes place, a subtle internode elongation is noted
17 to emit the next pair of leaves. From the V7 stage onwards, leaves appear in an alternate
18 sequence, and stem elongation follows a sigmoidal pattern (Seiler, 1997). As alternate leaves
19 appear and the R1 stage (bud appearance) approaches, the rate of internodes elongation
20 increases fastly until reaching their maximum length near the R4 stage. At the meantime,
21 there is an overlapping of the leaf development and the reproductive phase, so that the leaf
22 appearance continues after the R1 stage and the final leaf number occurs close to R4 stage,
23 when stem elongation stabilizes.

24 The change in leaf phyllotaxy (from opposite leaf pairs to alternate single leaves) at
25 V6 and V7 stages has been hypthesized to be in charge of the change in phyllochron in

1 sunflower (Sadras and Villalobos, 1993). We hypothesize that the change is phyllochron
2 may be or is also related to stem elongation, i.e. ontogeny represented by the change in leaf
3 phyllotaxy combined with stem elongation composes the main hypothesis to explain the
4 breakpoint and the difference between phylochron phases in cut sunflower. Garrison (1973)
5 concluded that ordely patterns of shoot growth reflect a close relationship between leaf and
6 stem in sunflower. the. In maize and others cereal crops, the changes in phyllochron occurs
7 around the time of the shift from vegetative to reproductive phase, indicating that there is a
8 relationship between the breakinpoint and the reproductive organ development (Xu et al.,
9 2023). In cut sunflower this relationship with the transition from vegetative to reproductive
10 phase and phyllochron is not clear, requiring more studies.

11 This study investigated deeper into leaf development dynamics in cut sunflower
12 genotypes. The novelty of our results is that phenological scales of sunflower, such as
13 Schneiter and Miller (1981) and even the BBCH scale (2001), may be too simplistic and do
14 not adequately describe the role of stem elongation on the growth and development of
15 sunflower, nor do they capture its nuances in relation to the dynamics of leaf appearance or
16 bud appearance. . Furthermore, our findings provide important insights for parameterizing
17 or enhancing leaf appearance models in sunflower. The bilinear relationship between leaf
18 number and thermal time indicates changes in leaf appearance rate (LAR), particularly since
19 the phyllochron from V7 upwards accelerates. Some existing sunflower simulation models
20 already employ a bilinear function to calculate leaf appearance using the phyllochron
21 approach (Chapman et al., 1993; Villalobos et al., 1996; Casadebaig et al., 2011). These
22 models rely on the concept of thermal time to link development with temperature and
23 compute leaf appearance. This approach serves as a simple and efficient descriptor when
24 compared to calendar days (Streck et al., 2002). However, the relationship between
25 development and temperature for calculating leaf appearance does not follow a single linear

1 pattern. Considering non-linear models to simulate leaf appearance may offer a more
2 accurate descriptor for cut sunflower, as already proposed for other species such as gladiolus
3 (Uhlmann et al., 2017), maize (Langner et al., 2018) and wheat (Paff et al., 2023). From this
4 perspective, one approach to enhance these models is to incorporate a chronological response
5 function into process-based models that takes into account a breakpoint in leaf appearance.
6 This adjustment would provide a more realistic and biologically meaningful prediction of
7 leaf appearance, as proposed for wheat by Streck et al. (2003).

8

9 **3.2.5 Conclusion**

10

11 We confirmed the existence of a breakpoint between the V6 and V7 stages, as
12 evidenced by the bilinear regression between leaf number and thermal time, in cut sunflower
13 genotypes. This led to identify two phyllochron phases: an early one of 34.87 °C day leaf⁻¹,
14 and a late one of 21.82 °C day leaf⁻¹. Phyllochron phases are consistency across genotypes,
15 and is affected by environmental influences, such as location variability. These results not
16 only enhance our knowledge about the phyllochron but also provide an ecophysiological
17 basis for developing a chronology response function in process-based models for cut
18 sunflower.

19

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21

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8

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10

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Figures captions

Fig. 1. Geographic location of the field on farm trials with cut sunflower genotypes conducted in three regions of Brazil, South America (A): (B) Northwest, (C) Midwest and (D) South, and in (E) Tuscany, Italy, Europe. World map in panel (F).

Fig. 2. Relationship between the accumulated leaf number on the stem (ALN, leaves stem⁻¹) and accumulated thermal time (ATT, °C day) for (A, C) the cut sunflower hybrid Amalfi Orange in the 06/08/2020 sowing date at Santa Maria - RS, Brazil, and (B, D) the cut sunflower hybrid Amalfi Golden in the 28/02/2023 sowing date at Pescia – Tuscany, Italy. Panels A and B indicate the Single phyllochron (yellow circles), and panels C and D indicate the Early phyllochron (green circles) and Late phyllochron (red circles).

Fig. 3. Box plots demonstrating the phyllochron phases for cut sunflower, in °C day leaf⁻¹, pooling data collected in field on farm trials in Brazil (2020-2023) and Italy (2023). Solid line inside the boxes indicate the median. Different lower-case letters are different according to Post Hoc Dunn Test at 5% probability.

Fig. 4. Median of phyllochron with interquartile range for cut sunflower, in °C day leaf⁻¹, comparing location for each phyllochron phase (A - single phyllochron, B – early phyllochron and C – late phyllochron), from data collected in field grown trials in Brazil (2020-2023) and Italy (2023). Locations: BVS (Boa Vista do Sul/RS), BZN (Bozano/RS), BNP (Brunópolis/RS), CNT (Canhotinho/PE), DRD (Dourados/MS), HDO (Herval D'Oeste/SC), JC (Júlio de Castilhos/RS), NX (Novo Xingú/RS), PSC (Pescia, Italy), PTR (Petrolina/PE), RS (Rio do Sul/SC), SM (Santa Maria/RS), TSN (Teresina, PI) and VCR (Vacaria/RS). The different lower-case letters within each panel are different according to Post Hoc Dunn Test at 5% probability.

Fig. 5. Median of phyllochron with interquartile range for cut sunflower, in °C day leaf⁻¹, comparing Seasons (Spring, Summer, Autumn, and Winter) for each phyllochron phase (A - single phyllochron, B – early phyllochron and C – late phyllochron), from data collected in field grown trials in Brazil (2020-2023) and Italy (2023). The different lower-case letters within each panel are different according to Post Hoc Dunn Test at 5% probability.

Fig. 6. Median of phyllochron with interquartile range for cut sunflower, in °C day leaf⁻¹, comparing genotypes for each phyllochron phase (A - single phyllochron, B – early phyllochron and C – late phyllochron), from data collected in field grown trials in Brazil (2020-2023) and Italy (2023). The different lower-case letters within each panel are different according to Post Hoc Dunn Test at 5% probability.

- 1 **Table 1.** Characterization of cut sunflower field on farm trials conducted during four years (2020 to 2023) in Brazil, South America, and
- 2 during one year (2023) in Italy, Europe, that composed the dataset used in this study.

| Country | Region | State | Locations | Latitude | Longitude | Year | Months of sowing dates | Genotypes | Environment | | |
|---------|----------------|----------|--------------------|--------------------|-------------|---------------|------------------------|-----------------------|--------------------|-------------|-------------|
| Brazil | Northeast | Piauí | Teresina | 5°5'S | 42°48'W | 2021 and 2022 | September and March | 1 | Tropical | | |
| | | | Pernambuco | Canhotinho | 8°52'S | 36°11'W | 2022 | November | 1 | Tropical | |
| | | | | Petrolina | 09°23'S | 40°30'W | 2022 | May | 1 | | |
| | | Midwest | Mato Grosso do Sul | Dourados | 22°13'S | 54°48'W | 2021 | October | 1 | Tropical | |
| | | Southern | Santa Catarina | Brunópolis | 27°18'S | 50°52'W | 2021 | March | 2 | Subtropical | |
| | Herval D'Oeste | | | 27°19'S | 51°49'W | 2021 | January to March | 7 | | | |
| | Rio do Sul | | | 27°12'S | 49°38'W | 2021 | March | 2 | | | |
| | | | | Rio Grande do Sul | Santa Maria | 29°43'S | 53°43'W | 2020 to 2023 | January to October | 12 | Subtropical |
| | | | | Júlio de Castilhos | 29°23'S | 53°68'W | 2021 | March | 2 | | |
| | | | | Novo Xingú | 27°43'S | 53°3'W | 2021 | September and October | 1 | | |
| | | | | Boa Vista do Sul | 29°21'S | 51°40'W | 2022 | March | 1 | | |
| | | | | Bozano | 28°22'S | 53°46'W | 2023 | January and February | 1 | | |
| | | | | Vacaria | 28°30'S | 50°55'W | 2023 | April | 1 | | |
| Italy | Tuscany | Pistoia | Pescia | 28°30'S | 50°55'W | 2023 | February | 8 | Temperate | | |

1 **Table 2.** Minimum (TMin), maximum (TMax) and mean air temperature (Mean), and photoperiod (P) across different locations, sowing date
 2 (dd/mm/yyyy), and transplant date (dd/mm/yyyy) in the trials in Brazil, South America, from 2020 to 2023, and Italy, Europe, in 2023.

3

| Country | Region | State | Locations | Sowing date | Transplant date | TMin (°C) | TMax (°C) | Mean (°C) | P (h) | | |
|---------|------------|--------------------|-------------------|-------------|-----------------|------------|------------|-----------|-------|-------|-------|
| Brazil | Northwest | Piauí | Teresina | 30/09/2021 | 07/10/2021 | 11.3 | 30.6 | 20.9 | 13.00 | | |
| | | | Canhotinho | 03/11/2022 | 16/11/2022 | 17.3 | 30.5 | 23.9 | 12.46 | | |
| | | | Pernambuco | Petrolina | 26/05/2022 | 03/06/2022 | 14.5 | 32.2 | 23.4 | 11.46 | |
| | Midwest | Mato Grosso do Sul | Dourados | | 15/10/2021 | 25/10/2021 | 14.7 | 35.7 | 25.2 | 12.82 | |
| | | | | | | | | | | | |
| | | Santa Catarina | Brunópolis | | 31/03/2021 | 04/14/2021 | 1.7 | 28.5 | 15.1 | 11.39 | |
| | | | | Curitibanos | 08/09/2022 | 05/10/2022 | 0.9 | 25.8 | 13.4 | 12.56 | |
| | | | Herval | | 29/01/2021 | 08/02/2021 | 9.5 | 33.3 | 21.4 | 12.63 | |
| | | | | D'Oeste | 26/02/2021 | 06/03/2021 | 4.0 | 33.3 | 18.6 | 12.00 | |
| | | | | | 22/03/2021 | 29/03/2021 | 4.0 | 30.5 | 17.2 | 11.31 | |
| | | | Rio do Sul | 29/03/2021 | 09/04/2021 | 2.8 | 27.4 | 15.1 | 12.00 | | |
| | | | Rio Grande do Sul | Santa Maria | | 06/08/2020 | 17/08/2020 | -0.9 | 34.5 | 16.8 | 11.64 |
| | | | | | | 22/01/2021 | 30/01/2021 | 12.7 | 35.0 | 23.8 | 13.09 |
| | | | | | | 26/02/2021 | 06/03/2021 | 9.8 | 35.0 | 22.4 | 12.05 |
| | | | | | | 11/05/2021 | 01/06/2021 | -0.6 | 35.1 | 17.2 | 10.21 |
| | | | | | | 08/06/2021 | 02/07/2021 | -0.6 | 35.1 | 17.2 | 10.60 |
| | 02/07/2021 | 16/07/2021 | | | -0.6 | 35.1 | 17.2 | 10.89 | | | |
| | 07/09/2021 | 24/09/2021 | | | -0.6 | 35.1 | 17.2 | 12.53 | | | |
| | 15/10/2021 | 26/10/2021 | 6.0 | 37.7 | 21.8 | 13.14 | | | | | |
| | 27/01/2022 | 04/02/2022 | 9.6 | 37.7 | 23.6 | 13.40 | | | | | |

| | | | | | | | | | |
|-------|---------|---------|---------------------|------------|------------|------|------|------|-------|
| | | | | 08/08/2022 | 25/08/2022 | 1.2 | 32.3 | 16.8 | 11.90 |
| | | | Júlio de | 17/03/2021 | 23/03/2021 | 0.0 | 30.3 | 15.1 | 11.29 |
| | | | Castilhos | 15/04/2021 | 25/04/2021 | 0.0 | 30.3 | 15.1 | 10.85 |
| | | | Novo Xingú | 23/09/2021 | 05/10/2021 | 16.0 | 30.0 | 23.0 | 12.81 |
| | | | | 09/10/2021 | 21/10/2021 | 16.0 | 30.0 | 23.0 | 13.10 |
| | | | | 25/10/2021 | 10/11/2021 | 16.0 | 30.0 | 23.0 | 13.51 |
| | | | | 16/11/2021 | 30/11/2021 | 16.0 | 30.0 | 23.0 | 13.73 |
| | | | Boa Vista do Sul | 12/03/2022 | 22/03/2022 | 4.3 | 32.0 | 18.1 | 11.52 |
| | | | Bozano | 27/01/2023 | 03/02/2023 | 6.6 | 36.4 | 21.5 | 13.01 |
| | | | | 03/02/2023 | 10/02/2023 | 6.6 | 36.4 | 21.5 | 12.75 |
| | | | | 10/02/2023 | 17/02/2023 | 6.6 | 36.4 | 21.5 | 12.57 |
| | | | | 17/02/2023 | 24/02/2023 | 6.6 | 34.3 | 20.4 | 12.30 |
| | | | Vacaria | 03/04/2023 | 13/04/2023 | -2.0 | 27.0 | 12.5 | 10.85 |
| Italy | Tuscany | Pistoia | Pescia | 28/02/2023 | 17/03/2023 | 0.5 | 32.7 | 16.6 | 12.96 |

1 **Table 3.** Means of phyllochron ($^{\circ}\text{C day leaf}^{-1}$) for the single factor planting methods and for
 2 the two-factor interaction genotype x phyllochron phases (from V1 to FLN ($\text{PHYL}_{\text{single}}$),
 3 phyllochron from V1 to V6 stages ($\text{PHYL}_{\text{early}}$), and phyllochron from V7 to FLN ($\text{PHYL}_{\text{late}}$))
 4 of six cut sunflower genotypes, in planting methods trial in Santa Maria, RS, Brazil, 2020.

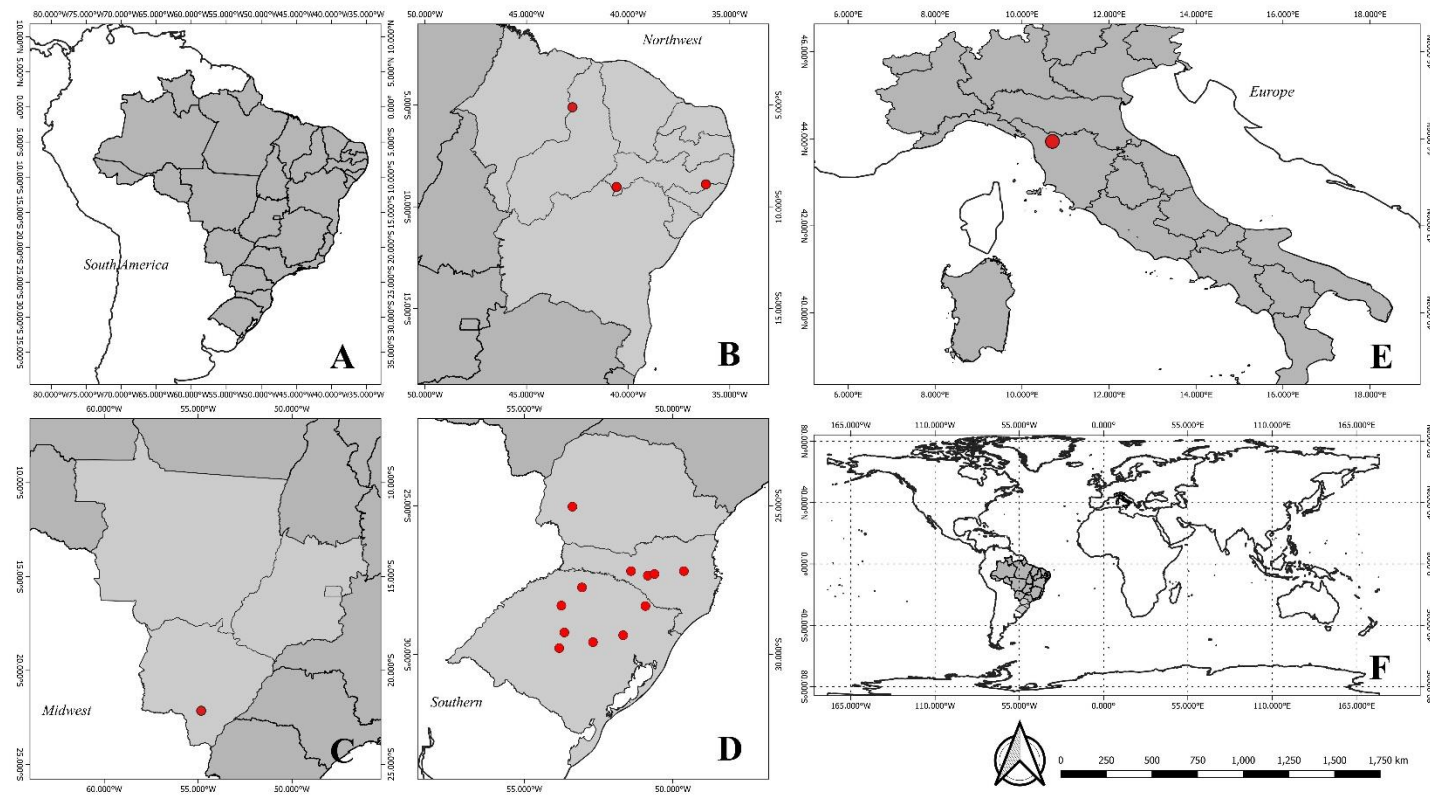
5

| Single factor: planting methods | | | |
|--|---|--|---|
| | Direct sowing | Transplanting | p-value |
| Phyllochron | 27.38 B | 29.15 A | 0.0 |
| Two-factor interaction: genotype x phyllochron phases | | | |
| Genotype | $\text{PHYL}_{\text{single}}$ | $\text{PHYL}_{\text{early}}$ | $\text{PHYL}_{\text{late}}$ |
| AG-01 | 25.93 aB* | 41.76 aA | 22.96 aC |
| AO-02 | 23.78 aB | 37.39 bcA | 22.17 abB |
| MO-03 | 25.75 aB | 39.64 abA | 22.39 abC |
| SO-06 | 25.07 aB | 38.55 abA | 23.91 aB |
| VO-09 | 23.87 aB | 34.34 cA | 19.36 bC |
| VT-11 | 24.49 aB | 34.11 cA | 23.28 aB |
| CV (%)** | 8.3 | p-value | 0.0005 |

6 * Means followed by the same lower case letters in columns and upper case letters in rows
 7 are note different according to the Tukey test at $p < 0.05$.

8 ** Coefficient of variation.

9



10

11 **Fig. 1.** Geographic location of the field on farm trials with cut sunflower genotypes conducted in three regions of Brazil, South America (A):

12 (B) Northwest, (C) Midwest and (D) South, and in (E) Tuscany, Italy, Europe. World map in panel (F).

13

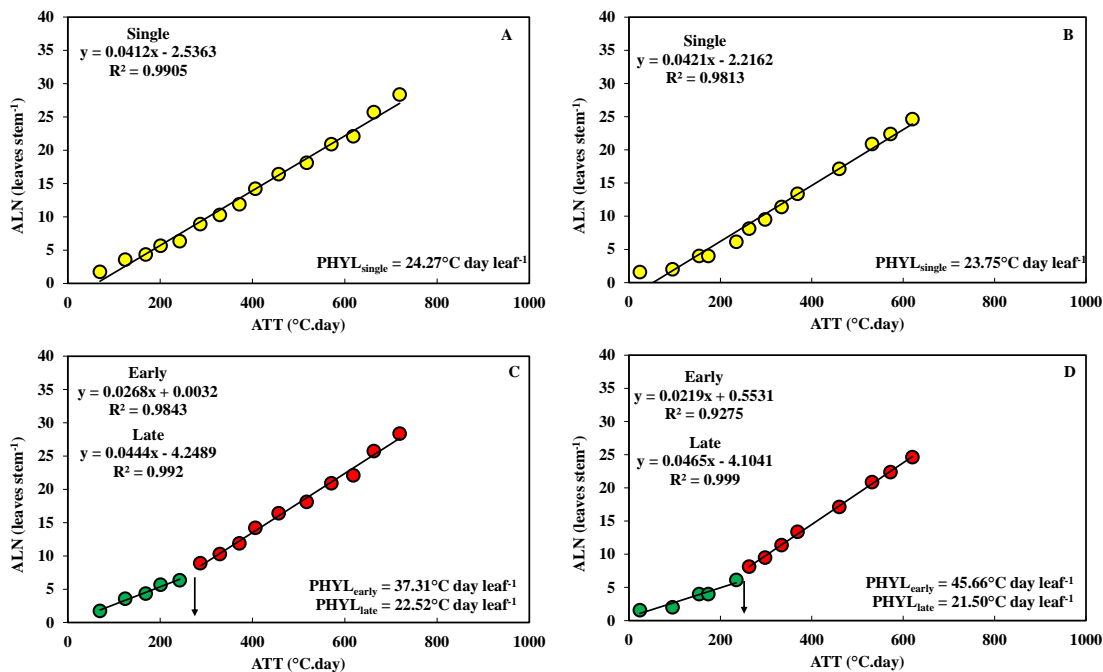


Fig. 2. Relationship between the accumulated leaf number on the stem (ALN, leaves stem⁻¹) and accumulated thermal time (ATT, °C day) for (A, C) the cut sunflower hybrid Amalfi Orange in the 06/08/2020 sowing date at Santa Maria - RS, Brazil, and (B, D) the cut sunflower hybrid Amalfi Golden in the 28/02/2023 sowing date at Pescia – Tuscany, Italy. Panels A and B indicate the Single phyllochron (yellow circles), and panels C and D indicate the Early phyllochron (green circles) and Late phyllochron (red circles).

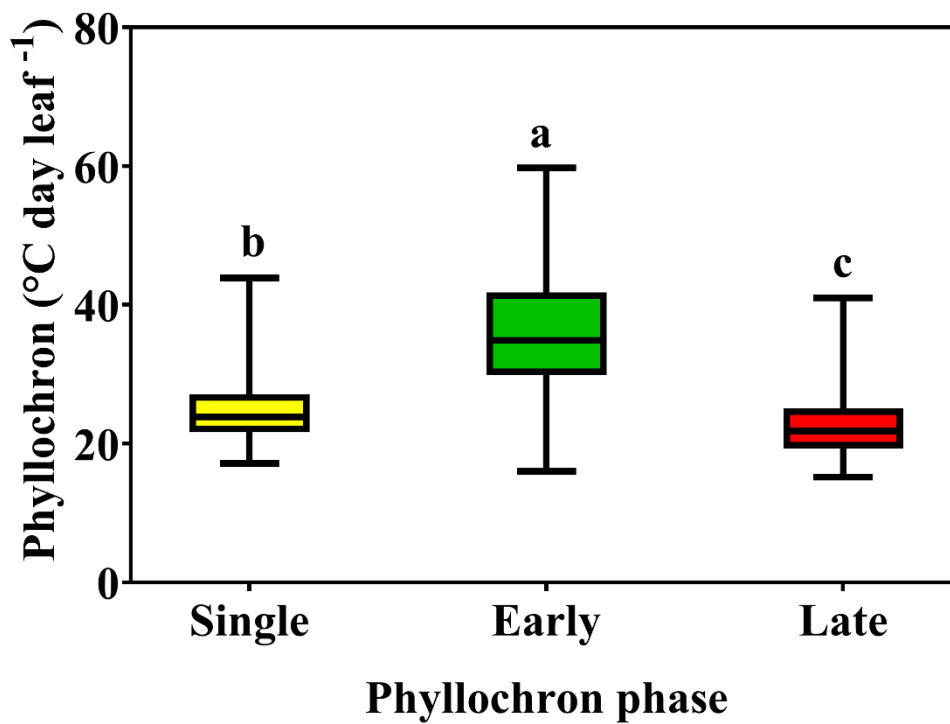


Fig. 3. Box plots demonstrating the phyllochron phases for cut sunflower, in °C day leaf⁻¹, pooling data collected in field on farm trials in Brazil (2020-2023) and Italy (2023). Solid line inside the boxes indicate the median. Different lower-case letters are different according to Post Doc Dunn Test at 5% probability.

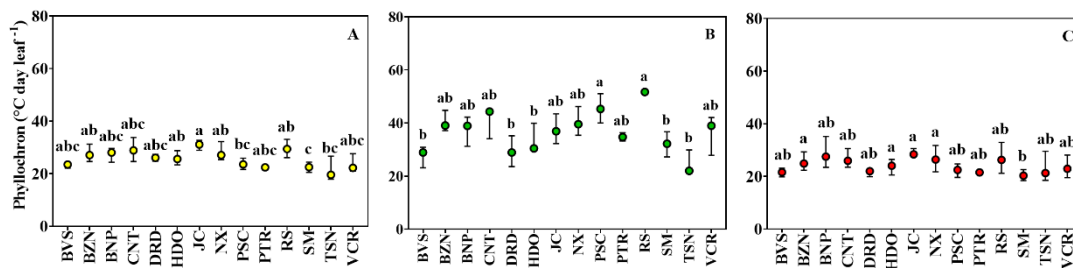


Fig. 4. Median of phyllochron with interquartile range for cut sunflower, in °C day leaf⁻¹, comparing location for each phyllochron phase (A - single phyllochron, B – early phyllochron and C – late phyllochron), from data collected in field grown trials in Brazil (2020-2023) and Italy (2023). Locations: BVS (Boa Vista do Sul/RS), BZN (Bozano/RS), BNP (Brunópolis/RS), CNT (Canhotinho/PE), DRD (Dourados/MS), HDO (Herval D'Oeste/SC), JC (Júlio de Castilhos/RS), NX (Novo Xingú/RS), PSC (Pescia, Italy), PTR (Petrolina/PE), RS (Rio do Sul/SC), SM (Santa Maria/RS), TSN (Teresina, PI) and VCR (Vacaria/RS). The different lower-case letters within each panel are different according to Post Doc Dunn Test at 5% probability.

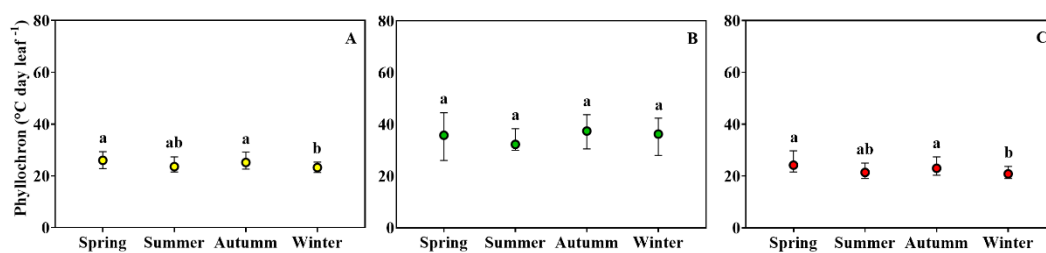


Fig. 5. Median of phyllochron with interquartile range for cut sunflower, in °C day leaf⁻¹, comparing Seasons (Spring, Summer, Autumn, and Winter) for each phyllochron phase (A - single phyllochron, B - early phyllochron and C - late phyllochron), from data collected in field grown trials in Brazil (2020-2023) and Italy (2023). The different lower-case letters within each panel are different according to Post Hoc Dunn Test at 5% probability.

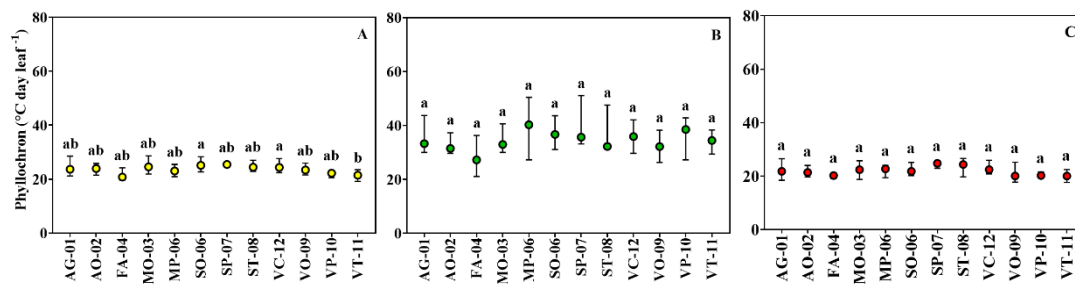


Fig. 6. Median of phyllochron with interquartile range for cut sunflower, in °C day leaf⁻¹, comparing genotypes for each phyllochron phase (A - single phyllochron, B – early phyllochron and C – late phyllochron), from data collected in field grown trials in Brazil (2020-2023) and Italy (2023). The different lower-case letters within each panel are different according to Post Doc Dunn Test at 5% probability.

3.3 CAPÍTULO 3 – FLOWER DEVELOPMENT AND YIELD OF FIELD GROWN
STATICE AS A FUNCTION OF TRANSPLANTING DATE AND LOCATION IN A
SUBTROPICAL ENVIRONMENT

(Será submetida à *South African Journal of Botany*, portanto as normas estão de acordo com as diretrizes exigidas)

1 **FLOWER DEVELOPMENT AND YIELD OF FIELD GROWN STATICE AS A**
2 **FUNCTION OF TRANSPLANTING DATE AND LOCATION IN A**
3 **SUBTROPICAL ENVIRONMENT**

4
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28 **Abstract**

29

30 *Limonium sinuatum* Mill., commonly known as statice or sea lavender, is widely
31 used as cut flower, fresh or dried. Flowering in statice occurs seasonally, requiring low
32 non-freezing temperatures for vernalization, followed by a long-day photoperiod and
33 temperatures between 22-27°C, being improved or prejudiced by the seedlings transplant
34 date. The objective in this study was to describe the flowering pattern of the statice crop
35 in the subtropics of Brazil, investigating factors that influence its reproductive phase.
36 Through field studies conducted across multiple transplant dates and locations during
37 2020 and 2021 in Southern Brazil, two cultivars of statice (QIS Yellow and QIS Purple)
38 were used to evaluate reproductive phase. Harvestings at R5 stage were performed weekly
39 and grouped per square meter by week, starting on Sundays ending on Saturdays. Stem
40 height, stem diameter, number of branches, and number of corymbs were recorded at each
41 harvest. Photoperiod and vernalization days (VD) for each transplant date and location
42 were calculated. The development of statice floral stems varied among cultivars,
43 transplant dates and locations in days to flowering (45 to 243 days), harvest period (5 to
44 18 weeks), yield (33 to 180 stems/m²) and parameters of quality in each harvest week.
45 There is a pattern in the weekly production of statice, with a peak production that can last
46 from 2 to 4 weeks, during which the production and quality of the flowers are higher.
47 Beyond this period, these factors begin to decline as the end of the cycle approaches. Our
48 results demonstrate that statice flower development and yield are intrinsically linked to
49 the species' vernalization and photoperiod requirements and help farmers to choose the
50 best moment to cultivate statice in subtropical environment. Furthermore, they provide
51 guidance for farmers on conducting statice harvests aimed at producing high-quality and
52 marketable flowers.

53

54 **Keywords:** *Limonium sinuatum* Mill., cut flower, temperature, vernalization, flowering,
55 harvesting.

56

57 **3.3.1 Introduction**

58

59 Statice (*Limonium sinuatum* Mill.) is an herbaceous plant native to the
60 Mediterranean Region, worldwide popular as a cut flower because of its high yield with

61 low technological inputs and the versatility of its floral stems, which can be used both as
62 fresh or dry flower in bouquets and flower arrangements, with long shelf life after harvest
63 (Nataraj et al., 2009; Ciotta and Nunes, 2011). Therefore, statice is an excellent flower
64 crop for diversifying flower production, providing resilience to farmers, and resulting in
65 a positive financial impact. For these agronomic features, statice was introduced in the
66 “Flowers for All” Project, the largest ongoing floriculture project in Brazil, which aims
67 to diversify crops and profit for small landholder family farmers (Streck and Uhlmann,
68 2021, Uhlmann et al., 2019).

69 Flowering is the visual expression of the reproductive phase, and in statice the
70 reproductive phase begins at the R1 stage (Heading), emitting numerous new stems
71 sequentially in the rosette (Buffon et al., 2022). Statice inflorescences are raceme-
72 corymbiform type (Blas, 1992), so that the main structure is characteristic of a raceme
73 with alternate branches on the main stem, and each branch has one or more corymbs. The
74 corymbs are the attractive part of the statice floral stem, composed of small spikes with
75 spikelets that support the calyxes, with a papery appearance, which can have several
76 colors such as yellow, white, blue, pink, and purple (Paparozzi, 1986; González-Orenga
77 et al., 2021; Xu et al., 2021).

78 Flowering in statice is the result of a complex interaction between temperature,
79 photoperiod, and vernalization (Wilfret, 1976; Pareja-Bonilla et al., 2024). As a
80 consequence, statice farmers have to choose the right time of the year for planting to
81 ensure flowering (Proietti et al., 2022). Air temperature is the main factor for the
82 development of statice throughout its cycle, from germination to flowering, and can affect
83 flowering in three different ways: through vernalization for flowering induction, by
84 accelerating flowering with increasing temperature up to the optimum temperature for
85 this phase, and by delaying the time to flowering with supra-optimal temperatures (Chen,

86 2005). Vernalization is the first pathway involved in static flowering. Vernalization is
87 defined as a requirement of plant exposure to low nonfreezing temperatures for inducing
88 or accelerating plants to flower (Chang et al., 2010; Kim, 2020). Usually, the
89 vernalization requirement for static is fulfilled by exposing the seedlings to temperatures
90 of 11 – 13 °C for three weeks (Shillo, 1976; Shillo and Zamski, 1985).

91 The second pathway involved in static flowering is photoperiod. Static is a
92 facultative long-day plant so that photoperiod longer than 13 hours accelerate flowering
93 (Shillo, 1976; Mattson and Erwin, 2005; Chen et al., 2010). The role of photoperiod in
94 inducing flowering in static only takes place after the vernalization requirements are met.
95 As a consequence, flowering of static will occur in the field when air temperature and
96 photoperiod conditions are favorable in Spring, when photoperiod is greater than 13 hours
97 and temperatures range from 22 to 27 °C (Shillo and Zamski, 1985).

98 Because of the vernalization and photoperiod requirements, static production in
99 Southern Brazil is seasonal. Sowing takes place in the Fall, plants remain in the vegetative
100 phase during the Winter when temperatures are low and vernalizing, and flowering takes
101 place in Spring (Cohen et al., 1995, Chang et al., 2010; Buffon et al., 2021). If the
102 vernalization and photoperiod requirements are not fulfilled, flowering may not occur or
103 may be delayed until environmental conditions are favorable.

104 Recent studies on flower development and yield of static were not found in the
105 literature and detailed studies on static floral production with practical applications to
106 farmers and consultants are necessary. Furthermore, as a cut flower, producing high-
107 quality floral stems is crucial. Consequently, in addition to knowing the harvesting period
108 of static floral stems, it is essential to understand whether the stems produced during this
109 period maintain a standard of quality or undergo changes throughout the harvest period
110 that may decrease their aesthetic value. Thus, the objective in this study was to describe

111 the flowering pattern of the statice cultivars in the subtropics of Brazil, investigating
112 factors that influence its reproductive phase.

113

114 **3.3.2 Material and Methods**

115

116 3.3.2.1 Field studies

117

118 Two field trials were conducted during 2020 and 2021 in eight locations in Rio
119 Grande do Sul (RS) and Santa Catarina (SC) States, Southern Brazil (Figure 1A). The
120 sites represent the main area of statice production in open field in Brazil and have
121 subtropical climates (Cfa and Cfb according to the Köppen climate classification) that
122 provide the necessary temperature and photoperiod conditions for flowering induction.

123 Seeds of *Limonium sinuatum* from Pan American Seeds® of two cultivars, QIS
124 Yellow and QIS Purple, were sown in 128-cell plastic trays containing commercial
125 substrate. After sowing the trays were kept in a protected and dark environment until
126 seedlings emerged (cotyledons were above the substrate surface) and afterwards were
127 transferred to a greenhouse covered with transparent plastic and open sides, where they
128 remained until transplanting. The seedlings were regularly irrigated and fertilized with
129 NP₂O₅K₂O 13-40-13 (1 g L⁻¹ of water) once a week.

130 The area for growing the statice in each location was previously prepared with pH
131 correction and beds of 1m wide, 9 m long and 0.25 m height were built. Seedlings were
132 transplanted to open field beds when they had 8 leaves and a well-structured root system.
133 Plant spacing was 0.5 m between rows and 0.3 m among plants within rows, with two
134 rows per bed and a plant density of 6.7 plants m⁻². On the day of transplanting, fertilization
135 was applied with NP₂O₅K₂O 05-20-20 at a rate of 250 kg ha⁻¹ and urea at a rate of 100
136 kg ha⁻¹. Irrigation was done by drip and management practices such as pest, disease, and

137 weed control were carried out as needed to keep plants free of biotic and abiotic factors.
138 The experimental design was a randomized complete block design with three replications
139 and two cultivars. Each replicate was a plot of 1.5 m in length with ten plants.

140 *Trial 1* was conducted during 2020 – 2021 at the Federal University of Santa
141 Maria located in Santa Maria/RS, Brazil, with 12 monthly sowing and transplanting dates,
142 from August 2020 until August 2021 (Table 1). The sowing was done 30 to 60 days before
143 the desired transplant date. The experimental design was a randomized complete block
144 design with three replications in a bifactorial scheme (twelve transplanting dates and two
145 cultivars).

146 *Trial 2* was a multilocation trial conducted in eight locations distributed across
147 the states of Santa Catarina and Rio Grande do Sul, in Southern Brazil (Figure 1). The
148 seedlings for this trial were produced in Santa Maria/RS, with sowing on May 29th, 2020.
149 When the seedlings reached 6 – 10 true leaves, approximately 60 days after sowing, they
150 were sent to each location where the transplanting was carried out. Transplanting dates
151 are presented in Table 1. In Dilermando de Aguiar/RS, cultivation was conducted in two
152 different farms. In Itaquí/RS, a trial was conducted with supplemental irrigation and
153 without irrigation, using the same experimental design, but in a bifactorial scheme (water
154 regime and cultivars).

155 3.3.2.2 Data collecting and statistical analysis

156

157 Six plants were tagged in each plot and the date of the phenological stages R1.1 (first
158 floral stem visible on the center of the rosette, Buffon et al., 2022) and R5.1 (all flowers
159 opened on the first floral stem, Buffon et al., 2022) were collected on the tagged plants. The
160 percentage of plants that initiated the reproductive phase (%R1.1) was calculated based on
161 the total number of tagged plants. This percentage was divided into two periods: plants that
162 initiated the reproductive phase until March 20, 2021, and plants that initiated the
163 reproductive phase after March 20, 2021. After, the total This date was chosen because is
164 the beginning of the Autumn Equinox in the Southern Hemisphere when temperatures and
165 photoperiod begin to decrease and are not favorable for statice flowering.

166 Floral stems were harvested when they reached the R5 stage (100% of the flowers
167 on the corymb are open, Buffon et al., (2022)). Harvests were carried out once or twice a
168 week depending upon the location and week of the year. Harvestings were grouped by week,
169 starting on Sundays ending on Saturdays. The amount of floral stems harvested per week
170 was accumulated and flower production was presented both as weekly number of floral stems
171 per square meter and accumulated number of floral stems per square meter. Week of harvest
172 was positioned as week of the year from 1 to 52 (2020) and 53 (2021). The harvests of *Trial*
173 *1* were all concluded in December 2021. In Júlio de Castilhos/RS, only phenological data
174 were collected and harvestings were not recorded.

175 At each harvest, flower production components such as the stem length, stem
176 diameter at 1 centimeter from the cutting, number of branches, and number of corymbs were
177 recorded. Figure 2 illustrates what was considered a corymb and a branch on the statice floral
178 stem. The flower production components were also assessed weekly to demonstrate their
179 variation throughout the harvests and in each transplanting date and location. The harvest
180 period was positioned from 1 to the last week of harvesting, independent of the week of the
181 year. Error bars were included for each harvest week. Linear regression was applied at 5%
182 of probability by Test F, to assess the relationship between the flower production
183 components.

184 At the end of the harvest period, the yield per square meter (stems/m²) was
185 calculated. Likewise, the yield per plant (stems/plant) was also calculated and assessed by
186 ANOVA and compared using Tukey's test, at a 5% probability of error, using SAS Analytics
187 Software.

188

189 3.3.2.3 Photoperiod and vernalization

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200

201 $f_{vn}(T) = [2(T-T_n)^\alpha (T_{ot}-T_n)^\alpha - (T-T_n)^{2\alpha}] / (T_{ot}-T_n)^{2\alpha}$ for T_n, T, T_x 202 $f_{vn}(T) = 0$ for $T < T_n$ or $T > T_x$ 203 $\alpha = \ln 2 / \ln [(T_x - T_n) / (T_{op} - T_n)]$

204

205 where T_n , T_{ot} , and T_x are the minimum, optimum, and maximum cardinal vernalization206 temperatures, T is the daily average temperature at which vernalization was performed, and207 \ln is the natural logarithm. Since there is no information available on the cardinal208 vernalization temperatures for *statice* in the literature, the cardinal vernalization209 temperatures for lily cultivation were considered, which are $T_b = -1.5^\circ\text{C}$, $T_{ot} = 5^\circ\text{C}$, and T_B 210 $= 21^\circ\text{C}$ (Streck 2002, Streck and Schuh, 2005). The VD was calculated by accumulating the

211 daily vernalization rates.

212 VD was calculated for three different periods during *statice* development. First, VD

213 was calculated from the day after sowing until the day of transplanting [VD(SO-TP)]. The

214 *statice* seedlings for *Trial 2* were produced in Santa Maria and VD (SO-TP) was calculated

215 using the meteorological data from this location. From transplanting onwards, data from the

216 corresponding location of cultivation were used. Then, VD was calculated from the day after

217 transplant until the occurrence date of the R1 stage [VD(TP-R1)]. Finally, the total VD was

218 calculated from the day after sowing until the occurrence date of the R1 stage [VD(SO-R1)].

219 Standard deviation (SD) and coefficient of variation (%) were calculated for VD values.

220 The maximum and minimum air temperature data used for VD calculation were
221 collected from May 2020 to December 2021 (*Trial 1*) and July 2020 to January 2021 (*Trial*
222 2), from the automatic weather stations of the National Institute of Meteorology (INMET)
223 in Santa Maria/RS, Bento Gonçalves/RS, Uruguaiana/RS, Joaçaba/SC, Curitibanos/SC, and
224 Ituporanga/SC. Meteorological station located in Santa Maria, represented the climate for
225 *Trial 1* at Santa Maria and *Trial 2* at Santa Maria/RS and Dilermando de Aguiar/RS farms.
226 Bento Gonçalves represents the climate in Pinto Bandeira/RS, and Uruguaiana represents
227 the climate of Itaqui/RS. Joaçaba, Curitibanos/SC and Ituporanga represent the climates in
228 Concórdia/SC, Curitibanos/SC and Rio do Sul/SC, respectively. Daily precipitation was also
229 registered for each transplanting date and location. Accumulated precipitation was calculated
230 through the sum of daily precipitation from transplanting date to the last harvest week.

231

232 3.3.3 Results

233

234 3.3.3.1 Meteorological data

235

236 The two field trials in different locations and transplanting dates exposed static
237 plants to a wide range of temperature, precipitation, and photoperiod (Figure 3). In Santa
238 Maria/RS during *Trial 1* air temperature varied from -1.8 to 38.8°C, and photoperiod varied
239 from 10h to 14h during Winter and Summer, respectively (Figure 3A). In *Trial 2* air
240 temperature ranged from -0.9 to 38.8°C in Santa Maria/RS and Dilermando de Aguiar/RS
241 (Figure 3B), from -2.4 to 38.8°C in Júlio de Castilhos/RS (Figure 3C), from -0.6 to 38.3°C
242 in Itaqui/RS (Figure 3D), from -1.3 to 35.3°C in Pinto Bandeira/RS (Figure 3E), from 1.1 to
243 36.8°C in Concórdia/SC (Figure 3F), from 1.1 to 33.2°C in Curitibanos/SC (Figure 3G), and
244 from -2 to 34.5°C in Rio do Sul/SC (Figure 3H). Since cultivation occurred with plantings
245 at the end of July or early August 2020 in all locations, the same trend of increasing both
246 average air temperature and photoperiod was observed in all locations. This is the time of
247 year when conditions are favorable for static flowering in Southern Brazil.

248

249 Precipitation also plays an important role in static production, so that three or more
250 rainy days in a row may decrease flower quality and favor diseases like Botrytis.
251 Accumulated precipitation in *Trial 1* varied among transplanting dates. In August/2020,
252 September/2020, and October/2020 the precipitation was 263.3 mm, 189.8 mm, and 175.83,
252 respectively. November/2020 and December/2020 accumulated 126.0 mm and 284.9 until

253 the last harvest of the cultivar QIS Yellow, and 898.9 mm and 776.9 until the last harvest of
254 the cultivar QIS Purple, respectively. From January/2021 to August/2021, the precipitation
255 decreased steadily as 1271.8 mm, 1062.2 mm, 1016.8 mm, 921.2 mm, 694.2 mm, 565.2 mm,
256 and 531.6 mm. In *Trial 2*, the meteorological station that represents Santa Maria/RS and
257 Dilermando de Aguiar/RS registered an accumulated precipitation of 629.3 mm. In Júlio de
258 Castilhos/RS, 632 mm was registered and 674.8 mm in Pinto Bandeira/RS. Itaquí/RS
259 registered the lowest precipitation, with 579.4 mm. Locations in Santa Catarina state
260 registered the highest precipitation, with 950.4 mm, 945.4 mm, and 881.0 mm for
261 Concórdia/SC, Rio do Sul/SC e Curitiba/SC, respectively.

262

263 3.3.3.2 Vernalization days

264

265 In *Trial 1* there was a reduction in vernalization days (VD) from the sowing to the
266 transplanting (SO-TP) phase from August/2020 (34.57 VD) to March/2021 (6.55 VD) and
267 an increase from March/2021 to August/2021 (48.82), with coefficient of variation equal to
268 67.76% (Table 2). The VD from the transplanting to R1 phase [VD(TP-R1)] decreased from
269 August/2020 (9.95 VD) to November/2020 (1.91 VC) for the cultivar QIS Yellow and from
270 August/2020 (22.19 VD) to October/2020 (11.85 VD) for the cultivar QIS Purple. This
271 occurred because statice was cultivated during a transition period from Winter to Spring
272 (August and September) and subsequently from Spring to Summer (October, November, and
273 December). In Spring, air temperature began to rise, accumulating fewer days with
274 vernalizing temperatures towards the end of each crop cycle. In December/2020 (3.94 VD),
275 there was a slight increase in VD(TP-R1) compared to November/2020 (1.91 VD) for QIS
276 Yellow.

277

278 Sowing and transplanting of statice in November/2020, December/2020, and
279 January/2021, February/2021 were considered "off-season" as the air temperature was high,
280 not providing temperatures low enough for vernalizing statice plants, and the photoperiod
281 was already inductive (higher than or equal to 13 hours). Therefore, plants transplanted
282 during these months required a longer period to accumulate the necessary vernalization days
283 to enter the reproductive phase, thus extending the developmental cycle. March/2021 and
284 April/2021 are again positioned in a transition period, this time from Summer to Autumn.
285 This is a time of the year when conditions in Southern Brazil reverse so that photoperiod and
temperature begin to decrease, providing the necessary conditions for vernalization.

286 Transplanting during the "off-season" exhibited high VD(SO-R1), such as 94.29 VD for QIS
287 Purple in November/2020.

288 In *Trial 2*, VD(SO-TP) varied among locations, from 31.54 to 36.20 VD (CV = 4.60%),
289 due to the different transplanting dates. After transplanting, VD(TP-R1) presented the
290 highest variations, from 10.36 to 19.86 for QIS Yellow (CV = 25.92%) and from 18.09 to
291 25.99 for QIS Purple (CV = 12.40%). Thus, VD(SO-R1) varied from 43.73 to 52.48 VD for
292 QIS Yellow (CV = 6.81%) and 52.44 to 59.93 VD for QIS Purple (CV = 5.17%). In general,
293 QIS Purple demonstrated higher vernalization requirements compared to QIS Yellow, except
294 for January/2021 (50.73 VD) and March/2021 (54.76 VD). This indicates that the QIS Purple
295 cultivar is more demanding in vernalization compared to the QIS Yellow cultivar.

296

297 3.3.3.3 Days to flowering

298

299 Time from transplanting to flowering varied between transplanting dates (Figure 4A)
300 and locations (Figure 4B). Transplanting during "off-season" had a longer vegetative phase
301 compared to the transplanting during Autumn-Winter season. Consequently, more days
302 until the first harvest were observed. Variations across cultivars were also verified, especially
303 on November/2020 and December/2020 transplanting, when time to flowering was 45 and
304 50 days for QIS Yellow and, 260 and 191 days for QIS Purple, respectively. Variations were
305 also observed between locations and cultivars (*Trial 2*) so that QIS Purple presented need
306 longer time to flowering than QIS Yellow in Rio do Sul/SC, Curitiba/SC, Júlio de
307 Castilhos/RS, and Itaquí/RS – Irrigated.

308

309 3.3.3.4 Percentage of plants reaching R1 Stage

310

311 Table 3 presents the percentage of plants that reached the reproductive phase (%R1) at
312 two times of the year: until March 20 and after March 20. The %R1 in *Trial 1* varied between
313 cultivars and transplanting date. In transplantings during the months of August/2020,
314 September/2020, October/2020, and June/2021, July/2021 and August/2021, 100% of the
315 plants of both cultivars reached the reproductive phase. However, plants transplanted from
316 November/2020 to February/2021 did not reach 100% R1 until March 20th. All QIS Yellow
317 plants from November/2020 and December/2020 flowered by March 20th, indicating that
318 the vernalization requirements were met for these plants. However, for QIS Purple plants,

319 only 16.6% in November/2020 and 33.3% in December/2020 reached the reproductive phase
320 until March 20th. The highest percentage of flowering for this cultivar occurred after March
321 20th, totaling 72.2% and 61.1%, respectively.

322 The low %R1 for "off-season" crops occurred due to the extended period that the plants
323 remained in the field. The static plants underwent senescence, primarily due to the heat in
324 the early days after transplanting during the Summer months. Therefore, the low %R1
325 percentage is more related to plant survival than to vernalization itself. The QIS Yellow
326 cultivar was the most affected by plant loss, indicating its greater sensitivity compared to
327 QIS Purple. In *Trial 2*, 100% of the plants entered the R1 stage at all sites. In Itaqui/RS, in
328 the non-irrigated cultivation, the percentage of plants in R1 was 88.9% for QIS Yellow and
329 94.4% for QIS Purple, due to plant mortality. In Santa Maria/RS, there was a loss of only
330 one QIS Purple plant.

331

332 3.3.3.5 Static flowers development

333

334 Morphologically, a static plant presents several axillary buds that will give rise to
335 floral stems, which develop successively, alternating at the center of the rosette (Buffon et
336 al., 2021a). Thus, floral stems reach the harvest point (R5 stage) at different times during
337 flowering, which can last for several weeks. This period is referred to as the "harvest period",
338 which is depicted for different transplant dates in Figure 5. The duration of this period will
339 depend on the transplant date and the number of floral stems emitted by the plant.

340 Harvest period for August/2020, September/2020 and October 2020 started at the
341 week 43, 45, 47 of the year 2020 and last 14 and 15, 13 and 13, 13 and 10 weeks, respectively,
342 for QIS Yellow and QIS Purple (Figure 5A, 5B and 5C). Transplanting in November/2020
343 and December 2020 presented two harvest periods (Figure 5D and 5E). The first one started
344 right after transplanting QIS Yellow, at the week 51 of 2020 for November/2020, lasting 15
345 weeks, and at the week 5 of 2021 for December/2020, lasting 12 weeks. The second one
346 started at week 35 and 36 for the QIS Purple cultivar, lasting 18 and 18 weeks, respectively.
347 This division in the harvest period during these two times of the year occurred due to the
348 vernalization requirement of each cultivar and whether it was met or not. Additionally, the
349 weekly production was lower than in the initial three months of cultivation.

350 The next months synchronized the flowering period after winter. Harvests from
351 transplants in January/2021 (Figure 5F), February/2021 (Figure 5G), March 2021 (Figure

352 5H) began only at weeks 36, 35, 35 of the year 2021, remaining 9 and 17, 9 and 17, 12 and
353 18 weeks for QIS Yellow and QIS Purple, respectively. Throughout the period, the plants
354 remained vegetative and excessively increased the number of leaves (data not shown).
355 Harvests of April/2021 began at week 36 and lasts 13 weeks for QIS Yellow (Figure 5I).
356 June (Figure 5J) and July/2021 (Figure 5K) harvesting started at week 39 and 40, remaining
357 12 and 11 weeks for QIS Yellow and 14 and 13 weeks for QIS Purple. Agosto/2021 (Figure
358 5L) was less productive and with a shorter harvest period than August/2020. Therefore, it is
359 observed that the main effect in the production of statice floral stems is the distribution of
360 production. Weekly production starts small, increasing until it reaches a peak, and then
361 gradually decreases until floral stem production completely ceases.

362 The same response was observed in the trials conducted during 2020 in seven locations
363 of *Trial 2* (Figure 6). In Júlio de Castilhos/RS, data about harvesting was not recorded. In
364 the on-farm trial conducted Itaquí/RS with irrigation (Figure 6A) and without irrigation
365 (Figure 6B), the harvest period started at week 42 and lasted for eight weeks for irrigated
366 statice and started at week 43 and lasted for seven weeks for non-irrigated, with a reduction
367 in production from the fourth week onwards. Curiously, the harvest period also starts at week
368 43, from 18 to 24 October/2021, for all other locations. Dilermando de Aguiar/RS, Pinto
369 Bandeira/RS, and Santa Maria/RS, the experiments were conducted on-farm, with the first
370 location on two different farms. At Farmer 01 (Figure 6C) in Dilermando de Aguiar/RS, the
371 harvest period lasted for 10 weeks, unlike Farmer 02 (Figure 6D), where this period lasted
372 only seven weeks.

373 Pinto Bandeira/RS presented a reduced harvest period (Figure 6E), with only five
374 weeks. However, in this location, harvests were conducted every two weeks, justifying the
375 reduction in the harvest period. Figure 6F demonstrates that in Santa Maria/RS, the harvest
376 period was eleven weeks. Despite the difference in the harvest period among locations in
377 Rio Grande do Sul, the same peak and decline production behavior can be observed
378 throughout the weeks. The harvest period in the trials conducted in Santa Catarina are
379 presented in Figures 6G, 6H and 6I. Concórdia/SC showed eight weeks of harvest (Figure
380 6G) and Curitiba/SC, eleven weeks (Figure 6H). In Rio do Sul/SC statice flowers were
381 harvested during seven weeks (Figure 6I). Similar to Rio Grande do Sul, the peak and decline
382 production behavior in Santa Catarina followed the same pattern.

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384 3.3.3.6 Yield

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387 Just as the %R1 and VD decreased with the increase in mean air temperature, a
388 reduction in the production of static flowers was also observed (Figure 7A). To identify the
389 occurrence of this reduction, only harvests conducted until March 20 were considered. For
390 QIS Yellow, there was a reduction from 176 stems/m² to 18 stems/m², and for QIS Purple,
391 from 146 stems/m² to 4 stems/m² for the August/2020 and December/2020 periods,
392 respectively. The slope of the linear regression shows that for each month the transplant date
393 is delayed, there may be a reduction of 43.18 stems/m² for QIS Yellow and 38.56 stems/m²
394 for QIS Purple. This reduction in stem production is related to the reduction in VD for each
395 period; thus, the lower the VD, the lower the production of floral stems in the crop. An
396 important observation is that even if 100% of the plants in the August/2020, September/2020,
397 and October/2020 periods initiated the reproductive phase, the production differed in these
398 three periods.

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407 On the other hand, an increase in productivity was identified starting from March 20
408 in the off-season plantings, from November/2020 to March/2021 (Figure 7B). Productivity
409 increased from 5 to 85.33 stems/m² for QIS Yellow and from 60 to 175.67 stems/m² for QIS
410 Purple. The angular coefficient of the linear regression shows that for each month the
411 transplant date advance, there may be an increase of 17.87 stems/m² for QIS Yellow and
412 28.2 stems/m² for QIS Purple. This increase in production occurred because the plants that
413 remained in the field only entered the reproductive phase after the passage of winter, during
414 which the remaining plants naturally underwent vernalization, producing floral stems
415 starting from week 35 of 2021.

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417 The yield of static (stems/m²) varied significantly among transplant date and locations
418 (Figure 8). ANOVA analysis showed significative interaction between transplant dates x
419 cultivars ($p = 0.044$) and locations x cultivars ($p = 0.0039$). QIS Yellow yield for August
420 2020 (176 stems/m²) and QIS Purple yield for March 2021 (179 stems/m²), demonstrate to
421 be significantly superior compared to the others transplant dates carried out. Yield during
422 off-season was the lowest, in November/2020 and December/2020. Tukey's test also
423 demonstrated that the highest productivities in Rio Grande do Sul were Santa Maria/RS and
424 in Santa Catarina, in Concórdia/SC. In Dilermando de Aguiar/RS, differences in the harvest
425 period were observed between Farmer 01 and Farmer 02, but yields were similar for QIS
426 Yellow and differ for QIS Purple. The lowest yield was verified in Rio do Sul/SC for both

417 cultivars. Yield did not differ statistically between irrigated and non-irrigated field grown in
418 Itaqui/RS. Thus, a longer harvest period may not necessarily be synonymous with higher
419 yield. Among yield per plant, significant interaction between transplanting dates x
420 cultivars ($p = 0.0223$) and locations x cultivars ($p = 0.0234$) was founded by ANOVA. The
421 number of stems per plant showed differences through Tukey's test, varying from 6.63 stems
422 and 30.52 stems/plant for QIS Yellow and 11.76 stems to 35.67 stems/plant in *Trial 1*. As
423 expected, locations in *Trial 2*, also demonstrated differences significantly. The lowest yield
424 was calculated in Rio do Sul/SC (9.73 and 5.20 stems/plant) and the highest yield in Santa
425 Maria/RS (31.60 and 26.04 stems/plant) for both cultivars studied.

426

427 3.3.3.7 Flower production components

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429 The flower production components studied are presented heatmaps demonstrating their
430 variation along the harvest weeks for *Trial 1* (Figure 9) and *Trial 2* (Figure 10). Just as there
431 is variation in production throughout the harvest period, flower production components also
432 varied. The highest values of production components are observed during the period
433 considered as the peak of production. During this peak, the production components are
434 potentialized, but the timing of this peak differs between transplant dates, cultivars, and
435 locations. For example, stem length for August/2020, September/2020, October/2020, this
436 moment occurs during the 3rd to 6th harvest weeks for both QIS Yellow (Figure 9A) and
437 QIS Purple (Figure 9B). However, for other transplant dates such as March/2021 and
438 April/2021, this peak was later, occurring between 3rd to 8th harvest weeks for QIS Purple.
439 Production peaks also occurred between locations, with higher values of stem length, stem
440 diameter, number of branches, and number of corymbs mostly during the 2nd to 6th harvest
441 weeks (Figure 10), varying with the number of harvest weeks. As the harvest period
442 progresses, these values begin to decrease until the latest harvest.

443

444 The relationship between flower production components is presented in Figure 11,
445 When relating production components, it is observed that there is a significant positive linear
446 relation between stem diameter and stem length, despite the low coefficient of determination
447 (QIS Yellow = 0.545, Figure 11A and QIS Purple = 0.4396, Figure 11B). The same
448 significant result was also verified among the other relationships tested, as: number of
449 branches and stem length (QIS Yellow = 0.4151, Figure 11C and QIS Purple = 0.3779,
Figure 11D), number of corymbs and number of branches on the floral stem (QIS Yellow =

450 0.7506, Figure 11E and QIS Purple = 0.8044, Figure 11F) and between number of branches
451 and stem diameter (QIS Yellow = 0.4519, Figure 11G and QIS Purple = 0.4683, Figure 11F).

452

453 **3.3.4 Discussion**

454

455 Brazil is a country with a wide territorial extension, and statice can only be field-
456 grown in the Southern region or in areas with altitudes above 1000 m, where nighttime
457 temperatures are lower than or equal to 15°C, meeting the vernalization requirements of
458 statice. The different transplanting dates provided a wide variation of temperature and
459 photoperiod throughout the years 2020 and 2021, both during the "off-season" and during
460 the optimal season for producing statice in the Subtropics of Brazil. Our results confirm that
461 long days and cool night temperatures, by vernalization, play an important role in regulating
462 the flowering process in statice (Semeniuk and Krizek, 1972). There was a penalty in the
463 percentage of flowering plants, due to factors such as delayed flowering and plant death.

464

465 According to our outcomes, vernalization requirements of statice cultivar are
466 established and demonstrate the differences between cultivars. QIS Yellow require less
467 vernalization days than QIS Purple to flowering, been able to flower at high temperatures
468 contrasting with the QIS Purple cultivar that requires more vernalization days to flower, as
469 demonstrate by the November/2020 and December/2020 transplanting dates. Different
470 colored cultivars of statice differ in vernalization requirements: yellow and white cultivars
471 can flower at high temperatures while pink, lavender and blue cultivars require greatest
472 vernalization period, as stated by Shillo and Zamski (1985).

472

473 Days to flowering are variable depending on cultivars and growing conditions, along
474 with temperature increases during growth, a delay in the time to flowering occurs. (Wilfret
475 et al., 1973; Semeniuk and Krizek, 1973). Yellow cultivars demonstrated to need less time
476 for the first harvest. Transplanting in "off-season," from November/2020 to February/2021,

476 prolonged the vegetative phase due to temperature increase, delaying the flowering. Growing
477 statice during this period was demonstrated to be not recommended, because developmental
478 cycle is prolonged and there was a decrease in flowers production components and yield.
479 High day/night temperatures inhibited or greatly reduce flowering while cool/night
480 temperature promote flowering in statice (Krizek and Semeniuk, 1972). Cold requirement is
481 one of the greatest challenges faced in tropical climate countries, and in Brazil, only Southern
482 region have conditions, due to the subtropical climate, to naturally meet this demand. One
483 option for this time of year is to provide vernalization artificially, as indicated by Buffon et
484 al. (2021).

485 Yield demonstrated to be affected by environmental conditions, decreasing with
486 exposure to high temperatures and increasing again after being appropriately vernalized with
487 the onset of winter. Thus, off-season statice crops synchronize the flowering with in-season
488 crops, with flowering in Spring. Synchronous flowering in all individual plants of a
489 population at the same time of each year is controlled mostly by photoperiodic conditions,
490 which is independent of seasonal and inter-annual variations in climate, and by vernalization
491 response, a form of biological ensuring that flowering is repressed until the end of the winter
492 months (Corbesier et al., 1996; Samach and Coupland, 2000; Borchert et al., 2005). Exposure
493 to low temperatures not only promotes flowering through vernalization but also increases
494 flower production as a consequence of a greater number of floral primordia being initiated
495 (Semeniuk and Krizek, 1973), confirmed with our findings about yield per plant. A high
496 number of floral primordia also contributes to the extension of the harvest period, as the
497 onset of the R5 stage in each stem does not occur simultaneously, but rather sequentially
498 (Buffon et al., 2022).

499 The low yield in transplanting in June/2021, July/2021, and August/2021 was related
500 to the high accumulated precipitation during the harvest period in 2021 compared to the same

501 period in 2020. For field grown statice, large amount of precipitation during the harvest
502 period is the biggest problem associated with low yields and flower production components.
503 Statice floral stems are extremely sensitive from the R4 stage (First flowers are open, Buffon
504 et al., 2022) onwards. Due to the calyxes papery appearance, high humidity hinders the
505 flower opening and reduces the production of marketable floral stems. Low plastic tunnels
506 with metal-conduit hoops tall enough to cover the plant canopy and plastic-film possible to
507 lift and clip the sides to venting, enable farmers to protect the statice flowers during this
508 period for a low-cost investment (Rauter et al., 2021).

509 The flower production components are important in statice to define the quality of
510 the marketable floral stems. A high or low floral stems quality depends on these components,
511 varying throughout the harvest weeks and cultivars were found. In general, QIS Yellow has
512 the longest stems than QIS Purple. Similar variations among cultivars were found by
513 Whipker and Hammer (1994), Nataraj (2009) and Basumatary and Hatibarua (2023). A peak
514 of production was observed, where in addition to producing more floral stems, stem length
515 is also higher, as well as other production components such as stem diameter, number of
516 branches, and number of corymbs. As demonstrated in Figs. 9 and 10, the stems meet
517 Brazilian market standards for length, ranging from 40 cm to 90 cm (Veiling Holambra,
518 2020). This relationship is confirmed by the results presented in Figure 11. After this peak
519 in production, there is a decrease in weekly production as well as a reduction in flower
520 production components. Thus, statice floral stems harvested late have lower quality,
521 corroborating with the results found by Whipker and Hammer (1994). This reduction in
522 flower production components in later harvestings is associated with the natural life cycle of
523 statice. Later harvestings occur at the end of the cycle, a moment when the number of leaves
524 decreases significantly, reducing the leaf area for intercepting solar radiation, triggering a
525 sequence of effects that will reduce the photosynthetic capacity of statice plants, resulting in

526 lower yield and quality (Pettigrew, 2008). The exhaustion of nutrients available in the soil at
527 the end of the harvest period also can be one factor that is interfering with the flower
528 production components. When adequate nutrients, as phosphorus, are not provided, stem
529 length is severely affected (Verdelin and McDonald, 2007), and consequently the other
530 production components dependent on stem length, such as stem diameter and number of
531 branches.

532 The findings from our study indicate practical results about flowering pattern of
533 static crop that can be applied to various realities of worldwide static farmers, especially
534 in Brazil. Brazilian flower market requires static floral stems that meet the commercial
535 quality standards (Ciotta and Nunes, 2007). These standards consider mainly stem length
536 and number of branches for selecting the floral stems, by color or mixed, to compose the
537 package for selling. Number of branches demonstrate to be dependent on stem length. Thus,
538 stems with greater length have greater diameter, as well as stems with a greater number of
539 branches have a greater number of corymbs, regardless of the cultivar. In general, larger
540 stems with a higher ratio of branching to corymbs are desired, for improving the quality of
541 the marketable stems. To meet these demands, the floral stems produced during the peak of
542 production are better suited attending the commercial standards.

543 On the other hand, farmers from Southern Brazil, specially of Rio Grande do Sul and
544 Santa Catarina states, produce field-grown static and commercialize flowers locally, in
545 floricultures, fairs or small markets. The distance and transportation costs to send their
546 production to the major cooperatives are not viable for Southern producers, who are mostly
547 small-scale family farmers with limited production areas. Therefore, the flowering pattern
548 of static and the versatility of its floral stems make its production a profitable business for
549 these farmers. Local markets often do not require a specific commercial quality standard,
550 and both shorter and longer stems can be marketed, of course maintaining the aesthetic

551 quality and uniformity of these floral stems. In this way, the entire production can be utilized,
552 from the first to the last harvest. The ability to use dried statice floral stems allows farmers
553 to store them for a longer period, reducing losses if they are not sold quickly. Furthermore,
554 shorter stems that are grown in the lasts weeks of harvesting can offer farmer other
555 possibilities to supplement income, as the composing floral arrangements with dry flowers,
556 opening up another business opportunity to sell the production and generate more profits for
557 the family. For these reasons, statice has been a huge success in the “Flowers For All Project”
558 (Streck and Uhlmann, 2021).

559

560 **3.3.5 Conclusion**

561

562 Challenges for statice flower production posed by subtropical climates, especially
563 during the "off-season" were found. The development of statice floral stems varied among
564 cultivars, transplanting dates, and locations. Harvest period revealed a peak in production
565 that can last from 2 to 4 weeks, characterized by higher yield and production components,
566 followed by a decline in later harvests. Overall, This study highlights the flowering pattern
567 of the statice crop and underscores the importance of considering environmental factors in
568 maximizing the statice production. It also provides guidance for farmers on choosing the
569 best moment to cultivate statice in subtropical environment, facilitating statice harvests
570 aimed at producing high-quality and marketable flowers.

571

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573

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578

579 **Conflict of interest statement**

580 The authors declare no conflicts of interest.

581

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596 **3.3.6 References**

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703 **Tables**

704 **Table 1.** Location and their respective characteristics of biome, latitude, longitude, altitude, sowing date (dd/mm/yyyy), transplant date
 705 (dd/mm/yyyy), and institution.

706

| Trial | Location | Biome | Latitude | Longitude | Altitude | Sowing date | Transplant date | Institution |
|------------|-----------------|-----------------------|----------|-----------|----------|-------------------------|-----------------|-------------|
| 1 | Santa Maria, RS | Atlantic Forest/Pampa | 29°43' S | 53°43'W | 95 m | 25/05/2020 | 05/08/2020 | UFSM |
| | | | | | | 06/07/2020 | 10/09/2020 | |
| | | | | | | 04/08/2020 | 05/10/2020 | |
| | | | | | | 17/09/2020 | 06/11/2020 | |
| | | | | | | 21/10/2020 | 16/12/2020 | |
| | | | | | | 26/11/2020 | 13/01/2021 | |
| | | | | | | 17/12/2020 | 08/02/2021 | |
| | | | | | | 21/01/2021 | 10/03/2021 | |
| | | | | | | 03/03/2021 ^a | 14/04/2021 | |
| | | | | | | 08/04/2021 | 17/06/2021 | |
| | | | | | | 26/04/2021 | 08/07/2021 | |
| 12/05/2021 | 04/08/2021 | | | | | | | |
| 2 | Concórdia, SC | Atlantic Forest | 27°14' S | 52°1'W | 578 m | 25/05/2020 | 07/08/2020 | IFC |
| | Curitibanos, SC | Atlantic Forest | 27°16' S | 50°30'W | 992 m | 25/05/2020 | 08/08/2020 | UFSC |
| | Rio do Sul, SC | Atlantic Forest | 27°12' S | 49°38'W | 332 m | 25/05/2020 | 10/08/2020 | IFC |
| | | Pampa | | | 54°12'W | 133 m | 25/05/2020 | 03/08/2020 |

| | | | | | | | |
|--------------------------|-----------------------|-------------|---------|-------|------------|------------|---------|
| Dilermando de Aguiar, RS | | 29°42' S | | | 25/05/2020 | 03/08/2020 | On farm |
| Itaqui, RS | Pampa | 29°16' S | 59°51'W | 57 m | 25/05/2020 | 31/07/2020 | On farm |
| Júlio de Castilhos, RS | Atlantic Forest/Pampa | 29°23' S | 53°68'W | 529 m | 25/05/2020 | 29/07/2020 | On farm |
| Pinto Bandeira, RS | Atlantic Forest | 29°05' S | 51°27'W | 638 m | 25/05/2020 | 30/07/2020 | On farm |
| Santa Maria, RS | Atlantic Forest/Pampa | 29°43' S | 53°43'W | 95 m | 25/05/2020 | 01/08/2020 | On farm |

707

^a Only cultivar QIS Yellow cultivated.

708 **Table 2.** Sowing date (mm/dd/yyyy), transplanting date (mm/dd/yyyy), vernalization days (VD, days) from the sowing to transplanting phase
 709 [VD(SO-TP)], VD from the transplanting to R1 phase [VD(TP-R1)], and VD from the sowing to R1 phase [VD(SO-R1)] of field trials with
 710 two stative cultivars QIS Yellow and QIS Purple in the *Trial 1* and *2* during 2020-2021 in eight locations in Southern Brazil.

| Trial | Locations | Sowing date | Transplanting date | VD(SO-TP) | VD(TP – R1) | | VD(SO-R1) | | |
|-------|-------------------------------------|-------------|--------------------|------------|---------------|---------------|---------------|---------------|---------------|
| | | | | | QIS Yellow | QIS Purple | QIS Yellow | QIS Purple | |
| 1 | Santa Maria | 25/05/2020 | 05/08/2020 | 34.57 | 9.95 | 22.19 | 44.52 | 56.76 | |
| | | 06/07/2020 | 10/09/2020 | 32.06 | 8.38 | 13.64 | 40.44 | 45.70 | |
| | | 04/08/2020 | 05/10/2020 | 26.81 | 5.48 | 11.85 | 32.29 | 38.66 | |
| | | 17/09/2020 | 06/11/2020 | 17.61 | 1.91 | 76.68 | 19.52 | 94.29 | |
| | | 21/10/2020 | 16/12/2020 | 10.2 | 3.94 | 39.57 | 14.14 | 49.77 | |
| | | 26/11/2020 | 13/01/2021 | 5.85 | 53.40 | 44.88 | 59.25 | 50.73 | |
| | | 17/12/2020 | 08/02/2021 | 6.57 | 51.46 | 70.07 | 58.03 | 76.64 | |
| | | 21/01/2021 | 10/03/2021 | 6.55 | 57.35 | 48.21 | 63.9 | 54.76 | |
| | | 03/03/2021 | 14/04/2021 | 7.90 | 47.53 | - | 55.43 | - | |
| | | 08/04/2021 | 17/06/2021 | 31.23 | 27.47 | 33.37 | 58.7 | 64.6 | |
| | | 26/04/2021 | 08/07/2021 | 41.05 | 16.68 | 24.26 | 57.73 | 65.31 | |
| | | 12/05/2021 | 04/08/2021 | 48.82 | 11.44 | 16.24 | 60.26 | 65.06 | |
| | | | | CV% | 67.76% | 88.26% | 60.50% | 36.03% | 25.81% |
| | | | | SD | 15.20 | 21.70 | 22.05 | 16.94 | 15.54 |
| 2 | Concórdia/SC | 25/05/2020 | 07/08/2020 | 35.18 | 12.45 | 18.09 | 47.63 | 53.27 | |
| | Curitibanos/SC | 25/05/2020 | 08/08/2020 | 35.49 | 10.76 | 24.16 | 46.25 | 59.65 | |
| | Rio do Sul/SC | 25/05/2020 | 10/08/2020 | 36.20 | 15.18 | 23.73 | 51.38 | 59.93 | |
| | Dilermando de Aguiar/RS – Farmer 01 | 25/05/2020 | 03/08/2020 | 33.83 | 10.36 | 21.28 | 44.19 | 55.11 | |

| | | | | | | | |
|--|------------|------------|--------------|---------------|---------------|--------------|--------------|
| Dilermando de Aguiar/RS – Farmer 02 | 25/05/2020 | 03/08/2020 | 33.83 | 10.69 | 19.96 | 44.52 | 53.79 |
| Itaqui/RS - Irrigated | 25/05/2020 | 31/07/2020 | 32.62 | 19.86 | 24.34 | 52.48 | 56.96 |
| Itaqui/RS – Non-irrigated | 25/05/2020 | 31/07/2020 | 32.62 | 18.79 | 25.99 | 51.41 | 58.61 |
| Júlio de Castilhos/RS | 25/05/2020 | 29/07/2020 | 31.54 | 15.28 | 21.39 | 46.82 | 52.93 |
| Pinto Bandeira/RS | 25/05/2020 | 30/07/2020 | 32.11 | 17.65 | 25.91 | 49.76 | 58.02 |
| Santa Maria/RS | 25/05/2020 | 01/08/2020 | 33.04 | 10.69 | 19.40 | 43.73 | 52.44 |
| | | CV% | 4.61% | 25.92% | 12.40% | 6.81% | 5.17% |
| | | SD | 1.55 | 3.67 | 2.78 | 3.26 | 2.90 |

711 CV (%) = coefficient of variation, SD = standard deviation

712 **Table 3.** Percentage of field grown statice plants that reached the reproductive phase (%R1) until March 20, after March 20 for QIS Yellow and
 713 QIS Purple in the *Trial 1* and 2 during 2020-2021 in eight locations in Southern Brazil.

| Trial | Location | Transplanting date | %R1 until March 20 | | %R1 after March 20 | |
|------------|-------------------------------------|-------------------------|--------------------|------------|--------------------|------------|
| | | | QIS Yellow | QIS Purple | QIS Yellow | QIS Purple |
| 1 | Santa Maria | 05/08/2020 | 100 | 100 | 0 | 0 |
| | | 10/09/2020 | 100 | 100 | 0 | 0 |
| | | 05/10/2020 | 100 | 100 | 0 | 0 |
| | | 06/11/2020 | 66.7 | 16.7 | 0 | 72.2 |
| | | 16/12/2020 | 66.7 | 33.3 | 0 | 61.1 |
| | | 13/01/2021 | 0 | 5.6 | 66.7 | 94.4 |
| | | 08/02/2021 | 0 | 0 | 44.4 | 61.6 |
| | | 10/03/2021 | 0 | 0 | 61.1 | 77.8 |
| | | 14/04/2021 ^a | 0 | - | 88.1 | - |
| | | 17/06/2021 | 0 | 0 | 100 | 100 |
| | | 08/07/2021 | 0 | 0 | 100 | 100 |
| 04/08/2021 | 0 | 0 | 100 | 100 | | |
| 2 | Concórdia/SC | 07/08/2020 | 100 | 100 | 0 | 0 |
| | Curitibanos/SC | 08/08/2020 | 100 | 100 | 0 | 0 |
| | Rio do Sul/SC | 10/08/2020 | 100 | 100 | 0 | 0 |
| | Dilermando de Aguiar/RS – Farmer 01 | 03/08/2020 | 100 | 100 | 0 | 0 |
| | Dilermando de Aguiar/RS – Farmer 02 | 03/08/2020 | 100 | 100 | 0 | 0 |
| | Itaqui/RS - Irrigated | 31/07/2020 | 100 | 100 | 0 | 0 |
| | Itaqui/RS – Non-irrigated | 31/07/2020 | 89.9 | 94.4 | 0 | 0 |
| | Júlio de Castilhos/RS | 29/07/2020 | 100 | 100 | 0 | 0 |

| | | | | | | |
|--|-------------------|------------|-----|------|---|---|
| | Pinto Bandeira/RS | 30/07/2020 | 100 | 100 | 0 | 0 |
| | Santa Maria/RS | 01/08/2020 | 100 | 94.4 | 0 | 0 |

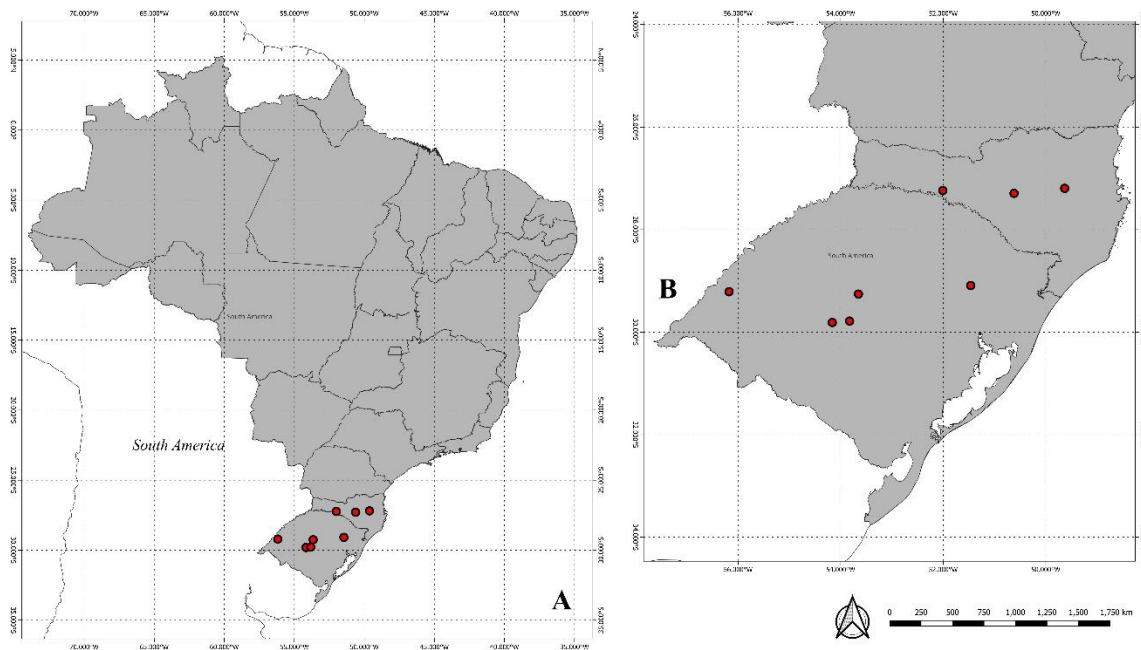
714 ^a Only cultivar QIS Yellow cultivated.

715 **Table 4.** Yield per plant (stems/plant) for QIS Yellow and QIS Purple in the *Trial 1* and 2
 716 during 2020-2021 in eight locations in Southern Brazil.

717

| <i>Trial 1</i> | | |
|-------------------------------------|-------------------|-------------------|
| Transplant date | QIS Yellow | QIS Purple |
| 05/08/2020 | 30.52 a | 24.56 abc |
| 10/09/2020 | 28.10 ab | 18.12 abc |
| 05/10/2020 | 21.11 abc | 11.76 c |
| 06/11/2020 | 13.00 bc | 12.21 c |
| 16/12/2020 | 6.63 c | 12.55 c |
| 13/01/2021 | 17.83 abc | 15.11 bc |
| 08/02/2021 | 20.83 abc | 35.67 A |
| 10/03/2021 | 23.88 abc | 32.4 ab |
| 14/04/2021 | 21.86 abc | - |
| 17/06/2021 | 17.72 abc | 21.83 abc |
| 08/07/2021 | 16.31 abc | 21.16 abc |
| 04/08/2021 | 16.01 abc | 15.89 bc |
| <i>Trial 2</i> | | |
| Location | QIS Yellow | QIS Purple |
| Concórdia/SC | 26.00 abA | 14.70 bB |
| Curitibanos/SC | 20.77 bcA | 15.23 bA |
| Rio do Sul/SC | 9.73 dA | 5.20 cA |
| Dilermando de Aguiar/RS – Farmer 01 | 15.83 cdA | 17.50 bA |
| Dilermando de Aguiar/RS – Farmer 02 | 15.13 cdA | 10.37 bcA |
| Itaqui/RS - Irrigated | 15.03 cdA | 9.93 bcA |
| Itaqui/RS – Non-irrigated | 15.73 cdA | 11.20 bcA |
| Pinto Bandeira/RS | 18.50 bcA | 18.27 abA |
| Santa Maria/RS | 31.60 aA | 26.07 aA |

718 Lowercase letters in columns compare one cultivar between transplant dates or locations and
 719 uppercase letters in lines compare cultivars in the same location, according to Tukey's test
 720 ($p < 0.05$).



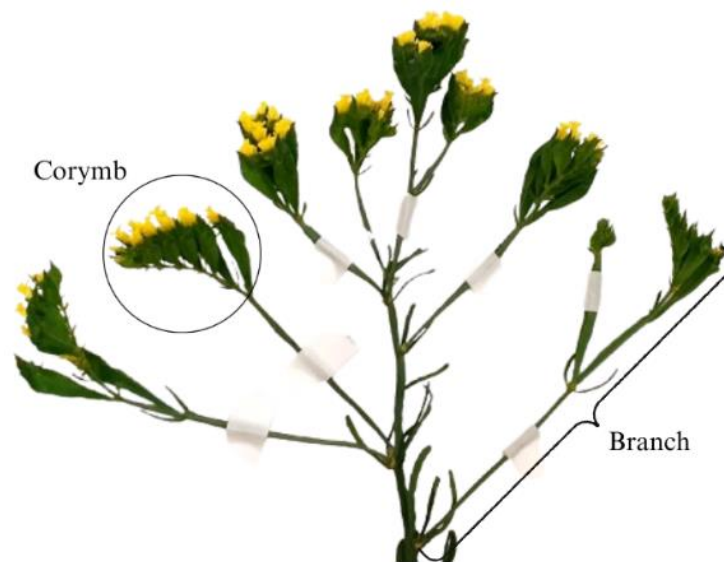
721

722 **Figure 1.** Geographic location of the field trials with stative conducted in eight locations in

723 Rio Grande do Sul and Santa Catarina States, Southern Brazil, during 2020 and 2021.

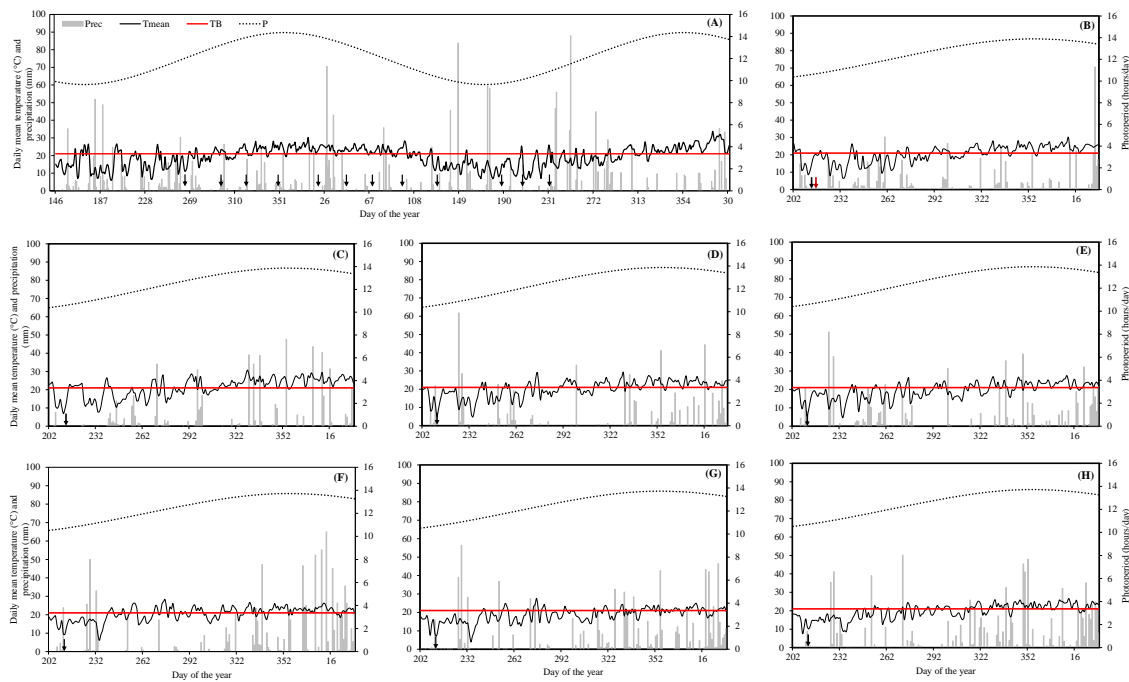
724 Locations are presented from the point of view of Brazil (A) and Southern Brazil (B).

725



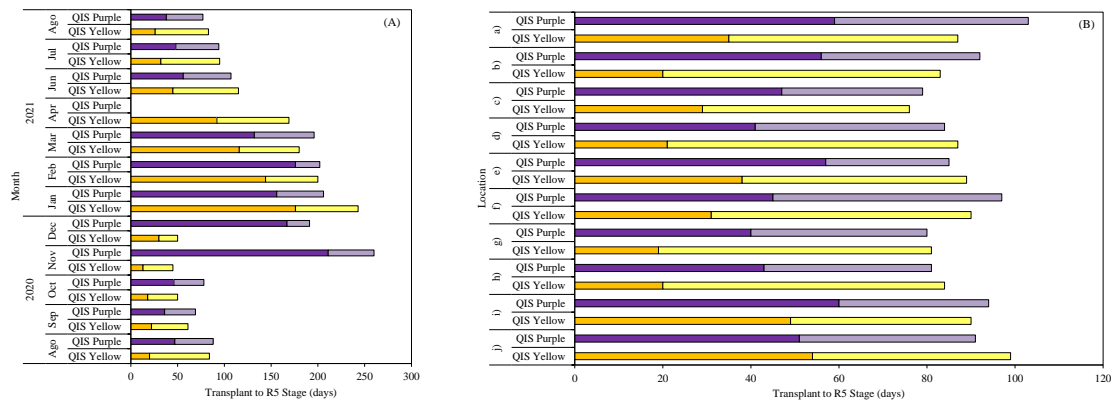
726

727 **Figure 2.** Illustration of the composition of a statice floral stem with corymbs and branching.



728

729 **Figure 3.** Daily variation of the mean air temperature (Tmean), precipitation (Prec) and
 730 photoperiod (P) during field trials with static conducted in eight locations in Rio Grande
 731 do Sul and Santa Catarina States, Southern Brazil, during 2020 and 2021: (A) *Trial 1*
 732 conducted in Santa Maria/RS. Each arrow indicates a transplanting date: August/2020,
 733 September/2020, October/2020, November/2020, December/2020, January/2021,
 734 February/2021, March/2021, April/2021, June/2021, July/2021, and August/2021; (B) *Trial*
 735 *2* in Santa Maria/RS and Dilermando de Aguiar/RS (indicated by arrow red), (C) Júlio de
 736 Castilhos/RS, (D) Itaqui/RS, (E) Pinto Bandeira/RS, (F) Concórdia/SC, (G) Curitibanos/SC
 737 and (H) Rio do Sul/SC. Each arrow indicates a transplanting date in each location. The
 738 horizontal red line indicates the upper base cardinal temperature for vernalization (21°C).



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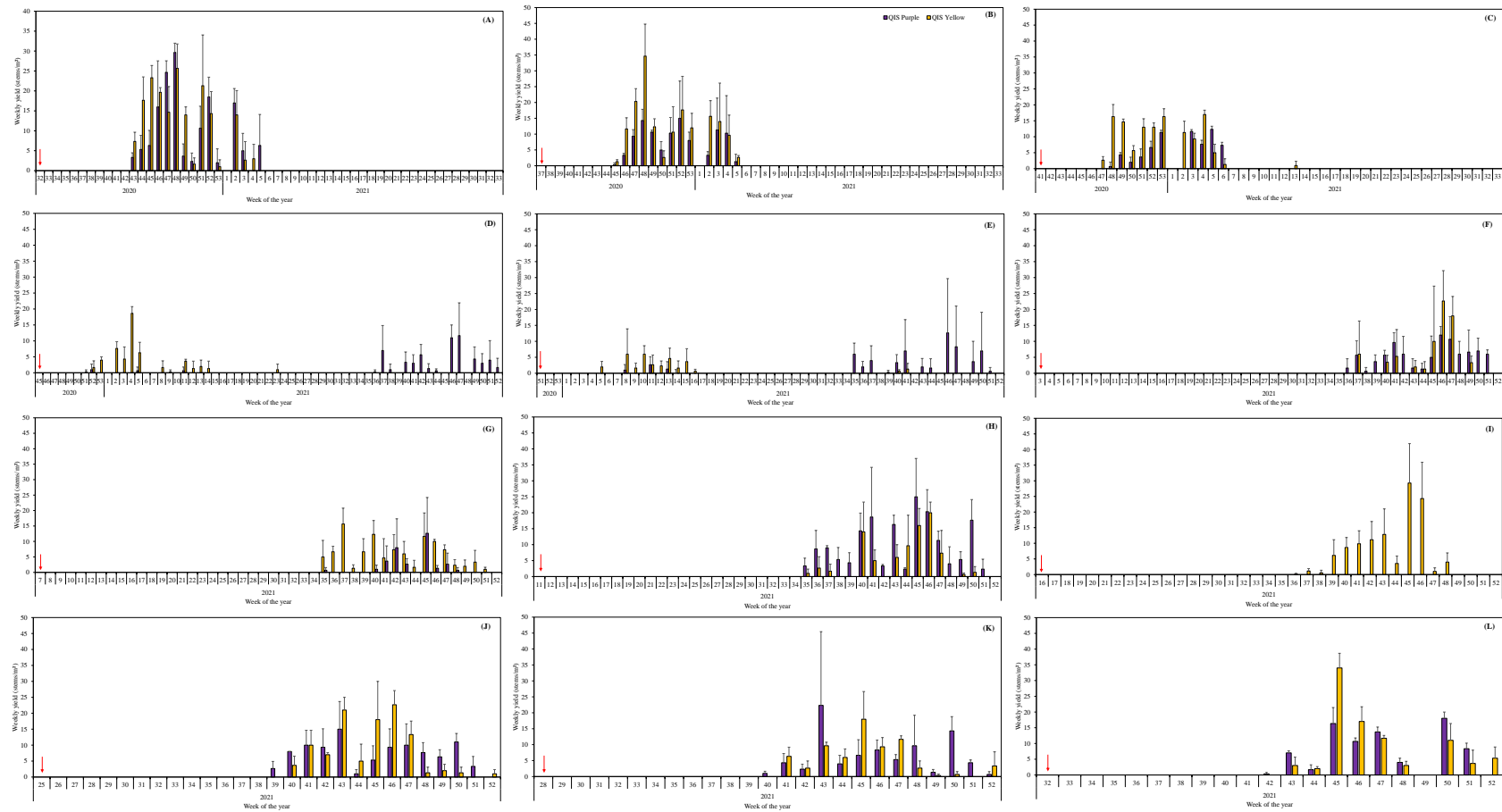
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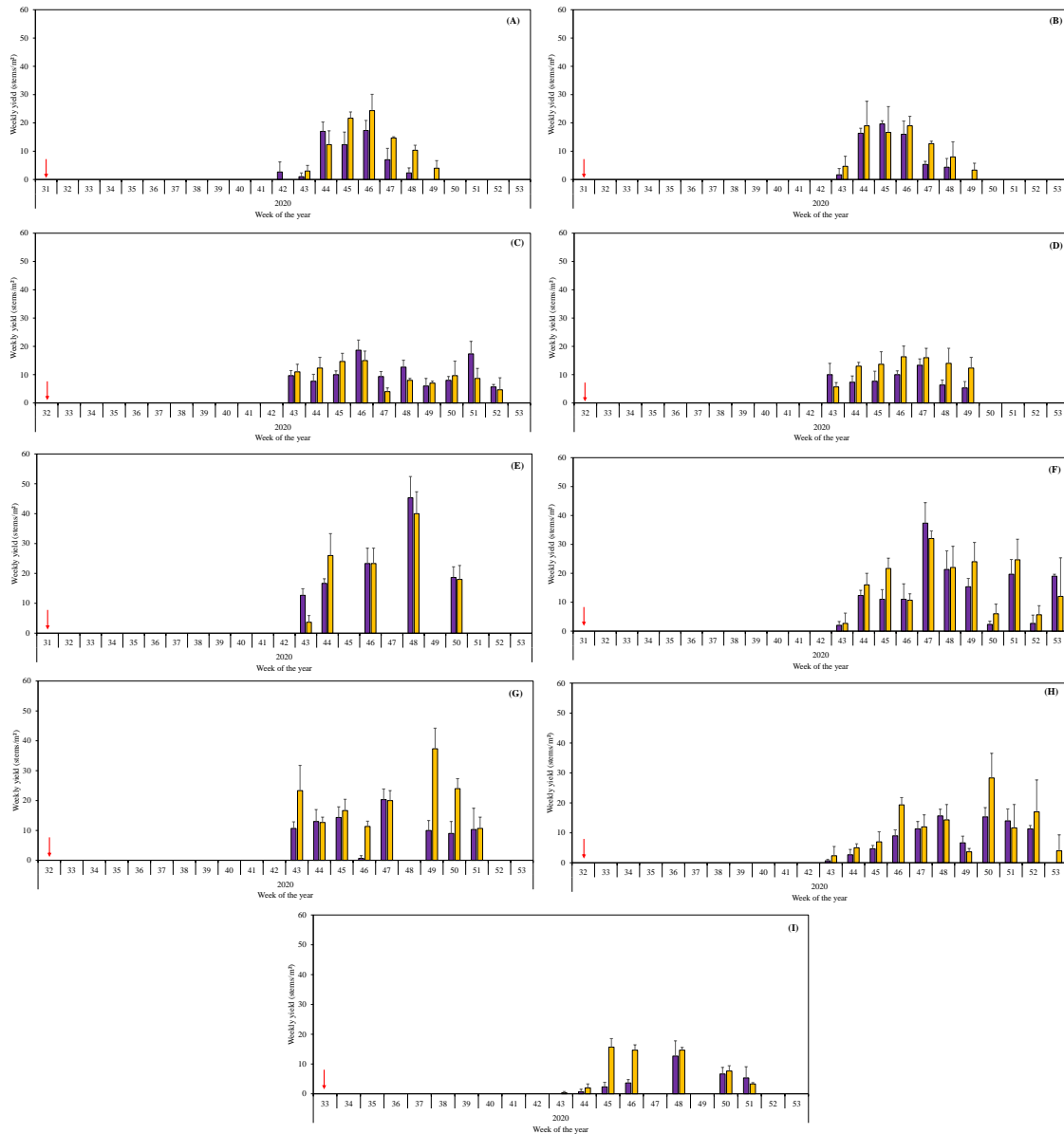
Figure 4. Days from transplanting to flowering (R5 stage) (bars) divided into vegetative phase (TP-R1, left end of the bars) and reproductive phase (R1-R5, right end of the bars) of field grown statice in Trial 1 (A) and Trial 2 (B) in eight locations in Rio Grande do Sul and Santa Catarina States, Southern Brazil, during 2020 and 2021. Locations in panel (B) are represented by letters as follows: a) Rio do Sul/SC, b) Curitibaanos/SC, c) Concórdia/SC, d) Santa Maria/RS, e) Pinto Bandeira/RS, f) Júlio de Castilhos/RS, g) Dilermando de Aguiar/RS – Farmer 1, h) Dilermando de Aguiar/RS – Farmer 2, i) Itaqui/RS – Non irrigated, and j) Itaqui/RS – Irrigated.



748

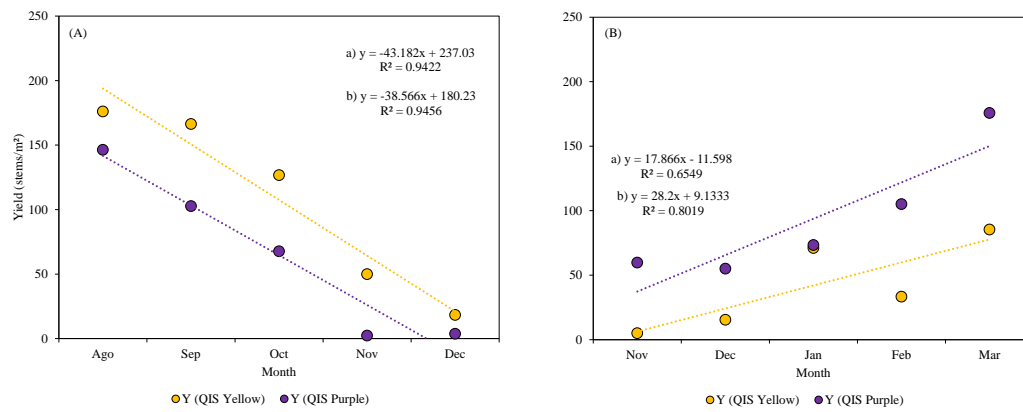
749 **Figure 5.** Weekly yield during the harvest period of field grown statice for each transplanting date in Santa Maria, RS, Brazil during 2020 and
 750 2021: (A) August/2020, (B) September/2020, (C) October/2020, (D) November/2020, (E) December/2020, (F) January/2021, (G)
 751 February/2021, (H) March/2021, (I) April/2021, (J) June/2021, (K) July/2021, and (L) August/2021. Error bars were included for each harvest.
 752 Each arrow indicates the week of transplanting in each month.

753



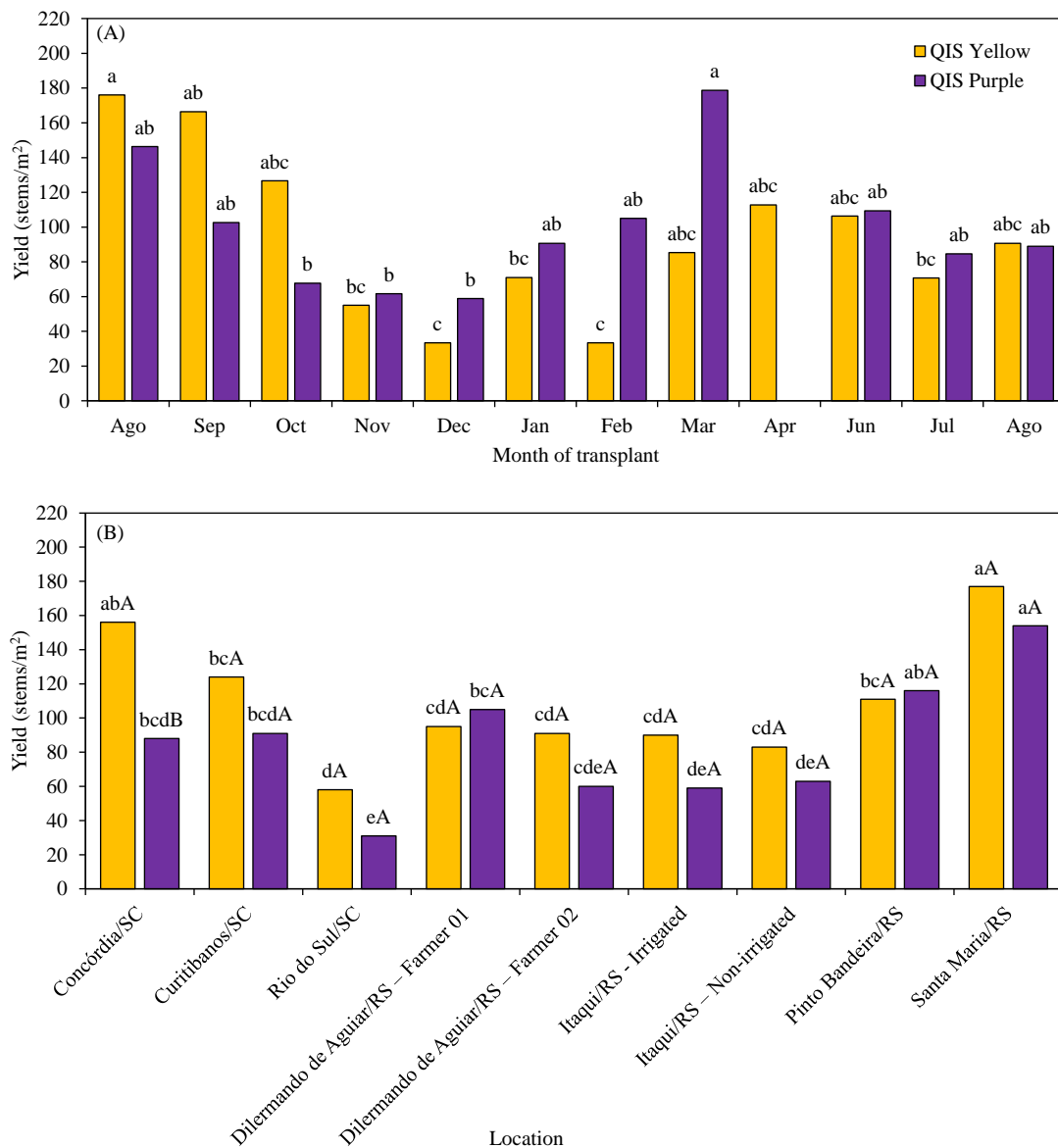
754

755 **Figure 6.** Weekly yield during the harvest period of field grown static in seven locations in
 756 Southern Brazil in 2020: (A) Itaqui/RS – Irrigated, (B) Itaqui/RS – Non irrigated, (C)
 757 Dilermando de Aguiar/RS – Farmer 1, (D) Dilermando de Aguiar/RS – Farmer 2, (E) Pinto
 758 Bandeira/RS, (F) Santa Maria/RS, (G) Concórdia/SC, (H) Curitiba/SC, and (I) Rio do
 759 Sul/SC. Error bars were included for each harvest. Each arrow indicates the week of
 760 transplanting in each location.



761

762 **Figure 7.** Floral stems yield (stems/m²) of field grown statice as a function of the
 763 transplanting date until March 20 (A) and after March 20 (B) in Santa Maria/RS, Brazil for
 764 *Trial 1* during 2020 and 2021. Equation a) corresponds to QIS Yellow and b) QIS Purple
 765 cultivars.



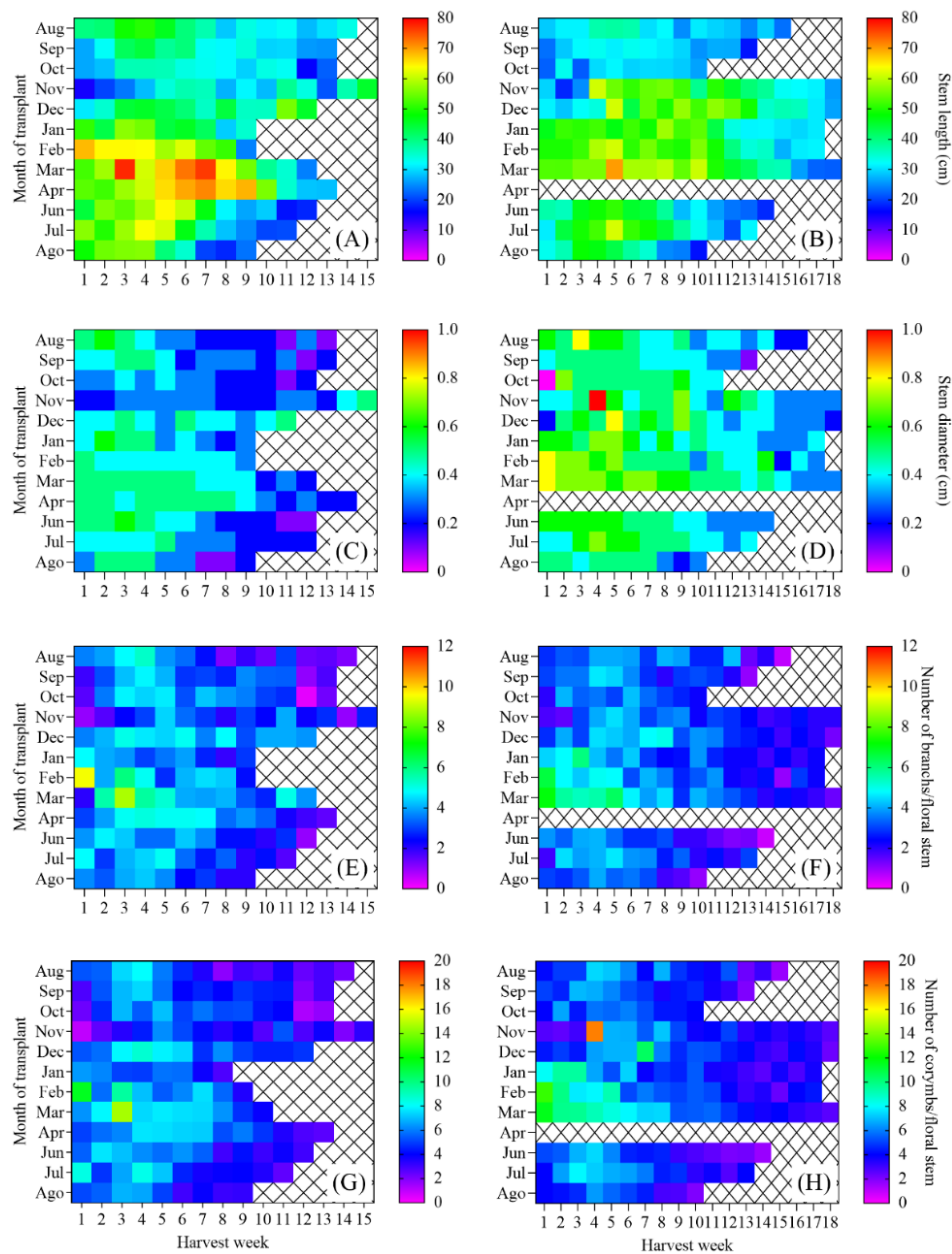
766

767 **Figure 8.** Floral stems yield (stems/m²) of field grown stative in different (A) transplanting768 dates for *Trial 1* and (B) locations for *Trial 2* in Southern Brazil during 2020-2021.

769 Lowercase letters compare the same cultivar between transplanting dates or locations and

770 uppercase letters compare cultivars in the same location, according to Tukey's test ($p < 0.05$).

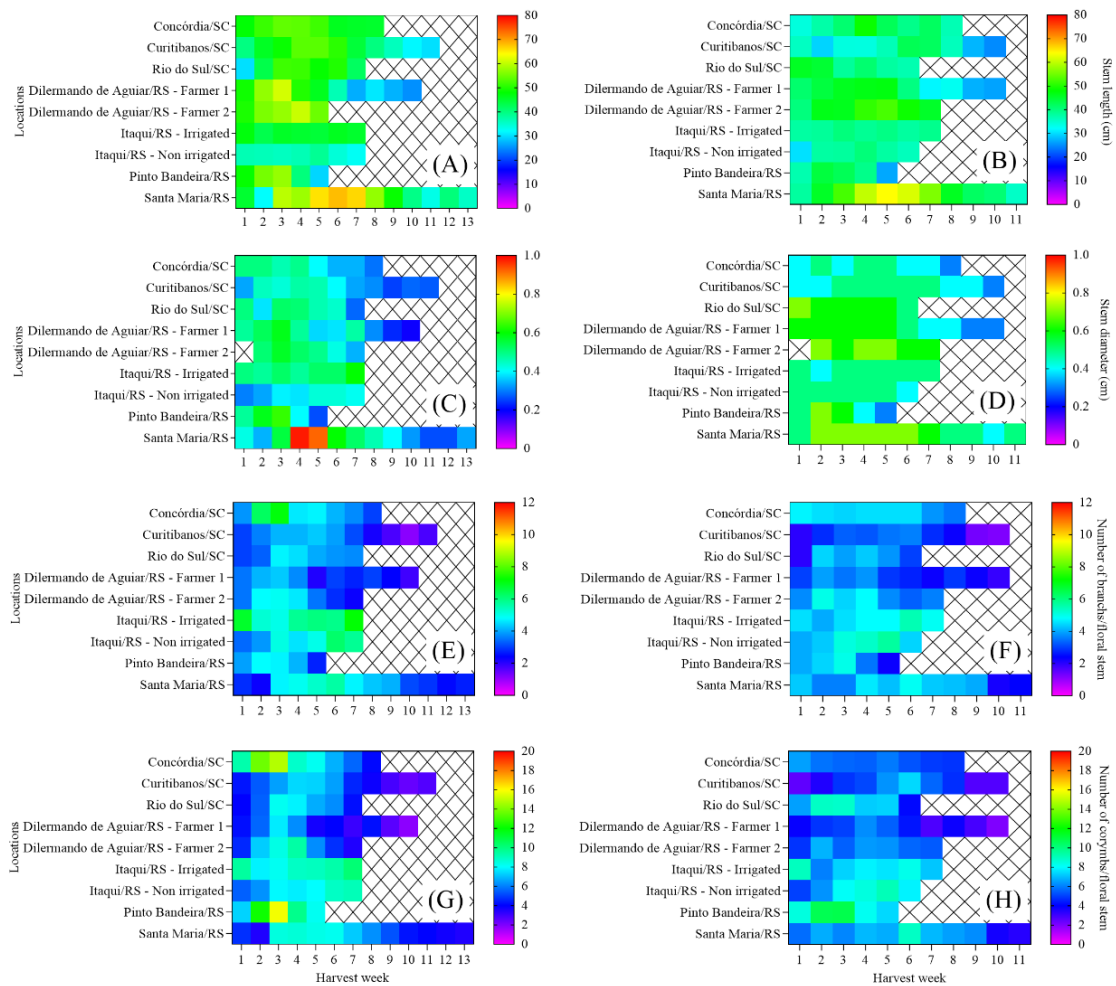
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772

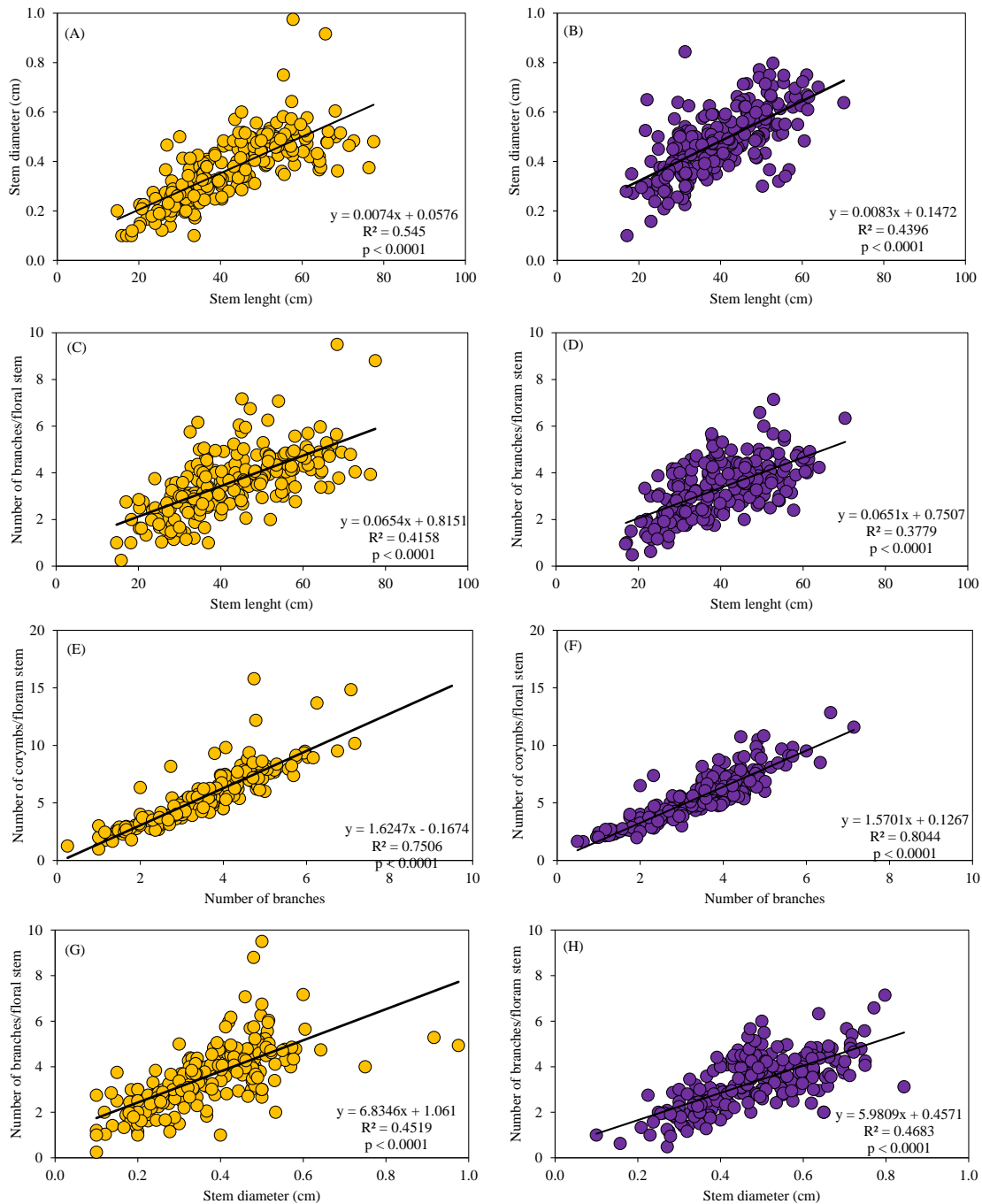
773 **Figure 9.** Flower production components of field grown statice floral stem throughout the
 774 harvest period for *Trial 1* in Santa Maria/RS, Brazil, during 2020-2021. Graphics (A, C, E,
 775 G) correspond to QIS Yellow, and Graphics (B, D, F, H) correspond to QIS Purple. The
 776 flower production components presented are (A, B) stem length (cm), (C, D) stem diameter,
 777 (E, F) number of branches/floral stem and (G, H) number of corymbs/floral. Color gradient
 778 in a heatmap goes from cold colors, representing low values, to hot colors, representing high
 779 values.

780



781

782 **Figure 10.** Flower production components of field grown static floral stems throughout the
 783 harvest period for *Trial 2* in seven locations in Rio Grande do Sul and Santa Catarina States,
 784 Southern Brazil, during 2020. Graphics (A, C, E, G) correspond to QIS Yellow, and Graphics
 785 (B, D, F, H) correspond to QIS Purple. The flower production components presented are (A,
 786 B) stem length (cm), (C, D) stem diameter, (E, F) number of branches/floral stem and (G,
 787 H) number of corymbs/floral. Color gradient in a heatmap goes from cold colors,
 788 representing low values, to hot colors, representing high values.



789

790 **Figure 11.** Linear regression between floral stem diameter (cm) and stem length (cm),

791 number of branches/floral stem and stem length (cm), number of corymbs/floral stem and

792 number of branches /floral stem, number of branches/floral stem and stem diameter (cm) for

793 QIS Yellow (A, C, E, G) and QIS Purple (B, D, F, H), respectively. Data of field grown

794 statice floral stems in *Trial 1 and 2* in seven locations in Rio Grande do Sul and Santa

795 Catarina States, Southern Brazil, during 2020 and 2021.

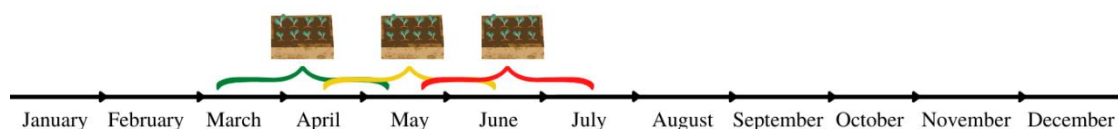
796

797 **Graphical abstract**

(A) SOWING



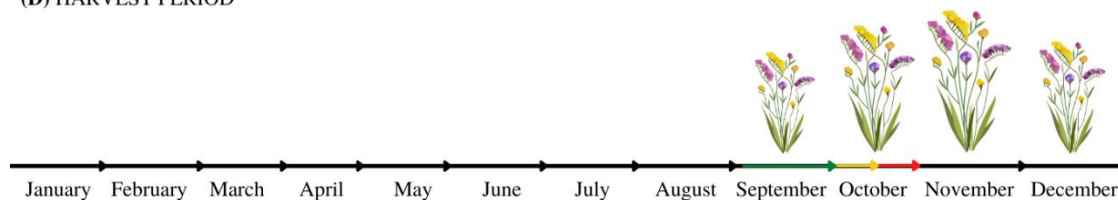
(B) SEEDLINGS GROWTH



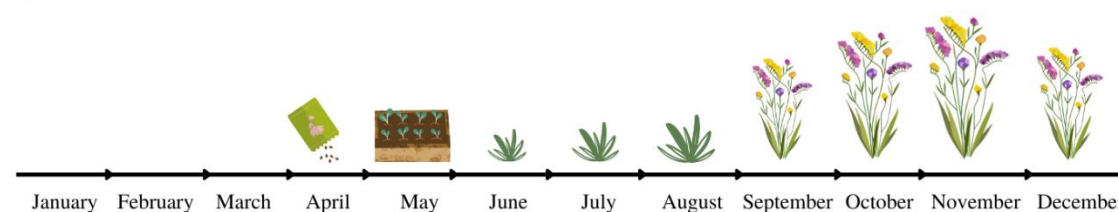
(C) TRANSPLANT



(D) HARVEST PERIOD



(E) GROWING SEASON EXEMPLE



798

799 This study investigated the flowering of static in subtropical environment with practical
 800 applications to the farmers. The harvest period can vary from 5 to 18 weeks depending on
 801 cultivar, transplant date and location. Among harvest weeks, the highest values of yield and
 802 flowers quality is achieved during about 3 to 5 weeks. Transplants during the off-season
 803 prolong the developmental cycle and reduce yield due to the vernalization requirements.

4 DISCUSSÃO

Demonstramos que ensaios de campo realizados multi-anos e multi-locais proporcionam uma melhor compreensão da interação entre espécies e ambientes. A colaboração estabelecida entre as diferentes instituições de ensino e pesquisa espalhadas pelo Brasil em pesquisas com o girassol de corte e statice, e com o Centro de Pesquisa em Horticultura e Floricultura (CREA) na Itália, com o girassol de corte, proporcionaram um banco de dados robusto para compreensão da interação genótipo x ambiente x manejo e produtor. Raros são os trabalhos na área da floricultura que conseguem atingir esse patamar de conexão entre instituições em prol de um mesmo objetivo: avançar na barreira do conhecimento para gerar resultados práticos e aplicados às reais demandas do produtor de flores. A riqueza dos resultados gerados nesta tese graças a essa imensa colaboração é notória.

No Capítulo 1, foi possível estabelecer relações ecofisiológicas entre variáveis dependentes e independentes, como por exemplo: ciclo de desenvolvimento em função da duração da fase vegetativa e reprodutiva em tempo térmico ($^{\circ}\text{C}$ dia), número de folhas no estágio R1 em função do número final de folhas e componentes de produtividade (comprimento da planta, espessura da haste e espessura do capítulo) em função do índice de área foliar. Poucos estudos sobre a espécie *Helianthus annuus L.* e principalmente para genótipos com finalidade ornamental abordam este nível de informações complexas e de grande relevância para a compreensão dos fatores ecofisiológicos que influenciam o crescimento, desenvolvimento e produção de hastes florais de girassol de corte.

Os resultados evidenciam que os genótipos de girassol de corte utilizados para este estudo são bem adaptados nos ambientes tropical, subtropical e temperado, apesar das variações proporcionadas pelas diferentes condições ambientais. Ademais, um grande avanço a partir deste primeiro capítulo é o estudo com genótipos de origem italiana que ainda não estão disponíveis para cultivo comercial no Brasil, mas que apresentam grande potencial para serem introduzidas no mercado brasileiro de flores.

No Capítulo 2, aprofundou-se a discussão sobre o desenvolvimento foliar em genótipos de girassol de corte, através do filocrono, variável também abordada no Capítulo 1, mas de forma simplificada. Observou-se que ocorre uma “quebra” na emissão de folhas entre os estágios V6 (seis folhas) e V7 (sete folhas), dividindo-a em duas fases. Com isso, a

relação entre a emissão de folhas e o tempo térmico não pode ser mais considerada linear, exigindo uma bi-linearidade entre as fases. Ao aplicar duas regressões lineares, foi possível determinar dois valores de filocrono, um para cada fase. O filocrono da fase “*early*” é maior ($34.87^{\circ}\text{C dia folha}^{-1}$) do que o filocrono na fase “*late*” ($21.82^{\circ}\text{C dia folha}^{-1}$), ou seja, até a sexta folhas a velocidade de emissão de folhas é menor do que a partir da sétima folha.

Em um primeiro momento, levantou-se a hipótese de que o método de plantio (semeadura direta ou transplante) poderia influenciar nessa mudança. O método mais utilizado por produtores e pelos participantes do Projeto Flores para Todos, é o transplante das mudas. As sementes são semeadas em bandejas com substratos e transplantadas apenas quando as folhas cotiledonares apresentam uma abertura de 180° e o primeiro par de folhas verdadeiras já está visível entre as folhas cotiledonares. Ao utilizar mudas transplantadas, quaisquer limitações no desenvolvimento das mudas e um possível estresse no transplante, pode prejudicar o estabelecimento inicial do stand. No entanto, os resultados demonstram que nem a semeadura direta nem o transplante são capazes de causar este retardamento no início do desenvolvimento foliar.

Descartada essa hipótese, observou-se clinicamente como ocorre a emissão de folhas em girassol de corte e sua morfologia. Constatou-se que essa mudança na velocidade de emissão de folhas está principalmente relacionada com a ontogenia da planta de girassol, visto que a quebra na emissão de folhas ocorre exatamente quando a filotaxia foliar se altera, de folhas opostas para folhas alternadas e o alongamento do caule se torna mais proeminente. Mudanças na velocidade de emissão de folhas relacionadas com a ontogenia também foi verificada para gramíneas, como o trigo (BAUMONT et al., 2019). O estudo do desenvolvimento foliar e a identificação de particularidades como essa, são de extrema importância para a calibração de modelos de simulação do desenvolvimento baseados em processos. Considerar essa mudança na velocidade de emissão de folhas no girassol de corte indica a necessidade da inclusão de uma função de resposta à cronologia no modelo de emissão de folhas para aumentar o desempenho do modelo em simular a emissão de folhas de girassol de corte.

Outra espécie de grande importância na floricultura brasileira e mundial é a *statice*. Esta espécie foi introduzida no Projeto Flores Para Todos em 2020 e tem grande aceitabilidade entre os produtores rurais, principalmente pela sua versatilidade e alta produtividade. Por isso, no Capítulo 3 abordou-se especificamente a produção de hastes florais de *statice*. Um dos grandes problemas enfrentados pelos produtores é o local de

cultivo e a sazonalidade da produção. No Brasil, o cultivo limita-se na Região Sul com transplantes a partir de maio e colheitas a partir de setembro, devido aos requerimentos de vernalização e fotoperíodo longo da espécie.

Os resultados demonstraram que a *statice* pode ser muito produtiva quando respeitada a sua época de cultivo e que em períodos de entressafra, a produção de flores atrasará e reduzirá significativamente. O florescimento da *statice* segue um padrão, no qual durante o período de colheita há uma janela de duas a quatro semanas em que a produtividade em hastes por metro quadrado é maior, bem como há um incremento nos componentes de produtividade (comprimento da haste, diâmetro da haste, número de ramificações por haste e número de corimbos por haste). Posteriormente a esta janela, estes parâmetros começam a cessar, reduzindo constantemente até o final do ciclo.

Estes resultados fornecem diretrizes para produtores de flores de *statice* para o planejamento da comercialização das hastes florais de *statice* em função da produtividade ao longo do período de produção. Por exemplo, sabendo que no plantio realizado no mês de Agosto a primeira colheita ocorrerá a partir da semana 43 do ano, e que o período de colheita pode durar onze semanas consecutivas, com a maior produtividade e qualidade das flores a partir da segunda ou terceira semana de colheita, o produtor pode agendar a entrega das flores para seus clientes para depois desta janela. Dada a possibilidade do uso da *statice* como flor seca, não havendo a venda do produto fresco, esta pode ser armazenada e comercializada posteriormente, diminuindo as perdas na produção. Este estudo preliminar sobre o padrão de florescimento da *statice* poderá servir se base a construção de um modelo que além de simular o desenvolvimento da planta, indicando a ocorrência dos estágios fenológicos, poderá simular o período de colheita das hastes florais de *statice* em resposta à vernalização e ao fotoperíodo e a frequência com que podem ser realizadas as colheitas das hastes florais. Esta funcionalidade em um modelo de simulação permitirá ao usuário realizar o planejamento da produção de *statice* com maior regularidade, precisão e praticidade.

Do ponto de vista da modelagem, espera-se que os resultados desta tese forneçam uma base sólida para o desenvolvimento de tecnologias na área da floricultura, como modelos de simulação do desenvolvimento baseados em processos para as culturas do girassol de corte e da *statice*. Para desenvolver um modelo com boas funcionalidades e alta taxa de acerto, estudos preliminares como esse são necessários, demandando tempo, recursos humanos e financeiros. Na área da floricultura, o uso de modelos ainda é muito limitado em função da grande diversidade de espécies e genótipos. No entanto, tecnologias como essa

tem ganhado espaço a cada dia, tornando-se importantes e indispensáveis ferramentas de gestão, auxiliando os produtores de flores na tomada de decisão para escolha de cultivares, época de cultivo, realização de práticas culturais e planejamento da colheita. Um excelente exemplo do uso de modelos na área da floricultura é o Aplicativo PhenoGlad Mobile, principal ferramenta de gestão utilizada no Projeto Flores Para Todos para o cultivo de gladiolos (UHLMANN, 2017; UHLMANN, 2019).

Sobretudo, espera-se que estes resultados forneçam informações importantes aos produtores de flores sobre ambiente e seus efeitos nos processos ecofisiológicos no cultivo de girassol de corte e statice. Apesar de utilizar como exemplo o Projeto Flores Para Todos, devido a sua importante abrangência nacional, os impasses relacionados à produção de cada uma das espécies estudadas vão além, e são realidade para a grande maioria dos produtores de flores no Brasil e ao redor do mundo. O setor de flores e plantas ornamentais brasileiro só progredirá, principalmente na diversidade de produtos ofertados (diferentes espécies), com o aumento de estudos como este, com aplicação prática que atendam as demandas de quem produz e que contribuam efetivamente para a resolução dos reais problemas enfrentados em todas as camadas do setor. Pesquisas na área da floricultura estão aumentando a cada dia, mas ainda há muito para avançar no conhecimento sobre flores e plantas ornamentais, dada a vasta diversidade de biomas existentes em solo brasileiro. Espera-se que esses resultados sirvam de embasamento para pesquisas futuras na área da floricultura, não apenas sobre *Helianthus annuus* L. e *Limonium sinuatum* Mill., mas entre outras espécies exóticas e nativas.

5 CONCLUSÕES

Há um impacto significativo dos ambientes tropicais, subtropicais e temperados no desenvolvimento, crescimento e produção de flores do girassol de corte. A duração da fase vegetativa tem uma relação linear positiva com o ciclo de desenvolvimento, assim como a fase reprodutiva, e é determinada pelo número final de folhas. Por outro lado, o índice de área foliar tem uma relação linear negativa com as datas de semeadura, assim como o filocrono e o número final de folhas. Os componentes de produção de flores são definidos pelo índice de área foliar e pelo número final de folhas. Em resumo, cultivares com ciclo de desenvolvimento mais longo têm alto número final de folhas, baixo filocrono e maior altura de planta do que cultivares com ciclo mais curto.

Há um ponto de quebra entre os estágios V6 e V7, evidenciado pela regressão bi-linear entre o número de folhas e o tempo térmico ($^{\circ}\text{C dias}$). Duas fases distintas de filocrono foram identificadas: uma inicial, a $34,87^{\circ}\text{C dia folha}^{-1}$, e uma tardia, a $21,82^{\circ}\text{C dia folha}^{-1}$. As fases do filocrono apresentam consistência entre os genótipos e são influenciadas pelo ambiente, como variabilidade de local de cultivo. Essas descobertas não apenas aprimoram nossa compreensão sobre o filocrono, mas também representam um avanço substancial, fornecendo uma base sólida para incluir uma função de resposta de cronologia no desenvolvimento de futuros modelos baseados em processos para o girassol de corte.

Há uma forte dependência da época de cultivo de *statice* às condições flutuantes de vernalização, fotoperíodo e temperatura. O desenvolvimento das hastes florais de *statice* variou entre cultivares, datas de transplante e locais nos dias até o início da fase reprodutiva (45 a 243 dias), no período de colheita (5 a 18 semanas), no rendimento (33 a 180 hastes/m²) e componentes de produção de flores em cada semana de colheita. O período de colheita revelou um pico na produção que pode durar de 2 a 4 semanas, caracterizado por um maior rendimento e componentes de produção, seguido por uma diminuição em colheitas posteriores, caracterizando um padrão no florescimento da cultura de *statice*.

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APÊNDICES

APÊNDICE A

Supplementary 1. Exploratory analysis of developmental cycle, phyllochron, final leaf number, plant height, stem diameter and capitulum diameter for tropical, subtropical and temperate environments for cut sunflower in 4 trials in Brazil and 1 in Italy.

| Environme nt | Variable | Descriptive statistics | | | | | | | | | |
|--------------------|---------------------|------------------------|--------|-----------|--------|--------|--------|-------|-------|--------|--------|
| | | Min | Max | \bar{x} | Med | Q1 | Q3 | SD | Skew | Kurt | CV% |
| Tropical | Dev. Cycle (°C day) | 792.8 | 1431.0 | 1067.0 | 1042.0 | 936.80 | 1179.0 | 152.7 | 0.26 | -0.60 | 14.31% |
| | Dev. Cycle (days) | 43.0 | 78.20 | 54.26 | 52.40 | 48.85 | 58.18 | 8.202 | 0.95 | 0.38 | 15.12% |
| | Phyllochron | 20.00 | 37.70 | 27.60 | 26.30 | 23.90 | 31.30 | 5.07 | 0.40 | -0.89 | 18.39% |
| | FLN | 15.60 | 32.50 | 24.69 | 24.70 | 22.80 | 28.20 | 4.35 | -0.59 | -0.19 | 17.63% |
| | LN at R1 | 7.80 | 26.90 | 18.21 | 18.80 | 14.80 | 21.60 | 4.77 | -0.41 | -0.75 | 26.20% |
| | FLN – LN at R1 | 2.20 | 15.20 | 7.29 | 7.40 | 5.30 | 8.80 | 3.03 | 0.60 | 0.12 | 41.53% |
| | Plant Height | 72.40 | 217.1 | 140.3 | 140.90 | 119.10 | 162.50 | 28.82 | 0.05 | -0.19 | 20.54% |
| | Stem diameter | 0.60 | 1.70 | 1.18 | 1.10 | 0.60 | 1.30 | 0.19 | 0.43 | 0.60 | 16.90% |
| Capitulum diameter | 3.50 | 8.90 | 5.29 | 5.20 | 4.70 | 5.78 | 0.98 | 0.92 | 2.21 | 18.44% | |
| Subtropical | Dev. Cycle (°C day) | 714.7 | 1457.0 | 1003.0 | 999.0 | 911.1 | 1075.0 | 142.5 | 0.66 | 0.86 | 14.21% |
| | Dev. Cycle (days) | 40.0 | 110.8 | 66.90 | 63.70 | 54.20 | 76.65 | 15.61 | 0.86 | 0.07 | 23.34% |
| | Phyllochron | 18.60 | 38.50 | 26.65 | 26.25 | 23.00 | 29.65 | 4.47 | 0.50 | -0.27 | 16.75% |
| | FLN | 13.0 | 40.40 | 24.45 | 24.65 | 20.30 | 28.13 | 5.42 | 0.27 | -0.12 | 22.14% |
| | LN at R1 | 6.80 | 30.70 | 17.02 | 16.75 | 13.53 | 20.98 | 4.93 | 0.11 | -0.57 | 28.94% |
| | FLN – LN at R1 | 0.90 | 14.00 | 6.80 | 6.70 | 4.70 | 8.80 | 2.78 | 0.23 | -0.39 | 40.93% |

Cont. **Supplementary 1**

| | | | | | | | | | | | |
|-----------|---------------------|-------|--------|--------|--------|--------|--------|------------|-------|-------|--------|
| | Plant Height | 54.5 | 217.4 | 127.2 | 126.0 | 101.40 | 151.70 | 36.70 | 0.23 | -0.31 | 28.85% |
| | Stem diameter | 0.60 | 2.8 | 1.36 | 1.30 | 1.13 | 1.50 | 0.34 | 1.16 | 3.68 | 28.14% |
| | Capitulum diameter | 2.80 | 10.30 | 5.81 | 5.60 | 5.00 | 6.60 | 1.27 | 0.69 | 0.86 | 21.88% |
| | Dev. Cycle (°C day) | 903.5 | 1383.0 | 1140.0 | 1131.0 | 1050.0 | 1247.0 | 140.6 0 | 0.40 | -0.25 | 12.34% |
| | Dev. Cycle (days) | 81.00 | 108.0 | 94.96 | 94.40 | 90.20 | 101.2 | 7.86 | 0.27 | -0.19 | 8.27% |
| | Phyllochron | 23.80 | 31.30 | 26.68 | 26.40 | 25.35 | 27.75 | 2.03 | 0.75 | 1.02 | 7.62% |
| Temperate | FLN | 17.50 | 32.30 | 26.25 | 26.10 | 23.60 | 30.60 | 4.55 | -0.47 | -0.39 | 17.33% |
| | LN at R1 | 12.40 | 25.50 | 18.25 | 18.00 | 14.65 | 21.35 | 4.06 | 0.45 | -0.77 | 22.24% |
| | FLN – LN at R1 | 5.00 | 13.20 | 8.04 | 7.40 | 5.10 | 13.20 | 2.96 | 0.39 | -1.43 | 36.84% |
| | Plant Height | 113.8 | 195.70 | 158.50 | 159.40 | 146.50 | 172.50 | 20.60 | -0.43 | 1.04 | 12.99% |
| | Stem diameter | 1.20 | 1.80 | 1.44 | 1.40 | 1.35 | 1.50 | 0.15 | 0.96 | 1.97 | 10.44% |
| | Capitulum diameter | 4.90 | 7.40 | 6.16 | 6.20 | 5.65 | 6.70 | 0.71 | 0.06 | -0.26 | 11.49% |

Min = minimum value; Max = maximum value, \bar{x} = mean, Med = median, Q1 = first quartile, Q3 = third quartile, SD = standard deviation, Skew = coefficient of Skewness, Kurt = coefficient of kurtosis, CV (%) = coefficient of variation.

APÊNDICE B

Supplementary 2. Minimum, maximum and mean temperature across different locations, sowing (mm/dd/yyyy), transplant date (mm/dd/yyyy) for each trial.

| Trial | Location | Sowing date | Transplant date | TMin (°C) | TMax (°C) | Tmean (°C) |
|--------------|--------------------------------|--------------------|-------------------------|------------------|------------------|-------------------|
| 1 | Santa Maria, RS, Brazil | 08/06/2020 | 08/17/2020 | -0.9 | 34.5 | 16.8 |
| | Santa Maria, RS, Brazil | 01/22/2021 | 01/30/2021 | 12.7 | 35.0 | 23.8 |
| | | 02/26/2021 | 03/06/2021 | 9.8 | 35.0 | 22.4 |
| 2 | Júlio de Castilhos, RS, Brazil | 2/25/2021 | 03/06/2021 | 0.0 | 30.3 | 15.1 |
| | Herval D'Oeste, SC, Brazil | 1/29/2021 | 02/08/2021 | 9.5 | 33.3 | 21.4 |
| | | 2/26/2021 | 03/06/2021 | 4.0 | 33.3 | 18.6 |
| 3 | Cáceres, MT, Brazil | 09/02/2022 | 09/12/2022 | 13.4 | 41.5 | 27.4 |
| | Capanema, PA, Brazil | 09/10/2022 | 09/17/2022 | 21.4 | 34.4 | 27.9 |
| | Curitibanos, SC, Brazil | 09/16/2022 | 10/17/2022 | 1.0 | 34.6 | 17.8 |
| | Dois Vizinhos, PR, Brazil | 01/27/2023 | 02/04/2023 | 10.8 | 33.8 | 22.3 |
| | Dourados, MS, Brazil | 01/19/2023 | 01/27/2023 | 13.7 | 35.7 | 24.7 |
| | Petrolina, PE, Brazil | 08/08/2022 | 08/16/2022 | 14.7 | 37.1 | 25.9 |
| | Santa Maria, RS, Brazil | 08/08/2022 | 08/25/2022 ^a | 1.2 | 32.3 | 16.8 |
| | São João Del Rei, MG, Brazil | 09/13/2022 | 09/23/2022 | 8.0 | 33.5 | 20.8 |
| 4 | Pescia, PT, Italy | 02/28/2023 | 03/17/2023 | 0.5 | 32.7 | 16.6 |
| | Morro do Chapéu, BA, Brazil | 04/17/2023 | 04/25/2023 | 16.6 | 33.9 | 25.2 |
| | Dourados, MS, Brazil | 10/15/2021 | 10/15/2021 | 14.7 | 35.7 | 25.2 |
| 16/08/2021 | | 08/25/2021 | 16.8 | 39.5 | 28.1 | |
| 5 | Teresina, PI, Brazil | 09/30/2021 | 10/07/2021 | 11.3 | 30.6 | 20.9 |
| | | 03/09/2022 | 03/22/2022 | 21.7 | 33.8 | 27.7 |
| | Areia, PB, Brazil | 09/11/2022 | 09/22/2022 | 17.5 | 30.0 | 23.7 |
| | Solânea, PB, Brazil | 09/11/2022 | 09/22/2022 | 17.5 | 30.0 | 23.7 |

| | | | | | |
|--------------------------------------|------------|------------|------|------|------|
| Canhotinho, PE, Brazil | 11/03/2022 | 11/16/2022 | 17.3 | 30.5 | 23.9 |
| Garanhuns, PE, Brazil | 04/03/2023 | 04/12/2023 | 18.5 | 34.5 | 26.5 |
| Jupí, PE, Brazil | 04/05/2023 | 04/15/2023 | 18.5 | 34.5 | 26.5 |
| Petrolina, PE, Brazil | 02/03/2022 | 02/11/2022 | 19.1 | 34.9 | 27.0 |
| | 05/26/2022 | 06/03/2022 | 14.5 | 32.2 | 23.3 |
| Cascavel, PR, Brazil | 09/29/2021 | 10/12/2021 | 12.5 | 33.8 | 23.1 |
| Santa Tereza do Oeste, PR, Brazil | 03/17/2022 | 03/28/2022 | 13.7 | 27.1 | 20.4 |
| Aurora, SC, Brazil | 10/05/2021 | 10/15/2021 | 11.3 | 32.9 | 22.1 |
| Brunópolis, SC, Brazil | 03/17/2021 | 03/31/2021 | 1.7 | 28.5 | 15.1 |
| | 03/31/2021 | 04/14/2021 | 1.7 | 28.5 | 15.1 |
| Concórdia, SC, Brazil | 10/06/2021 | 10/25/2021 | 9.6 | 32.5 | 21.0 |
| Curitibanos, SC, Brazil | 09/08/2022 | 10/05/2022 | 0.9 | 25.8 | 13.4 |
| Ituporanga, SC, Brazil | 10/18/2021 | 10/27/2021 | 10.0 | 32.9 | 21.4 |
| Herval D'Oeste, SC, Brazil | 03/22/2021 | 03/29/2021 | 4.0 | 30.5 | 17.2 |
| Rio do Sul, SC, Brazil | 03/29/2021 | 04/09/2021 | 2.8 | 27.4 | 15.1 |
| | 10/05/2021 | 10/15/2021 | 11.3 | 32.9 | 22.1 |
| Seara, SC, Brazil | 09/26/2022 | 10/13/2022 | 4.4 | 29.6 | 17.0 |
| Trombudo Central, SC, Brazil | 10/18/2021 | 10/26/2021 | 10.0 | 32.9 | 21.4 |
| Cachoeira do Sul, RS, Brazil | 03/20/2021 | 03/29/2021 | 2.7 | 36.0 | 19.3 |
| | 04/13/2021 | 04/27/2021 | 2.7 | 33.9 | 18.3 |
| Júlio de Castilhos, RS, Brazil | 04/15/2021 | 04/25/2021 | 0.0 | 30.3 | 15.1 |
| | 10/13/2021 | 10/22/2021 | 9.1 | 35.8 | 22.4 |
| | 11/04/2021 | 11/16/2021 | 11.3 | 39.0 | 24.1 |
| | 01/08/2022 | 01/20/2022 | 11.2 | 31.3 | 25.1 |
| | 04/12/2022 | 04/26/2022 | -1.0 | 31.3 | 15.1 |
| Dilermando de Aguiar, RS, Brazil | 03/15/2021 | 03/24/2021 | 5.6 | 33.8 | 19.7 |
| | 10/07/2022 | 10/25/2022 | 9.4 | 37.7 | 23.5 |
| | 10/24/2022 | 11/04/2022 | 9.6 | 37.7 | 23.6 |
| Santa Maria, RS, Brazil | 03/05/2021 | 03/13/2021 | 3.2 | 35.0 | 19.1 |

Cont. Supplementary 2

| | | | | | |
|----------------------------------|------------|------------|------|------|------|
| | 03/05/2021 | 03/16/2021 | 3.2 | 35.0 | 19.1 |
| | 03/16/2021 | 03/23/2021 | 3.2 | 35.0 | 19.1 |
| | 05/11/2021 | 06/01/2021 | -0.6 | 35.1 | 17.2 |
| | 06/08/2021 | 07/02/2021 | -0.6 | 35.1 | 17.2 |
| | 07/02/2021 | 07/16/2021 | -0.6 | 35.1 | 17.2 |
| | 07/13/2021 | 08/10/2021 | -0.6 | 35.1 | 17.2 |
| | 09/07/2021 | 09/24/2021 | 6.0 | 37.7 | 21.8 |
| | 10/15/2021 | 10/26/2021 | 9.6 | 37.7 | 23.6 |
| | 01/27/2022 | 02/04/2022 | 10.4 | 37.7 | 24.0 |
| | 04/20/2022 | 04/29/2022 | 1.1 | 33.2 | 17.1 |
| | 09/16/2022 | 09/29/2022 | 4.6 | 34.9 | 19.7 |
| Caiçara, RS, Brazil | 09/27/2021 | 10/08/2021 | 15.8 | 27.4 | 21.6 |
| Venâncio Aires, RS, Brazil | 03/15/2021 | 03/23/2021 | 3.2 | 35.0 | 19.1 |
| | 04/05/2021 | 04/13/2021 | 1.3 | 34.6 | 17.9 |
| Novo Xingú, RS, Brazil | 09/23/2021 | 10/05/2021 | 16.0 | 30.0 | 23.0 |
| | 10/09/2021 | 10/21/2021 | 16.0 | 30.0 | 23.0 |
| | 10/25/2021 | 11/10/2021 | 16.0 | 30.0 | 23.0 |
| | 11/16/2021 | 11/30/2021 | 16.0 | 30.0 | 23.0 |
| Seberi, RS, Brazil | 09/27/2021 | 10/08/2021 | 7.8 | 33.9 | 20.8 |
| | 10/22/2021 | 11/04/2021 | 7.8 | 36.0 | 21.9 |
| | 10/21/2021 | 11/04/2021 | 7.8 | 36.0 | 21.9 |
| Vale do Sol, RS, Brazil | 10/20/2021 | 11/12/2021 | 9.1 | 36.9 | 23.0 |
| Boa Vista do Sul, RS, Brazil | 03/12/2022 | 03/22/2022 | 4.3 | 32.0 | 18.1 |
| Novo Cabrais, RS, Brazil | 02/22/2022 | 03/02/2022 | 6.4 | 37.7 | 22.0 |
| | 03/11/2022 | 03/19/2022 | 6.4 | 34.4 | 20.4 |
| Picada Café, RS, Brazil | 03/12/2022 | 03/28/2022 | 6.3 | 35.4 | 20.8 |
| Santa Bárbara do Sul, RS, Brazil | 03/14/2022 | 03/26/2022 | 2.9 | 33.8 | 18.3 |
| Teutônia, RS, Brazil | 03/14/2022 | 03/23/2022 | 6.7 | 34.9 | 20.8 |
| Piratini, Brazil | 03/11/2022 | 03/24/2022 | 6.5 | 31.6 | 19.0 |

Cont. Supplementary 2

| | | | | | |
|------------------------------|------------|------------|------|------|------|
| Rio Pardo, RS, Brazil | 02/04/2022 | 02/14/2022 | 12.3 | 38.0 | 25.1 |
| | 02/14/2022 | 02/24/2022 | 8.0 | 38.0 | 23.0 |
| Bom Princípio, RS | 02/22/2022 | 03/02/2022 | 6.3 | 37.8 | 22.0 |
| Cândido Godói, RS | 03/11/2022 | 03/21/2022 | 6.0 | 36.2 | 21.1 |
| Maçambará, RS, Brazil | 03/10/2022 | 03/20/2022 | 7.1 | 35.2 | 21.1 |
| Estrela Velha, RS, Brazil | 03/10/2022 | 03/31/2022 | 1.2 | 34.4 | 17.8 |
| São Marcos, RS, Brazil | 09/15/2022 | 10/07/2022 | 4.2 | 30.1 | 17.1 |
| Lajeado, RS, Brazil | 09/10/2022 | 10/01/2022 | 6.0 | 33.6 | 19.8 |
| Faxinalzinho, RS, Brazil | 09/29/2022 | 10/17/2022 | 3.6 | 29.8 | 16.7 |
| Barra do Ribeiro, RS, Brazil | 10/17/2022 | 10/28/2022 | 9.3 | 35.6 | 22.4 |
| Colinas, RS, Brazil | 09/20/2022 | 10/03/2022 | 7.2 | 35.7 | 21.4 |
| Santa Rosa, RS, Brazil | 09/17/2022 | 09/27/2022 | 3.4 | 35.0 | 19.2 |
| Alegrete, RS, Brazil | 02/23/2023 | 03/02/2023 | 11.2 | 36.4 | 23.8 |
| | 03/02/2023 | 03/09/2023 | 6.1 | 35.4 | 20.7 |
| | 03/09/2023 | 03/16/2023 | 6.1 | 35.4 | 20.7 |
| | 03/16/2023 | 03/23/2023 | 6.1 | 35.1 | 20.6 |
| Bozano, RS, Brazil | 01/27/2023 | 02/03/2023 | 6.6 | 36.4 | 21.5 |
| | 02/03/2023 | 02/10/2023 | 6.6 | 36.4 | 21.5 |
| | 02/10/2023 | 02/17/2023 | 6.6 | 36.4 | 21.5 |
| | 02/17/2023 | 02/24/2023 | 6.6 | 34.3 | 20.4 |
| Vila Flores, RS, Brazil | 04/03/2023 | 04/18/2023 | 3.2 | 28.8 | 16.0 |
| Vacaria, RS, Brazil | 04/03/2023 | 04/13/2023 | -2.0 | 27.0 | 12.5 |
| Nova Petrópolis, RS, Brazil | 04/03/2023 | 04/14/2023 | 6.4 | 35.3 | 20.8 |
| Bento Gonçalves, RS, Brazil | 04/04/2023 | 04/22/2023 | 6.4 | 28.8 | 17.6 |
| Sapiranga, RS, Brazil | 04/05/2023 | 04/21/2023 | 1.9 | 34.7 | 18.3 |
| São Borja, RS, Brazil | 04/01/2023 | 04/14/2023 | 6.9 | 32.5 | 19.7 |