

# Oral Presentation abstracts

## Teasing apart the genetic basis of adaptation in a changing climate

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In a rapidly changing climate, there is an increased need to both quantify the threat of climate change and better understand populations' ability to respond to climate change. This ability to respond may in part depend upon the genetic makeup or genomic architecture underlying phenotypic traits important to adaptation in a changing climate. This research examines the genetic basis of adaptation in a changing climate focusing on long-lived forest trees linking phenotype, genotype and environment. Specifically, this research considers the value of forest tree hybrid zones in climate adaptation and considers the phenotypic and genomic consequences of porous species boundaries. In addition, this research asks whether climate pre-adaptation may facilitate colonization in novel environments. Using *Arabidopsis* as a model organism to test predictions of climate pre-adaptation, we suggest that genetic variation associated with climate may be harnessed to predict population-level responses to climate change. Finally, a major outstanding question for rare species asks whether rare species have the necessary variation to evolve. This research asks what are the consequences of intentional immigration of new alleles between populations, and how might that impact the evolutionary trajectory of long-lived species. Integrating genomic, phenotypic, and climatic information, this research suggests that to maintain resilience, manage widespread and diverse gene pools, and predict adaptation under future climates interdisciplinary approaches will be necessary, building bridges across the fields of plant biology.

## Sedges of Vietnam: species discoveries and insights into the evolution of *Carex* (Cyperaceae).

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Although Vietnamese *Carex* species represent a fraction of global sedge diversity (ca. 85 of 1966 spp.), their inclusion in systematic studies has led to unprecedented insights into the evolution and diversification of this genus. Our investigations have shown that *Carex* is comprised of two major evolutionary lineages, with over 98% of all species (1936 spp.) falling within the Major *Carex* Alliance. The remaining 30 species are highly diverse morphologically, and are restricted to East and Southeast Asia, and comprise the Minor *Carex* Alliance. Overall, our findings suggest that morphological diversification may have occurred in clades dominated by East and Southeast Asian species followed by canalization of a narrower range of phenotypes in species-rich, cosmopolitan lineages. Our results also suggest a Southeast Asian origin for the genus or that this region acted as a refugium for these early diverging groups. Vietnamese *Carex* have not only afforded unprecedented insights into deep patterns of relationship within the genus, they have also upon closer examination revealed species groups that are hidden under a single name. One example of this unrecognized diversity is seen in *Carex kucyniakii* s.l. (Minor *Carex* Alliance), a taxon that represents a complex of three distinct species. These taxa are endemic to Northern Vietnam and exhibit some of the most extraordinary morphological features found in the Cyperaceae including pseudopetiolate leaves, up to 12 cm wide, and pink or red inflorescences. We are aware of other

undescribed *Carex* species and propose that the sedge flora of Vietnam could reveal a comparable number of taxonomic novelties given a more focused attention by taxonomists.

### **Molecular aspects of traits of economic importance in wheat**

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Wheat is one of the most important cereal crops globally, and its seed serves as a basic unit of propagation, and as source of food, feed and raw material for a wide range of biobased industrial products. Understanding the molecular mechanisms underlying the control of traits of economic importance such as seed yield and quality is critical to design molecular strategies for improving wheat productivity. Seed yield in wheat is determined mainly by the deposition of starch in the endosperm, which is partly regulated by the allocation of photosynthetically fixed carbon to sucrose for transport to the developing seeds and the conversion of this sucrose into starch mainly during the seed filling phase. Consequently, the seeds enter into the maturation phase, which is characterized by the induction of dormancy and desiccation tolerance. Maintenance of moderate level of seed dormancy is crucial in cereal crops such as wheat as this trait is closely associated with tolerance to field sprouting, one of the major constraints encountered in wheat production. Results from our studies with respect to the molecular aspects of physiological processes related to seed yield and dormancy will be discussed.

### **CRISPR genome editing using Cas9 protein and optimized RNAs delivered as an RNP complex**

Integrated DNA Technologies

Precise genome editing by the CRISPR/Cas9 system has proven to be ground-breaking in basic research. The CRISPR/Cas9 system demonstrates unparalleled editing efficiency in a broad range of host species and cell types, but suffers from concerns related to target site specificity. Modified RNA guides and mutant Cas9 proteins have been developed to reduce off target editing by Cas9, but in many cases these remedies introduce significant on-target editing problems. Cas9 protein is increasingly being used for genome editing by direct transfection of an active guide RNA Cas9 ribonucleoprotein (RNP) complex into cells. Here we present optimized, chemically modified crRNA:tracrRNA guide complexes along with a novel mutant Cas9 protein that has been evolved to reduce off-target gene editing while maintaining on-target potency. These components are ideally suited for RNP delivery allowing highly efficient genome editing without the complications associated with delivering plasmid or viral DNA.

### **Latitudinal trends in pollination success of the rewardless orchid, *Cypripedium candidum*, and associated changes in the plant and insect community**

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Plants that do not offer a food reward for pollinators rely on the co-flowering community to sustain insects, but may compete with them for visitations. Extended growing seasons at southern latitudes may allow for rewardless plants to flower earlier than other species, and may decrease the number of co-flowering competitors. Early season insect diversity could also decrease at lower latitudes, but phenological variation among taxa may occur. Therefore, plants with restrictive flowers may experience latitudinal variation in reproductive success due to the abundance of effective pollinators. *Cypripedium candidum* is a rewardless orchid species that is endemic to tall grass prairies in North America. I conducted a survey of populations in four latitudinal study regions from northern Iowa to southern Manitoba, across *C. candidum*'s range. In each of these regions I quantified fruiting success and the abundance of co-flowering and pollinator species. Consistent with predictions, co-flowering species diversity was highest in Manitoba and lower in southern regions. Population fruiting success was low in Manitoba and ranged from 11-38%, whereas more southerly areas (southern Minnesota and Iowa) showed a greater range (2-84%). Regression analyses determined that fruiting success was significantly higher in Iowa, and was associated with an increased abundance of facilitative plant taxa (*Packera* and *Zizia*) and decreased abundance of competitive taxa (*Sisyrinchium* and *Pedicularis*). Large-bodied insects increased in abundance further north, potentially limiting the pollination of restrictive *C. candidum* flowers, but we did not detect a significant effect of the composition of the insect community on orchid fruiting success.

### **Sounding the alarm: characterization of early defense signaling in the canola-*L. maculans* pathosystem using dual RNA-seq and laser microdissection**

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*Leptosphaeria maculans*, the causative agent of canola blackleg disease, is a devastating fungal pathogen that affects the Canadian agriculture industry. Blackleg resistance is mediated by extracellular membrane receptors that rapidly modulate cellular signaling to prevent fungal invasion. Although studies have characterized global gene expression changes in gross leaf sections, none have looked at the plant defense pathways activated directly at the infection site and in the first cells responding to *L. maculans*. To accomplish this, we combined the depth of RNA sequencing with the specificity of laser microdissection. In susceptible and resistant canola, 100 micron tissue sections were collected from the infection site at 1 and 3 days post-inoculation, and sequenced on the Illumina platform. At 1 dpi, resistant plants showed notable changes to gene expression related to calcium signaling and redox state. Further, we identified transcription factor networks that were active specifically in resistant hosts following inoculation. Down-regulation of important defense elements in susceptible plants, such as jasmonic acid biosynthetic genes, suggests repression of host immune responses by fungal effectors. Current research aims to functionally validate these findings. Together, this study is improving our understanding of blackleg resistance in canola, and provides a suite of new targets that can be tested for their ability to bolster resistance in one of Canada's most valuable crops.

### **The effect of *Frankia* spp. and ectomycorrhizal fungi on *Alnus viridis* ssp. *crispa* growing in low fertility and saline soil**

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Green alder (*Alnus viridis* ssp. *crispa*) is known to form symbioses with *Frankia* and ectomycorrhizal fungi (ECMF), both of which are important for uptake of nutrients (N, P, respectively). The aim of our study was to examine the effect of these symbioses on green alder growing in nutrient-poor soil and saline conditions. The first experiment involved inoculating green alder growing in low fertility soil with three species of ectomycorrhizal fungi (*Lactarius torminosus*, *Lactarius theiogalus*, *Hebeloma crustuliniforme*) alone or in combination, with and without *Frankia*. The *Frankia* inoculated plants had significantly higher total biomass and root extracellular acid phosphatase activity compared with non-*Frankia* treatments. However, nodulated plant total biomass decreased with an increasing number of fungal species. The second experiment examined the effect of *Hebeloma crustuliniforme* and *Frankia* on green alder exposed to 0, 50 and 100 mM NaCl. *Frankia* inoculation showed significant increase in plant performance but *H. crustuliniforme* inoculation did not. Plant mass, nodule allocation and total nitrogen fixation decreased with NaCl exposure. With an increasing level of NaCl, there was a decrease in plant chlorophyll fluorescence in non-symbiont plant. However, plant chlorophyll fluorescence did not decrease with the increasing level of NaCl when plants were inoculated with either or both symbionts. Our study suggested that *Frankia* have positive effects on plant growth but the ectomycorrhizal fungi do not.

### **The effect of naringenin chalcone on cuticular wax loads in *Arabidopsis thaliana***

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The plant cuticle serves to protect non-woody tissues from evaporative water loss, microbial attack and deflects UV light. In defense from the latter, plant flavonoids are known to play a major role as well. The *TRANSPARENTTESTA5* gene codes for chalcone isomerase, a key step in the production of flavonoids. Many years ago, *tt5-1*, a knock-out mutant of *TT5*, was reported to have a reduction of epicuticular wax crystals. We confirmed that this reduction is concomitant with a ~25% reduction surface wax-loads, mainly caused by a reduction in products of the decarbonylation pathway. In contrast to the *tt5* mutant, the *tt4 tt5* double mutant shows normal wax loads. We also determined that *tt5* mutants accumulate a group of naringenin glycosides, which are not found in the *tt4* mutants. While there is an observed reduction in wax loads in *tt5*, we do not observe any significant changes in the expression of wax biosynthetic genes. This implies that there is “cross-talk” between flavonoids and the biosynthesis of cuticular waxes in *Arabidopsis*.

### **Phytoglobins maintain root meristem function and integrity during hypoxic conditions**

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Growth of *Zea mays* roots subjected to hypoxia is highly influenced by the expression of Phytoglobins (Pgbs), plant hemoglobin proteins. Relative to wild type (WT), growth of roots subjected to 4% oxygen is inhibited in maize plants suppressing one of the two Pgbs (*ZmPgb1.1*

or *ZmPgb1.2*), and encouraged in those in which the two Pgb's are over-expressed. The root inhibition phenotype following suppression of Pgb's is most likely the result of an elevation in nitric oxide (NO) at the meristem site altering auxin metabolism and flow. Perturbations in the expression of genes involved in auxin synthesis and transport, more evident in hypoxic root tips down-regulating Pgb's, cause an abnormal auxin localization pattern at the root apical meristem which alters the oxidized environment needed to preserve the integrity of the cells in the quiescent center (QC). A switch from an oxidized to a reduced environment, with a prevalent accumulation of reduced ascorbic acid and glutathione occurs in QCs suppressing Pgb's. These changes were most likely the result of an increased activity of ascorbate oxidase at the root tip. Hypoxic roots over-expressing Pgb's retained an oxidized environment in the QCs, which is a prerequisite for normal meristem function and root growth. Regeneration assays of QC cells further confirmed that the presence of Pgb's is needed to preserve cell viability and root integrity during conditions of hypoxia. Collectively, these data provide a novel model of meristem function during stress conditions integrating Pgb's with existing factors participating in root apical meristem maintenance.

### **Creating links among signalling pathways: the connection between microtubules and hormones in sustaining cell proliferation**

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The ability for plant growth to be sustained depends on an intricate balance between cell division and differentiation in specialized regions called meristems. In *Arabidopsis thaliana*, this balance is compromised in mutants lacking the microtubule-associated protein CLASP. Previous work showed that in roots, CLASP tethers sorting vesicles to microtubules, which fosters the recycling of the auxin transporter PIN2 and the brassinosteroid (BR) hormone receptor BRI1 to the plasma membrane. We recently performed RNA sequencing of wild-type and *clasp-1* null mutant root tips, which revealed transcriptional differences in hormone crosstalk and cell cycle progression. Since hormone signalling is perturbed in *clasp-1*, we investigated the involvement of CLASP in modulating the BR pathway in the root apical meristem. *clasp-1* mutants have impaired recycling of BRI1 receptors and decreased responses to treatment with exogenous BR. We show that increased BR signalling leads to reduced *CLASP* transcript and protein levels, as well as a discernible shift in microtubule organization. Notably, we discovered that two BR-activated transcription factors, BZR1 and BZR2, bind to the *CLASP* promoter. By mutating the promoter such that *CLASP* was no longer targeted by BZR1/2, we observed differences in meristem size and hypersensitivity to treatments with both BR and osmotic stress-inducing agents. Finally, green fluorescent protein-CLASP reporter lines driven by either the endogenous or mutated *CLASP* promoter revealed a gradient of CLASP fluorescence that was highest near actively dividing cells and diminished towards elongating cells. Together, these findings uncover a remarkable negative feedback loop that sustains meristem homeostasis.

### **Orchid monitoring and management in Manitoba Tall-grass Prairie: lessons learned**

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Endangered prairie orchids such as Small White Lady's-Slipper (*Cypripedium candidum*) and Western Prairie Fringed Orchid (*Platanthera praeclara*) are threatened by loss or alteration of habitat due to woody encroachment, hydrological alteration, and incompatible grazing and fire. Established in 1989, the 4,650 ha Manitoba Tall Grass Prairie Preserve is managed through a multi-parcel, multi-year planning approach that incorporates a recurrent prescribed grazing and fire disturbance rotation to maintain and recover multiple endangered species and the habitats and successional stages they depend upon. Management decisions have been informed by periodic species and ecosystem monitoring results, assessments of management effectiveness, research, a climate-change adaptation process, and feedback from local agricultural partners. Regular assessment and integration of new information has resulted in several changes to management & monitoring approaches. Here we present, from a practitioner's perspective, our evolving approach.

### **Phytoglobulin over-expression alleviates drought stress in maize seedlings**

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The effect of water stress generated by applications of 25% w/v polyethylene glycol (PEG) was evaluated in 3-leaf stage (V2) maize (*Zea mays* L.) seedlings over-expressing or down-regulating the *Zea* maize phytoglobulin 1.1 (ZmPgb1.1) gene. Over-expression of ZmPgb1.1 increased tolerance to drought and resulted in profound changes in the accumulation of ethylene and reactive oxygen species (ROS). Time course analysis of the lines over 0, 4, 8, and 16h showed an increased expression of genes participating in ethylene synthesis and response, as well as ROS production, in the line suppressing ZmPgb1.1. Relative to the wild type (WT) line, reduced expression of the same genes occurred in the ZmPgb1.1 over-expressor. The observed effects were regulated by nitric oxide (NO). While pre-incubation with the NO donor sodium nitroprusside (SNP) induced the transcription of ethylene and ROS generating genes in the WT and ZmPgb1.1 over-expressing line, application of the NO scavenger cPTIO had opposite effects in the WT and ZmPgb1.1 down-regulating line. These results are consistent with the role of Pgb as NO scavengers. Pharmacological treatments altering ethylene levels and ethylene measurements further showed that this hormone is integrated in the Pgb regulation of drought tolerance. A model is proposed in which suppression of NO by Pgb reduces ethylene and ROS accumulation, thereby alleviating drought stress.

### **Phytoglobulin is an important factor in soybean (*Glycine max*) flooding tolerance**

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Flooding has many detrimental effects on the growth of plants. The developmental plasticity allows plants to adapt and acclimate to flooding conditions such as partial or full submergence,

where the oxygen level is very limited. As oxygen binders and nitric oxide scavengers, Phytoglobins (Pgbs, plant hemoglobins) can facilitate morphological and/or physiological adaptation to various stresses especially hypoxic or flooding conditions. To assess how the expression level of Pgb1 improves flooding tolerance, we have generated soybean (*Glycine max*) plants over-expressing or down-regulating Pgb1, and evaluated their tolerance to partial and full submergence. Under conditions of partial submergence, plants over-expressing Pgb1 exhibited improved performance by retaining a higher photosynthetic rate and forming a larger number of adventitious roots relative to wild type plants. Plants down-regulating Pgb1 were more susceptible to excessive moisture and displayed reduced photosynthetic rates and a lower number of adventitious roots. This trend was more pronounced under full submergence, where the over-expression of Pgb1 ensured a greater survival and recovery rate. The relevance of Pgb1 in conferring flooding tolerance was also examined in commercial soybean varieties where a strong correlation existed between the ability to tolerate excessive water and the expression of Pgb1. Collectively, these studies indicate Pgb1 plays a key role in alleviating flooding stress, and can be a potential marker for flooding tolerance selection.

### **Profiling the gene expression landscape of canola seed: transcriptional and methylomic analysis of *Brassica napus* seed development**

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Canola (*Brassica napus*) is one of Canada's most economically valuable crops. The seed is the source of the two most valuable canola products: canola oil and seed meal. We still know very little about the genetic mechanisms that control valuable seed traits such as oil and protein content. Seed improvement by traditional breeding methods is laborious and time-consuming. To take a more targeted approach to seed improvement, we require a more thorough understanding of the mechanisms of gene expression that underlie seed development overall. To elucidate the transcriptional networks that underlie canola seed development, we used next generation RNA sequencing to study whole canola seeds at the ovule, globular, heart, mature green, and dry seed stages of seed development. Whole genome bisulfite sequencing was then used to profile the canola seed methylome at an early (globular) and late stage (mature green) of seed development. Both the transcriptional and methylation profiles of early and late seed development are significantly distinct. Methylation changes occur primarily in the CHH context between early and late seed development. Furthermore, several bZIP transcription factors are identified as potential regulators of energy metabolism and early development, and several bHLH transcription factors are potentially novel regulators of embryo maturation and seed dormancy. Using T-DNA insertional mutagenesis, RNA interference, and overexpression, we are characterizing key regulators in seed and early plant development. By understanding the patterns of DNA methylation and gene expression, we are finding new tools to profile seed development and improve the quality of canola seeds.

### **Do high diversity tallgrass prairie plant communities rely on niche partitioning?**



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The concept of niche partitioning is often invoked as an explanation of species coexistence in communities. Traditionally plant communities have been thought of as having less potential for niche partitioning than animal communities since plants rely on the same resources. However, recent studies have suggested that plant species can be distributed across gradients of soil moisture at small spatial scales, providing a mechanism for coexistence. Remnant, wet tallgrass prairie communities have a high species richness which can potentially be driven by soil hydrology niche partitioning. I quantified variation soil hydrology of tallgrass communities and the abundance of plant species over two growing seasons on three sites in the Tallgrass Prairie Preserve. Although sites varied in their hydrology, the majority of the species were found on all three sites, suggesting site to site differences in hydrology have little effect on community composition. Between sites, species position, relative to other species, along the hydrological gradients was similar. A comparison of the hydrological niches of species pairs using kernel density estimation and showed that less than 10% of the species pairs showed significant differences in their hydrological niches. This suggests that while soil hydrology influences the distribution of species in tallgrass prairies, niche partitioning is insufficient to explain species coexistence.

### **The application of RNAi biotechnology to control fungal necrotrophs *Sclerotinia sclerotiorum* and *Botrytis cinerea***

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*Sclerotinia sclerotiorum*, the causal agent of stem rot, infects over 500 plant species and causes severe crop losses globally. Traditional management tactics involve the use of agro-chemicals, which can have deleterious impacts on the surrounding agro-ecological landscape if not managed properly. Producers also use crop rotations to manage disease outbreaks from year to year, but can fail due to the large host range of this necrotrophic fungus and the formation of durable resting structures, termed sclerotia, which can persist in the soil for years. Therefore, new species-specific methods to mitigate aggressive fungal pathogens are needed. Our novel strategy exploits an inherent cellular defense mechanism, called RNA interference (RNAi), using double stranded RNA (dsRNA) molecules to trigger the specific degradation of transcripts within the invading fungus. Applying RNA-sequencing technology to observe global transcriptomic changes at the *Brassica napus*-*S. sclerotiorum* interface, we uncovered an array of genes to target using RNAi. DsRNA molecules first demonstrated reductions of specific transcript levels *in vitro*. Using synthesized dsRNAs, *S. sclerotiorum* disease pressure was then mitigated on the surface of *Brassica napus* and *Arabidopsis thaliana* leaves. Furthermore, molecules designed to target select homologues in *Botrytis cinerea* also imparted host protection, reducing lesion size on *B. napus* leaves. Finally, *A. thaliana* plants were created to express hairpin RNA (hpRNA), a dsRNA molecular mimic, and resisted fungal aggression. Taken together, the RNAi biotechnologies we developed represent the next-generation of environmentally-conscious, phytopathogenic management strategies.

### **Expression of Arabidopsis class 1 Phytoalbumin (*AtPgl1*) delays death and degradation of the root apical meristem during severe PEG-induced water deficit.**

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Maintenance of a functional root is fundamental to plant survival in response to some abiotic stresses such as water deficit. We have found that overexpression of Arabidopsis class 1 Phytoglobin (*AtPgb1*) alleviated the growth retardation of polyethylene glycol (PEG)-induced water stress by reducing programmed cell death (PCD). This was in contrast to PEG-stressed roots down-regulating *AtPgb1* that exhibited extensive PCD. The death program experienced by the suppression of *AtPgb1* in stressed roots was mediated by reactive oxygen species (ROS) and ethylene. Suppression of ROS synthesis or ethylene perception reduced PCD and partially restored root growth. The PEG-induced cessation of root growth was preceded by structural changes in the root apical meristem (RAM), including the loss of cell and tissue specification, possibly as a result of alterations in PIN1- and PIN4-mediated auxin accumulation at the root pole. These events were attenuated by the overexpression of *AtPgb1* and aggravated when *AtPgb1* was suppressed. Specifically, suppression of *AtPgb1* compromised the functionality of the *WOX5*-expressing quiescent cells (QCs), leading to the early and premature differentiation of the stem cells and to a rapid reduction in meristem size. Other root domain markers, such as *SCARECROW* (*SCR*), which demarks the endodermis and QCs, and *WEREWOLF* (*WER*), which specifies the lateral root cap, were also most affected in PEG-treated roots with suppressed *AtPgb1*. Collectively, the results demonstrate that *AtPgb1* protects roots exposed to lethal levels of PEG, and suggest a novel function of this gene in ensuring meristem functionality through the retention of cell fate specification.

### **Identification and characterization of an *Ephedra sinica* N-methyltransferase involved in ephedrine biosynthesis.**

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Plants produce an impressive diversity of alkaloids with potent biological activities. Many of these, including ajmaline, caffeine, morphine and ephedrine, require the action of N-methyltransferase (NMT) enzymes during biosynthesis. Studies concerning the origin of morphine and other related alkaloids found in the order Ranunculales have revealed the existence of a large monophyletic group of NMTs specialized for benzyloisoquinoline alkaloid (BIA) substrates. Unexpectedly, our recent work identified an NMT from a distantly-related plant species, *Ephedra sinica*, which shows substantial identity to the clade of known BIA NMTs and yet accepts phenylpropylamino alkaloids. *In vitro* characterization of recombinant *E. sinica* NMT (EsNMT) suggests that it participates in the biosynthesis of ephedrine, pseudoephedrine and related alkaloids. Furthermore, EsNMT accepts a range of phenylpropylamino alkaloids and related molecules, including the upstream pathway intermediate cathinone. The physiological role of the enzyme was further evaluated by analysis of correlations between alkaloid accumulation, enzyme activity and transcript abundance profiles across internodes and root tissues. The discovery of EsNMT, a putative heterofunctional homolog of BIA NMTs, provides an opportunity to study the evolutionary divergence of biocatalysts recruited to two very different plant alkaloid pathways. Crystallographic determination of the EsNMT structure is underway, and we anticipate that a comparison of physical features with previously studied BIA NMTs will provide leads for the rational engineering of catalytic function. To highlight the biotechnological utility of this enzyme,

we are developing microbial systems containing artificial pathways for the biosynthesis of ephedrine from commercially-available feedstocks.

### **Probing the molecular basis of lignin biosynthesis in wheat biomass**

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Lignin as an integral component of plant cell wall plays an important role in conferring mechanical strength and tolerance to biotic and abiotic factors. Despite all these benefits, it interferes to the use of biomass as a raw material for the production of biobased industrial products. Modification of the amount and quality of lignin without affecting its agronomic benefits is a possibility but it requires knowledge on the molecular mechanisms underlying its synthesis. This study identified candidate wheat lignin biosynthetic genes and investigated the transcriptional regulation of lignin synthesis in wheat biomass. Analysis of the expression of lignin biosynthesis candidate genes revealed the predominance of specific genes including *4-coumarate:CoA ligase1 (4CL1)*, *p-coumarate 3-hydroxylase1 (C3H1)*, *cinnamoyl-CoA reductase2 (CCR2)*, *ferulate 5-hydroxylase2 (F5H2)* and *caffeic acid O-methyltransferase2 (COMT2)*, in all vegetative tissues assayed, suggesting their significance in lignin formation in wheat biomass. Comparative analysis of lignin biosynthesis at both molecular and biochemical levels between two wheat genotypes that show difference in their resistance to lodging indicated a close association between stem strength and internode lignin content. Lignin content in the internode appeared to have a very close association with the level of cytokinin, which is reported to play a role in enhancing its formation.

### **Ureide metabolism in response to cadmium treatment in *Arabidopsis thaliana***

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Ureides are nitrogenous compounds derived from oxidative degradation of purine rings. Due to their high nitrogen content, ureides play an important role in nitrogen storage and recycling in plants. Amongst ureides, allantoin not only serves as a transportable nitrogen-rich compound, but also is involved in plant stress responses via scavenging reactive oxygen species (ROS). However, how allantoin minimizes ROS accumulation and alleviates oxidative damage is under debate. In order to clarify the potential role allantoin plays in plant stress tolerance, we investigated the effect of cadmium (Cd) on ureide metabolism in *Arabidopsis thaliana*. In response to Cd treatment, allantoin accumulates in wild-type (Col-0) plants due to elevated uricase (*UO*) along with reduced allantoinase (*ALN*) transcript abundance. These two genes encode enzymes important for production and degradation of allantoin, respectively. Allantoinase-negative (*aln-3*) *Arabidopsis* mutants show constitutive allantoin accumulation, and although *aln-3* mutants take up and store more Cd within their leaf tissue, they contain less damaging ROS and show more Cd resistance. The protective mechanism employed by *aln-3* mutants is mediated through antioxidant systems, enhancing the activity of antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase. To evaluate the effect of allantoin accumulation on Cd-resistance, *ALN-overexpressed Arabidopsis* lines (*ALN<sub>ox</sub>*) were constructed. Increased *ALN* transcript level and enhanced ALN

enzyme activity in *ALNox* lines, caused a Cd-sensitive phenotype. Together, these data provide evidence for a positive correlation between allantoin content and Cd tolerance in *Arabidopsis*.

### **The influence of soil nitrogen level on tomato defense to Whiteflies**

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Whiteflies are one of the leading causes of tomato yield loss worldwide. Phloem feeders, including whiteflies, prefer foliage with high nitrogen (N) content because organic N is a limiting factor for herbivores. Since soil N is commonly insufficient for adequate crop growth, farmers supplement soils with N-containing fertilizers. While an adequate supply of N and other nutrients is necessary for growth and fruit production, if plants with greater access to N have greater N content in their tissues, then soil N supplementation also makes plants less resistant to whiteflies. Therefore, there must be a level of fertilizer addition beyond which the losses in fruit production caused by greater attraction and feeding of pests, exceed the gains brought about by N availability. In this study, we grew four commercial tomato varieties under three levels of soil N fertilization and infested half of these plants with whiteflies after eight weeks of growth. After twenty weeks of growth, we compared the yield and resource allocation in whitefly infected and uninfected plants to find whether soil N supplementation results in greater susceptibility of tomato plants to whitefly infestation and if so, to what extent; and to find whether some varieties of tomato are more resistant or tolerant to whiteflies. From the results, we are expecting to suggest an optimum N fertilization level at which the losses in yield due to reduced N is outweighed by the benefits from the reduction of economic and environmental costs of using chemicals (fertilizers and pesticides) in tomato cultivation.

### **Pollination ecology in mixed grass Prairies**

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Research on plant-pollinator networks shows that there are “core” species that are involved in most of the interactions. These data are of potential interest to restoration ecologists as they suggest that including core flowering plant species is essential for establishment of healthy pollinator communities and long-term sustainability of restored sites. Insect visitation data from a mixed grass prairie (i.e. Yellow Quill Prairie Preserve) was collected to identify core plant species from this prairie type and allow comparison with data from fescue and tall grass prairies in Manitoba. The flowering peak was in May, possibly due to the fact this prairie is grazed, but the insect visitation peak was in late July. Mixed grass prairie was more similar to fescue than tall grass prairie in terms of the percentage of insect visits. Bees and wasps were responsible for only 21% of all visits in the tall grass prairies but 73% in the fescue and 72% in the mixed prairie. *Solidago nemoralis* was the only plant species that was commonly visited in all three prairie types. Differences in soil moisture may be affecting the composition with bees being more common in drier areas and flies more common in moister ones.

### **COBRA’s complicated journey to modulate cellulose production in plants**

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Plant shape is controlled by the mechanical properties of their cell walls, which depend on cellulose, a tension-bearing polysaccharide deposited by enzyme complexes spanning the plasma membrane, and often associated with microtubules. The *cob-1* mutant allele has a conditional phenotype: its roots undergo conspicuous swelling and reduced cellulose production when grown on high-sucrose. The *cob-4* null allele is seedling-lethal. In an effort to elucidate the mechanisms by which COBRA influences cellulose production, we engineered fluorescent protein- and affinity-tagged versions of COBRA for live cell imaging and biochemical analysis respectively. Using total internal reflectance fluorescence microscopy to follow yellow fluorescent protein-tagged COBRA (COB-YFP), we detected fluorescent puncta near the plasma membrane, a high proportion of which track along cortical microtubules in association with cellulose synthase complexes. This distribution is in stark contrast with previous immunofluorescence and immunogold localization studies, which detected COBRA epitopes in the cell wall and not in association with microtubules. Since COB-YFP does not fully rescue the *cob-4* phenotype, we used immunofluorescence to detect the distribution of 6XHistidine-tagged COBRA (HIS-COB), which fully rescues *cob-4*. We found a more or less identical distribution pattern. Intriguingly, immunoblotting of HIS-COB identified a smaller protein band. Based on these findings we propose that following secretion COB undergoes cleavage. The N-terminal cellulose binding fragment remains in the cell wall while the C-terminal cleavage product is endocytosed to transiently associate with cellulose synthase complexes. We postulate that this cleavage phenomenon senses and modulates cellulose production.

### **Controlling crop pests using RNA interference technologies**

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Insects are our most serious competitors for food and fibre, costing billions of dollars annually in crop losses and in insect control programs. Chemical insecticides remain our most powerful weapon to control the pests, but with increasing incidences of insecticide resistance and growing concerns about the off-target effects of many of the chemical insecticides, new, environmentally-safer methods of insect control are needed. RNA interference (RNAi) is a sequence-specific method of reducing gene expression in virtually all eukaryotes, and can be induced by delivery of double-stranded RNA (dsRNA) to a target organism. In recent years, a growing number of research groups have been developing dsRNA-based insecticides. Given the specificity of RNAi, it has the potential to generate species- or genus-limited insecticides that can selectively control the pests, without adversely affecting non-target beneficial species. Our research group has been developing dsRNA-based technologies to control a variety of pest insects, including chewing insects such as flea beetles, and sucking insects such as aphids. Here, we will report on our progress on the development of different methods of dsRNA delivery to plants, including both transgenic and topically-applied formulations, and will discuss the potential for this technology to provide a new generation of pest control methods that will have reduced impacts on the environment.

## **Discovery of an extraorgan freezing strategy in cold acclimated winter wheat and rye crowns**

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Extraorgan freezing as a mechanism for increasing freezing survival, was discovered in Norstar winter wheat (*Triticum aestivum* L.) and Hazlet rye (*Secale cereale* L.) crowns. Water content, NMR microimaging and diffusion weighted imaging were used to visualize and quantify shifts in tissue water. Cold-acclimation significantly decreased water mobility in the vascular transition zone (VTZ) and intermediate zone, located between the shoot apical meristem (SAM) and VTZ, to a greater degree in rye than wheat. Desiccation of the intermediate zone is hypothesized to form a barrier to ice propagation into the SAM. Differential thermal analysis revealed SAM supercooled, avoiding freezing, and slower cooling rates increased the supercooling ability of the SAM. During slow cooling ( $2^{\circ}\text{C h}^{-1}$ ), the water content of the SAM decreased while the leaf sheath increased. Moreover, the leaf sheaf had higher ice nucleation activity, resulting in subsequent preferential ice accumulation. Differential thermal analysis identified the second exotherm between  $-6$  and  $-8^{\circ}\text{C}$  which corresponded to cavity formation in the VTZ. A third exotherm ( $-20^{\circ}\text{C}$  in wheat and  $-28^{\circ}\text{C}$  in rye) corresponded with injury to the SAM. The SAM had a low ice nucleation activity and lower freezing survival rate than the VTZ when excised and frozen *in vitro*. Possible mechanisms of extraorgan freezing as a basis for differences in freezing resistance between rye and wheat will be postulated along with this finding.

## **Transcriptome analyses of canola (*Brassica napus*) treated with the plant growth promoting rhizobacterium *Pseudomonas chlororaphis* PA23 identifies differential expression of growth and defense related genes**

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The rhizosphere is a complex environment with a microbiome that contains bacterial and fungal species that promote plant growth. Recently, the use of microbes has become an emerging solution for improving productivity and sustainability of the agro-ecological landscape. However, the mechanisms underlying these intricate plant-microbe interactions are poorly characterized. *Pseudomonas chlororaphis* strain PA23 is a gram-negative rhizobacterium endogenous to the soils of Western Canada. Canola seedlings treated with *P. chlororaphis* as a soil drench were larger in size with more developed root and shoot systems. We used a combination of RNA sequencing and computational biology to uncover the genes and gene ontology terms responsible for the remarkable growth phenotype. We show that treatment of canola with *P. chlororaphis* increased the expression of genes associated with photosynthesis, nutrient transport, and growth regulation. Together, this study presents novel data into the global genetic and regulatory mechanisms responsible for improved plant performance and has applications for growing strategies using biological additives in Canada and abroad.

## **Testing phenotypic and genetic trade-offs between growth and drought resistance in lodgepole pine and white spruce in Alberta**

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Climate changes are threatening forest health in the Canadian boreal forest. Traditional tree breeding programs, with their long cycle times, cannot timely produce well-adapted seedlings. Moreover, it is unclear whether selection for drought-resistance trees would result in lowered productivity. Here, we present a study from the Genome Canada, Resilient Forests (RES-FOR): Climate, pests and policy, genomic applications project. The RES-FOR project applies genomics selection, a novel technology that can substantially speed up breeding cycles, to tree breeding programs. In this study, we are examining multiple phenotypic traits associated with drought resistance and growth, including gas exchange, specific leaf area, stomatal density, and integrated water use efficiency based on carbon isotope ratio in approximately 1,000 trees in 35+ year old progeny trials of lodgepole pine and white spruce in Alberta. Corresponding growth rate data has also been made available for these trials from our industrial and government partners. Our goals are to test whether families show phenotypic and/or genetic trade-offs between drought resistance and growth, and to explore the amount of genetic variation present within these breeding populations. The phenotypic data will also be used to develop genomic estimated breeding values. Preliminary analyses shows considerable phenotypic variations among families for all gas exchange traits measured. In spruce, families with higher intrinsic water use efficiency had lower rates of photosynthesis. Furthermore, families showed differential responses in gas exchange to vapor pressure deficit in both species. We are measuring the other traits and will incorporate them into the analysis in future.

# Poster abstracts



### **Role of RAE1 in *Arabidopsis thaliana* heat tolerance.**

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Plants need to adapt to environmental conditions. Here we examine heat stress response in *Arabidopsis thaliana*. Studies in our lab have implicated DDB1 in heat tolerance. DDB1 interacts with DWD proteins, and six heat induced DWD genes were identified as candidate genes. Analysis of heat sensitivity in mutant alleles of these genes revealed that a RNA EXPORT1 (*rae1*) promoter allele resulted in heat sensitivity in adults and seedlings. We examined the effect of this allele on the *RAE1* transcript and found that the transcript was still expressed in control conditions, however heat stress resulted in failure of intron 1 splicing. We examined stronger *rae1* alleles, however were unable to identify homozygotes, suggesting gametic or zygotic lethality. Additional analysis indicated that RAE1 is required in the female gamete. Nonetheless heterozygotes of one of these stronger alleles also exhibit increased heat sensitivity. YFP-RAE1 was found to be nuclear localized, however following heat treatment it was also detected in cytoplasmic speckles. Our findings on the role of RAE1 in Arabidopsis heat tolerance will be presented.

### **Effects of low soil nitrogen and salinity on the nitrogen fixing species *Shepherdia canadensis*.**

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The bitumen extraction and refinement process used in Alberta oil sands produce saline tailings. The effects of NaCl on the physiology of a native woody boreal species, Canadian buffalo berry [*Shepherdia canadensis* (L.) Nutt], a potential reclamation candidate, was examined. The experiment was conducted at two levels of soil nitrogen as low levels of nitrogen have been observed to increase rates of fixation in nitrogen fixing species. Seedlings were inoculated with *Frankia* and treated with 0, 100 or 200 mM NaCl and 0 or 1 mM nitrate for eight weeks. The results showed that photosynthetic rates decreased with increasing salinity. This decrease was due to a decrease in stomatal conductance (week 2) and then to ionic damage to photosystem II (shown by a change in chlorophyll fluorescence) (week 5). Sodium and chloride content in the tissues increased with increasing salinity, with higher accumulation in the leaves. Calcium, potassium and nitrogen content did not decrease with salinity. Nitrogen fixation rate decreased with increased salinity, but the level of nitrogen content in the tissues did not change between treatments. This suggests that even though nitrogenase activity decreased, *Shepherdia canadensis* was able to maintain its nitrogen level under NaCl stress. *Shepherdia canadensis* was found to be tolerant to NaCl up to 100 mM and less tolerant at 200 mM. The addition of 1 mM nitrogen into the soil did not have a significant effect on the salt tolerance of *Shepherdia canadensis*. Recommendations for reclamation will be discussed.

### **Identification of candidate genes operating in the piperine biosynthetic pathway of *Piper nigrum* L.**

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Decades of work have investigated the diversity of specialized metabolites in black pepper (*Piper nigrum* L.), but the biosynthetic pathways that produce these compounds are largely unknown. Piperine is a major flavour compound in black pepper. The last step in its biosynthesis is catalyzed by piperoyltransferase (PT), conjugating the piperidine ring onto a piperoyl moiety. We hypothesize that PT is a member of the BAHD family of acyltransferases. We further hypothesize the piperoyl moiety is produced from a phenylpropanoid precursor elongated by a polyketide synthase-like enzyme (PKS). Reads, but no published assemblies, are available from seven data sets in the NCBI short read archive: one fruit, two leaf, and four root. Given these reads, this study identifies a candidate set of BAHD and/or PKS coding genes with higher expression levels in fruits than leaves. Trimmed and normalized reads were assembled with BinPacker, SOAPdenovo-Trans, and TransABYSS, with  $k$ -mer sizes of 21-31, step 2 and 41-80, step 10; BinPacker only supports  $k \leq 32$ . The resulting assemblies were processed through the EvidentialGene pipeline and redundancy removed through non-redundant gene set comparisons, with redundancy set to 40-80%, step 10. Expression levels were evaluated and differentially expressed genes identified. BAHD and PKS genes were identified through BLAST searches of the assembly and compared with the differentially expressed sets to propose candidates involved in piperine biosynthesis. This approach demonstrates the utility of over-assembling *de novo* transcriptomes and the value of redundancy removal for differential expression analysis in polyploid organisms.

### **Spatial regulation of laccases and peroxidases during lignification in *Arabidopsis thaliana***

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Lignin is a critical biopolymer that is deposited in secondary cell walls of vascular plants during development. In the inflorescence stem of *Arabidopsis thaliana*, lignified secondary cell walls confer structural strength in fiber cells and efficient water transportation in xylem vessel elements. Secreted cell wall enzymes, laccases and peroxidases, oxidize monolignols leading to polymerization of the lignin biopolymer. These oxidative enzymes comprise large gene families in *A. thaliana*, with 17 laccase and 73 peroxidase gene members, making it difficult to ascertain which genes are involved in developmental lignification. Using fluorescently tagged proteins, we studied the distribution of two laccases, *LACCASE4* (LAC4) and LAC17, and a peroxidase, *PEROXIDASE64* (PRX64), in the inflorescence stem. LAC4-mCherry complemented the irregular xylem phenotype of *lac4/lac17* double mutants, indicating the fusion protein was functional. LAC4 and LAC17 were found distributed throughout the secondary cell wall layers in both vessel elements and fibers, but absent from the middle lamella region between adjacent cells. In contrast, PRX64 was restricted to the middle lamella and cell corners of interfascicular fibers and was not found in the vascular bundles. We hypothesize that plant cells control the localization of LAC and PRX to specific cell types and secondary cell walls regions to coordinate lignin deposition throughout development.

## **Allelic diversity and expression analysis of *abscisic acid insensitive 5* with respect to seed dormancy in wheat**

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Seed dormancy is an adaptive trait that blocks seed germination under favourable conditions, and the level of dormancy in cereal crops such as wheat is closely associated with preharvest sprouting (PHS), germination of the grain prior to harvest under moist conditions. Dormancy is a complex trait regulated by intrinsic such as plant hormones and environmental factors. The plant hormone abscisic acid (ABA) plays an important role in regulating the level of dormancy, and this role of ABA is mediated by its level and signaling. Abscisic acid insensitive 5 (ABI5) is one of the components of the ABA signaling pathway that regulate seed response to ABA and dormancy. To gain insights into the involvement of ABI5 in the regulation of dormancy in wheat, this study performed comparative analysis of its genomic nucleotide sequences and expression level in wheat genotypes that exhibit a range of difference in the level of seed dormancy (high, intermediate and low level of dormancy). Our gene sequence analysis showed allelic variations in the ABI5 homeologues across the different genotypes. Further experiments are being carried out to determine if the expression of the gene encoding *ABI5* is associated with the level of dormancy, and some of the results will be discussed during the presentation.

## **Physiological mechanisms in near isogenic spring wheat sister lines contributing to differences in grain yield and grain protein.**

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Wheat (*Triticum aestivum* L.) is the most widely grown cereal crop in Canada covering more than 10 million hectares. Canadian wheat has been well recognized by the industry and consumers all over the world for its quality. Wheat breeders aim to improve grain yield and grain protein concentration for better milling, gluten strength and other rheological characteristics. Grain yield and grain protein concentration are negatively correlated, influenced by a complex genetic relationship among the traits, which makes improving both very difficult. Five isogenic sister lines were developed at the Swift Current Research and Development Centre, Swift Current, SK, Canada from an elite spring wheat breeding population named 'B1018' (BW928/BW431//Carberry). These sister lines differ for grain yield, grain protein, and test weight although maturity and plant height, were indistinguishable in field trials. To better understand the mechanisms regulating the yield and protein relationship in these wheat sister lines, in-depth field and greenhouse experiments were conducted. Here we report preliminary findings with studies on spike morphological traits, yield fill rate, and N uptake, partitioning and post-anthesis

remobilization to decipher physiologically mechanisms in wheat sister lines carrying differential traits associated with grain yield and protein quality. Outcomes from the studies using various physiological, biochemical and molecular approaches are discussed.

### **Genetic modulation of cruciferin for the improvement of meal quality in *Brassica napus***

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Cruciferin is a seed storage protein that accounts for approximately 60 % of the total protein in canola seed. Cruciferin has unique functional characteristics that renders it a valuable additive for the food processing industry. Improvements in cruciferin content will improve the value and quality of canola meal to both producers and processors. Breeding for the improvement of cruciferin content in canola first requires knowledge of existing phenotypic variation and an understanding of the genetic control of the five genes that control cruciferin content. To assay phenotypic variation in cruciferin content, an ELISA-based approach was developed to facilitate the efficient screening of total cruciferin content in diverse canola germplasm. During phenotypic screening, significant variation in total cruciferin content was observed amongst genotypes. To understand the genetic control of cruciferin, quantitative RT-PCR was used to examine the distribution of cruciferin transcripts as well as their relative abundance across different tissues and throughout seed development. The accumulation of cruciferin transcripts was only detected in the developing seed with approximately equal abundance of each transcript class. Finally, a genome editing approach was employed to modify the seed storage protein profile of canola by targeting select cruciferin genes. Transgenic plants were identified and further characterization will be performed. Collectively, these experiments will facilitate the development of canola cultivars with specialized seed storage protein profiles.

### **Short-term limits to reproduction in two species of rewardless slipper orchids (*Cypripedium*): resources, pollinator abundance, or pollinator preference?**

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Species with rewardless flowers often suffer low reproductive success because visitors learn to avoid flowers without nectar or edible pollen. Thus, fruiting is usually limited by pollen receipt within a season. However, fruiting success can vary widely within and among species. Factors causing such variation are not well understood, particularly for species that do not mimic a “model” rewarding species or insect mate to reduce pollinator learning. We studied reproduction in the

common yellow and threatened small white lady's slipper orchids (*Cypripedium parviflorum* and *C. candidum*) and their hybrids in tall grass prairie remnants. These orchids achieve pollination by temporarily trapping floral visitors. The proportion of *C. parviflorum* plants setting fruit ranged from 22-89% over three years and four sites in Manitoba. Fruit set in *C. candidum* was more typical of a rewardless species, ranging from 3-35%. Pollen supplementation over two seasons increased whole plant fruit and seed production in *C. candidum* but not in *C. parviflorum*. Instead *Cypripedium parviflorum* experienced a balance between pollen and resource limitation. To account for the difference between species, we assessed foraging insects and analyzed fruiting in relation to segregating floral traits of hybrids. Yellow slippers and greater height may enhance the attractiveness of *C. parviflorum* to foraging insects in this yellow-dominated flowering community. In addition, larger flowers in this species may accommodate more insects. Thus, species with rewardless flowers may avoid pollen limitation, even without mimicry to slow pollinator learning.