Variation in the uptake of thiamethoxam, a neonicotinoid pesticide, in the the Nested Association Mapping (NAM) Founder inbreds of maize

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by

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Abstract

Neonicotinoid pesticides have recently come under fire for their negative impacts on pollinators. However, seed treatments of neonicotinoid compounds such as imidacloprid are regularly applied to over 79% of maize grown commercially in the US. Thiamethoxam is one of the least studied of these compounds. In this study, I examined the variation of systemic uptake of thiamethoxam by maize seedlings using a liquid chromatography - mass spectrometry (LC-MS) protocol to observe thiamethoxam concentration in the 26 founder inbred lines of the Nested Association Mapping (NAM) panel, along with the inbreds Mo17 and W22. I observed that the mean differences in concentration 16 days post planting between tropical and temperate lines are significantly different. Additionally, I calculated a minimum value for percent thiamethoxam uptake from seed treatment for 8 inbred genotypes.

Introduction

Neonicotinoid pesticides are some of the most widely used in US agriculture, especially in the cultivation of maize. According to data collected by the US Geological Survey in 2013, by 2007, at least 79% of maize hectares planted in the US were seed treated with neonicotinoids (Douglas et al 2015). Applied most frequently as a seed treatment, which solubilizes in water and is taken up by the plant, these systemic pesticides provide resistance to early stage chewing pests via blockage of nicotinic acetylcholine receptors (nAchR) in insect nervous systems. However, in recent years the negative environmental effects of neonicotinoids have begun to attract public attention, culminating in the recent European Union ban on outdoor use of thiamethoxam and two other neonicotinoid pesticides, clothianidin and imidacloprid, in 2018 (Butler 2018). This ban was based on a large-scale literature review and data collection analyzing the harm to pollinators caused by the routine commercial forms of neonicotinoid pesticide application: seed treatment and granules (European Food Safety Authority (EFSA) 2018). Thiamethoxam is a pesticide in the neonicotinoid class, but has been studied directly much less often than others such as imidacloprid. Thiamethoxam is known to be highly soluble in water and fairly stable, with a half-life of 12-14 days in alkaline water, and has been found in river water and drainages that collect runoff from agricultural areas (Sánchez-Bayo, F., & Hyne, R. V. 2014). Thiamethoxam also has been shown to contaminate plants grown to provide cover cropping for beneficial insects (Bredesen et al 2019), and some of its nitroguanidine moiety cleavage products been detected in maize pollen collected form beehives near treated fields (Sánchez-Hernández, L. et al 2016). These concerns are augmented by the low uptake rate of neonicotinoids into plant tissues. Studies of clothianidin and imidacloprid show no more than 5% uptake of the initial seed treatment by maize (Alford, A., & Krupke, C. H. 2017; Sur, R., & Stork, A. 2003). In the light of these concerning side effects of thiamethoxam, it is important that we understand the biological mechanisms behind the assimilation of treatment into plant tissue. In this study, I attempt to shed some light on the uptake and assimilation of thiamethoxam into maize tissue.

Thiamethoxam differs from other neonicotinoid pesticides in that thiamethoxam is not the com-

pound that acts against insects. Thiamethoxam has a binding affinity to relevant insect receptors that is 8000-fold less than that of clothianidin. However, thiamethoxam works as a pesticide because it is rapidly converted to clothianidin in plant and insect tissue, and shows similar efficacy as an insecticide to the other members of the neonicotinoid family (Nauen et al 2003). Structures of thiamethoxam and clothianidin can be seen in Figure 1.

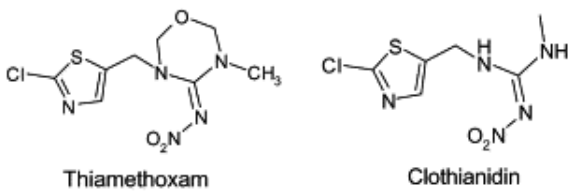

Figure 1. Chemical structures of thiamethoxam and clothianidin (structures from Nauen et al 2003).

In this study, I hypothesized that the rate of thiamethoxam uptake is genetically controlled. In order to study this phenomenon, I analyzed the 26 Nested Association Mapping founder lines, plus inbreds W22 and Mo17, for thiamethoxam concentration 16 days post planting with a seed treatment using an LC-MS protocol developed for this compound. The initial intent of this study was to identify putative genetic loci that are responsible for differences in uptake of the initial seed treatment by quantitative trait loci (QTL) mapping with the established population of NAM Recombinant Inbred Lines (RIL) that have the inbred B73 as ther ecurring parent. For this reason, I originally focused my experimental design on looking for differences between B73 and other inbreds. However, no significant difference between B73 and other lines was observed. Other significant differences were observed in posthoc analyses, especially between inbred subpopulation groups.

Materials and Methods

1. Plant growth and tissue collection

All seedling experiments were carried out with the 26 NAM founders, Mo17 and W22 (Table 1). Seeds were treated by the Seedcare institute in March 2016 with the Syngenta product Cruiser5FS at 0.5mg active ingredient (AI) per seed.

Inbred	Origin	Pedigree	NSS	SS	TS	Pop	Sweet	Subpopulation
Tx303	Texas	Yellow Surcropper	0.36	0.065	0.575	0	0	mixed
M37W	South Africa	21A**2 x Jellicorse	0.404	0.012	0.584	0	0	mixed
Mo18W	Missouri	Wf9*Mo22(2)	0.307	0.011	0.683	0	0	mixed
Mo17	Missouri	C.I.187-2*C103	0.998	0.001	0.001	0	0	nss
OH7B	Ohio	[(Oh07*38-11)Oh07]	0.967	0.031	0.002	0	0	nss
OH43	Ohio	Oh40B*W8	0.998	0.001	0.001	0	0	nss
MS71	Michigan	A619*R168	0.997	0.002	0.001	0	0	nss
Ky21	Kentucky	Boone County White	0.859	0.008	0.133	0	0	nss
B97	Iowa	BSCB1®C9	0.981	0.016	0.003	0	0	nss
M162W	South Africa	K64R**2 x B1138T	0.996	0.002	0.002	0	0	nss
W22	Wisconsin	III.B10*W25v	0.931	0.063	0.006	0	0	nss
HP301	Indiana	Supergold	0	0	0	1	0	popcorn
P39	Indiana	Purdue Bantam	0	0	0	0	1	sweet
IL14H	Illinois	White Narrow Grain Evergreen	0	0	0	0	1	sweet
CML228	Mexico	Suwan-1/SR	0.002	0.003	0.995	0	0	ts
Ki3	Thailand	Suwan 1(S)C4-S8-5-3	0.002	0.001	0.997	0	0	ts
CML52	Mexico	Pop. 79 = STA ROSA	0.01	0.002	0.988	0	0	ts
CML333	Mexico	Pop. 590	0.029	0.013	0.959	0	0	ts
CML322	Mexico	Recyc. US + Mex	0.005	0.01	0.985	0	0	ts
Ki11	Thailand	Suwan 1(S)C4-S8-18-7	0.017	0.003	0.98	0	0	ts
NC350	North Carolina	(H5*PioneerX105A)*H101	0.002	0.003	0.995	0	0	ts
CML103	Mexico	Pop. 44	0.005	0.003	0.992	0	0	ts
CML277	Mexico	Pop. 43 = La Posta (Tux.)	0.004	0.001	0.995	0	0	ts
NC358	North Carolina	TROPHY SYN	0.143	0.001	0.856	0	0	ts
CML247	Mexico	Pool 24 (Tuxpeño)	0.006	0.003	0.991	0	0	ts
CML69	Mexico	Pop. 36 = Cogollero (Caribbean)	0.028	0.006	0.966	0	0	ts
TZI8	Nigeria	TZB x TZSR	0.005	0.041	0.955	0	0	ts

Table 1. Classification of inbreds used in uptake experiments. NSS = non-stiff stalk, SS = stiff stalk, TS = tropical and subtropical, Pop = popcorn, Sweet = sweetcorn. The information in this table comes from Flint-Garcia et al 2005. Numbers in columns 4-8 represent the membership probabilities of a given inbred to that subpopulation, while column 9 represents subpopulation classification based on these probabilities. For more information on membership

probabilities and subpopulation classifications, see Table S1 in Flint-Garcia et al 2005.

1.1 Initial seedling screen

For the initial screen of the 26 founders, Mo17 and W22, I planted 9 seeds from each line at a depth of 2.5 cm in a corn-specific soil mixture. Flat design was as follows: seven 9x6 flats (cell dimensions 5 x 4 x 5 cm) were planted with 4 lines in each flat. One row of B73 was planted in a different row in each flat in order to control for micro-environmental variation, and the last row in every flat was left empty. This planting scheme was followed identically for a control planting of untreated seeds. Plants were cultivated in a growth chamber at 50% humidity with 12 hours light, 12 hours dark, at 28°C during lights-on and 24°C during lights-off. All flats were bottom watered to prevent treatment leeching. After 15 and 16 days, respectively, all above-soil tissue for the control group and the treatment group was harvested and transferred on dry ice to the -20°C freezer, with the exception of the first 20 control lines collected which were stored in the -80°C freezer, and maintained there until lyophilizing.

1.2 Seedling replicate

For the seedling replicate, all growth conditions except watering were replicated with the 7 inbred lines that took up the most thiamethoxam in the first screen, along with B73. These lines were: B97, B73, Ky21, M37W, M162W, Il14h, Mo18W, and CML103. Plants were watered from the top rather than inundated from below as in the first replicate. Seedlings were collected from the soil up and flash frozen in liquid nitrogen.

1.3 Pollen screen

Plants were grown to maturity in the greenhouse and pollen collected at anthesis. Pollen was flash frozen in liquid nitrogen then stored -80°C, moved to -20°C for 2 years, and back to -80°C until extraction due to logistical complications.

2. Liquid Chromatography-Mass Spectrometry

2.1 Initial seedling screen

For the first seedling screen, done in conjunction with the McArt lab, the extraction was performed using a 1:1 acetonitrile:water solvent. After lyophilizing the seedlings for 24 hours, samples were ground on a paint shaker in 50 mL Falcon tubes with ten 3.2mm stainless steel ball bearings (Grade 2000, AISI Type 430; Abbott Ball) in each until tissue was pulverized. One mL solvent mix was added, and samples were hand-shaken and centrifuged at 13,000 rpm for 15 minutes. Five hundred μL supernatant was conserved at -20°C until sample processing was complete for all NAM lines. Samples were then filtered and prepared for LC-MS analysis.

Sample analysis was carried out with a Vanquish Flex UHPLC system (Dionex Softron GmbH, Germering, Germany) coupled with a TSQ Quantis mass spectrometer (Thermo Scientific, San Jose, CA). The UHPLC was equipped with a Kinetex 2.6 μm EVO C18 100 Å column (150 mm x 2.1 mm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The temperature of the column was maintained at 35°C throughout the run and the flow rate was 500 μL/min. The elution program was the following: 2 min equilibration (99% B) prior to injection, 0-3 min (5%-99% B, linear gradient), 3-4.5 min (99% B, column wash), 4.5-4.7 min (99%-5% B, linear gradient), 4.7-5 min (5% B, re-equilibration). The flow from the LC was directed to the mass spectrometer through a Heated Electrospray probe (H-ESI). The settings of the H-ESI were: spray voltage 4000 V, Sheath gas 50 (arbitrary unit), Auxiliary gas 14 (arbitrary unit), Sweep gas 1 (arbitrary unit), ion transfer tube temperature 32°C, vaporizer temperature 350°C .The mass spectrometer was operated in positive mode and the detection was carried out using Selected Reaction Monitoring (SRM). Two transitions were monitored: one for quantification ($291.977 > 211.125$) and the other for confirmation ($291.977 > 181.071$). The SRM parameters for both transitions are summarized in Table 1. The resolution of both Q1 and Q3 was set at 0.7 FWHM, the dwell time for each transition was 100 ms and the pressure of the collision gas (argon) was set at 1.5 mTorr (Nicolas Jean-Baptiste Baert, personal communication,

March 2019).

2.2 Seedling replicate and pollen screen

The subset replicate of the seedling screen was done at the Boyce Thompson Institute. Tissue samples were homogenized in a Spex SamplePrep 1600 MiniG for 30 seconds 2 times at 1500 strokes per minute, and 50mg of tissue was taken from each sample. Extraction buffer for the seedling samples was 95% MeOH, 5% H_2O, and 1% formic acid. The extraction was performed by adding 200 μL of the extraction buffer to each ground sample, vortexing, and centrifugation for 30 seconds at 21,200 g.

After centrifugation, samples were sonicated with a Q Sonica, model CL-334. The sonicator was programmed with an amplitude of 100, process time of 25:00 with a pulse on time of 3 seconds and pulse off time of 2 seconds, with temperature monitoring on. After sonication, samples were centrifuged at 21,200 g for 10 minutes, then tubes were reoriented in the opposite direction and centrifugation was repeated. 50 μL supernatant was transferred to LC-MS vials in preparation for analysis.

The reversed-phase liquid chromatography was performed using a Dionex Ultimate 3000 Series LC system (HPG-3400 RS High Pressure pump, TCC-3000RS column compartment, WPS-3000TRS autosampler) controlled by Chromeleon Software (Thermo Fisher Scientific) and coupled to Orbitrap Q-Exactive mass spectrometer controlled by the Xcalibur software (Thermo Fisher Scientific). A Phenomenex Kinetex C18 30mm x 2.1mm at 30°C and the flow rate 0.5 mL/min of mobiles phases A (H_2O:0.1% Formic Acid) and B (AcN:0.1% Formic Acid) was used for separation of target molecular features. The gradient starting condition was 3%B at 0 min, rising to 18% B at 1.5 min, to 98% B at 1.8 min, then was held for 1 min, and followed by 0.2min re-equilibration at the starting condition. A heated electrospray ionization source (HESI-II) in positive mode was used for the ionization with parameters of spray voltage at 3.5 kV, capillary temperature at 380 °C, sheath gas and auxiliary gas flow at 60 and 20 arbitrary units respectively, and probe heater temperature 400 °C. The data were acquired in m/z range of 200–500, 140,000 FWHM resolution (at

m/z 200), AGC target 3e6, maximum injection time of 200ms, in profile mode. Metabolite quantification was estimated with Thermo Scientific Xcalibur™ Version 4.1.31.9 (Quan/Processing) (Navid Movahed, personal communication, April 2019). The pollen samples were processed with the same protocol, except that the 5% H_2O was omitted and replaced with MeOH.

3. Quantification and statistical analysis

3.1 Comparison of individual lines

Using uptake data from the first seedling screen, I compared all lines against each other using an ANOVA (Base R) with concentration thiamethoxam being predicted by sample, and subsequently examined pairwise comparisons with a Tukey's HSD test (Base R). I used a square root transformation on the data to meet assumptions of normality for ANOVA. The initial intent of this experiment was to map the trait of thiamethoxam uptake using existing NAM RIL with B73 as the reccurent parent, so I also compared all lines from the seedling replicate to B73 in R using the DunnettTest function from the DescTools package. For the seedling replicate, I log transformed the data in order to meet the assumptions of normality for ANOVA.

3.2 Subpopulation cluster analysis

I performed an unplanned analysis of uptake in subpopulations by clustering lines according to the classifications in Table 1. I clustered sweetcorn and popcorn lines with the temperate lines due to their clustering with non-stiff stalk lines in the STRUCTURE analysis from Flint-Garcia et al 2005. When I clustered mixed lines into other subpopulations, I clustered them based on the membership probabilities as determined by Flint-Garcia et al 2005. When the membership probability for a subpopulation was > 0.5, I included the mixed line in that subpopulation. Among the NAM Founders, the mixed lines are all classified into the tropical group by this metric. Table 2 shows the structure of clusters in the various analyses. Analyses were carried out in R with ANOVA followed by a Tukey's HSD test. I transformed the data by square root in order to meet

the assumptions of normality for ANOVA.

Analysis	Cluster Definitions
1	Subpopulations treated as separate categories
2	Temperate = sweet, popcorn, nss, ss; Tropical = ts; Mixed = mixed
3	Temperate = sweet, popcorn, nss, ss; Tropical = ts, mixed
4	Temperate = nss,ss; Tropical = ts; Mixed = mixed
5	Temparate = nss,ss; Tropical = ts, mixed

Table 2. Subpopulation clusters based on membership probabilities and outcomes of STRUCTURE analysis in Flint-Garcia 2005.

3.3 Pollen screen

I compared the concentration values in pollen with the same method as the seedling experiments. I transformed the data by square root in order to meet the assumptions for ANOVA.

3.4 Percent AI translocated

I used data from the seedling replicate, in which the mass of full seedlings was measured before extraction for thiamethoxam detection, to calculate the minimum percent AI translocated into seedling tissue after 16 days (Figure 7). I used the value of 0.5mg AI per seed given by the Seedcare Institute for the treatment of Cruiser5FS as the initial AI. I calculated the amount of thiamethoxam in individual seedlings using the ng/mg fresh tissue concentration values, converting this to mg/mg fresh tissue, and multiplying by the mass in mg of the whole seedling.

Results

1. Comparison of individual lines

The mean uptake across the inbred lines can be seen in Figure 2. Posthoc discovery that the concentration of the seed treatment on B73 used in first planting different from all other lines by a

factor of $1/2$ led to the exclusion of B73 from all analyses of the original seedling screen dataset. Using a cutoff of p = 0.05, statistically significant differences between pairs of lines were observed. Namely, M162W and B97 exhibited statistically significantly more uptake than various inbreds on the low end of the uptake spectrum, as summarized in Table 3.

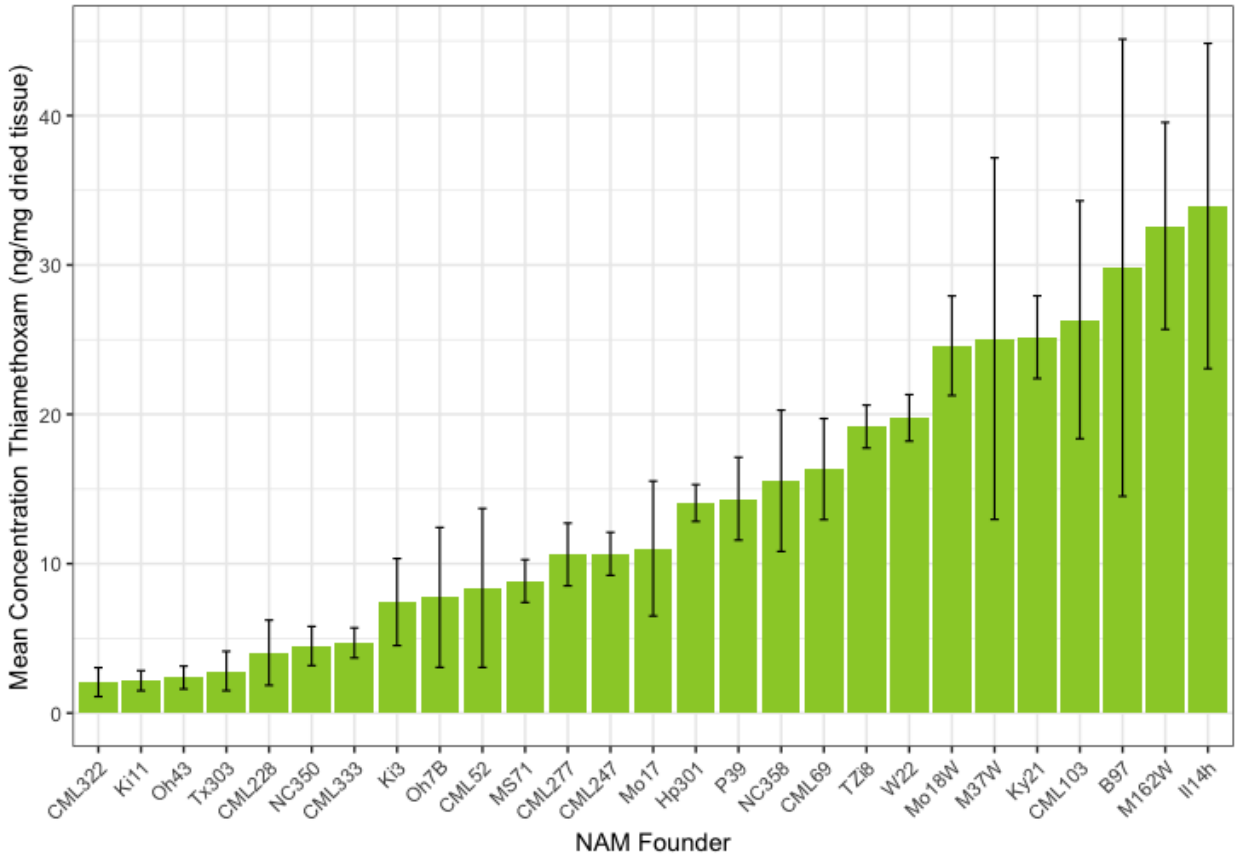

Figure 2. Mean thiamethoxam concentration in 2-week-old seedling tissue of the NAM Founders + Mo17 and W22. ANOVA on these samples gave a p-value of $4.92\text{x}10^{-6}$.

High uptake line	Low uptake lines	p-value
M162W	CML228	0.00105
	CML322	0.000726
	CML333	0.0100
	CML52	0.0264
	Ki11	0.000299
	Ki3	0.0460
	NC350	0.00743
	Oh43	0.000437
	Oh7B	0.00934
	Tx303	0.000377
B97	CML228	0.0340
	CML322	0.0212
	Ki11	0.044
	Oh43	0.0168
	Tx303	0.0149
CML103	CML228	0.0224
	CML322	0.0140
	Ki11	0.00777
	Oh43	0.0108
	Tx303	0.00949
Il14h	Ki11	0.0200
	Oh43	0.0251
	Tx303	0.0230
	CML228	0.0421
	CML322	0.0257
Ky21	Oh43	0.00749
	Tx303	0.00658
	CML228	0.0159
	CML322	0.0100
	Ki11	0.00536
M37W	CML322	0.0477
	Ki11	0.0303
	Oh43	0.0404
	Tx303	0.0362
Mo18W	CML228	0.0214
	CML322	0.0134
	Ki11	0.00737
	Oh43	0.0102
	Tx303	0.00901

Table 3. Summary of uptake differences among individual lines. P values were calculated by Tukey's HSD.

Given that the initial intent of this study was to map the trait of thiamethoxam uptake using ex-

isting NAM RIL with B73 as the recurrent parent, my initial aim was to determine if there were statistically significant differences in uptake in any inbreds from B73. The uptake data from the replicate seedling screen can be seen in Figure 3. There was no statistically significant difference observed between any lines.

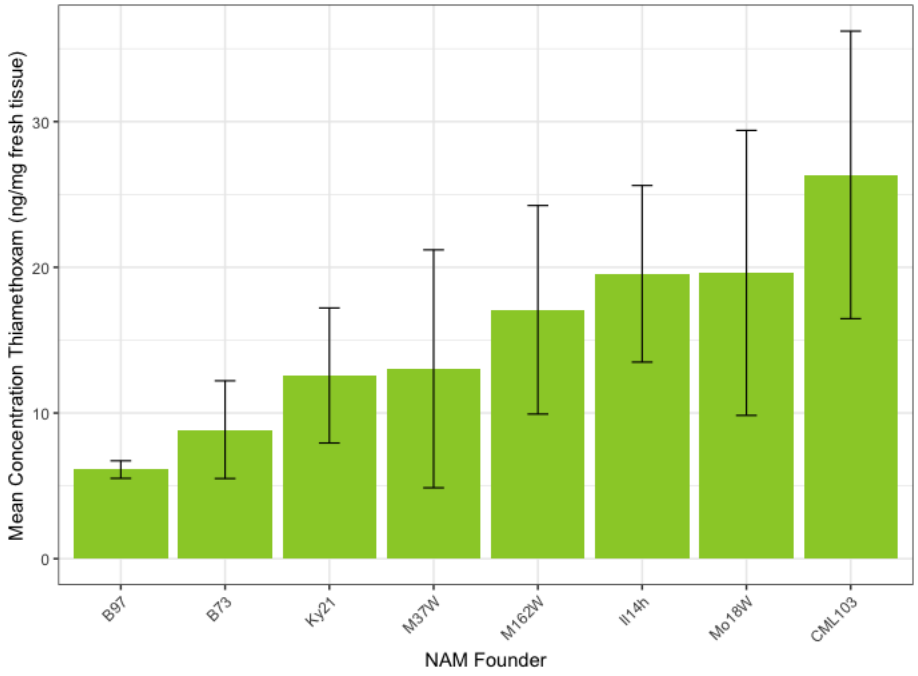

Figure 3. Mean thiamethoxam uptake in seedling tissue. An ANOVA with thiamethoxam concentration predicted by sample gave p = 0.407.

2. Supopulation cluster analysis

When subpopulation classifications from Table 1 were run through statistical analysis as separate groups, there was no statistically significant difference between subpopulations observed (Figure 4). However, In analyses 2-4, no matter how the lines were grouped, there was a statistically significant difference observed between the tropical and temperate subpopulations. Outcomes of all 4 analyses are shown in Figure 5.

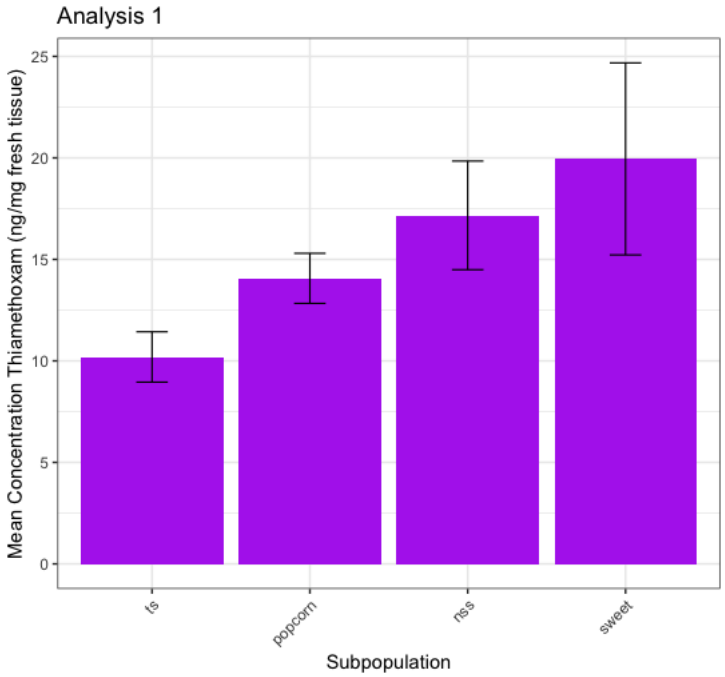

Figure 4. Mean thiamethoxam uptake by subpopulation group from Analysis 1 as defined in Table 2. The ANOVA returned a p-value of 0.0604.

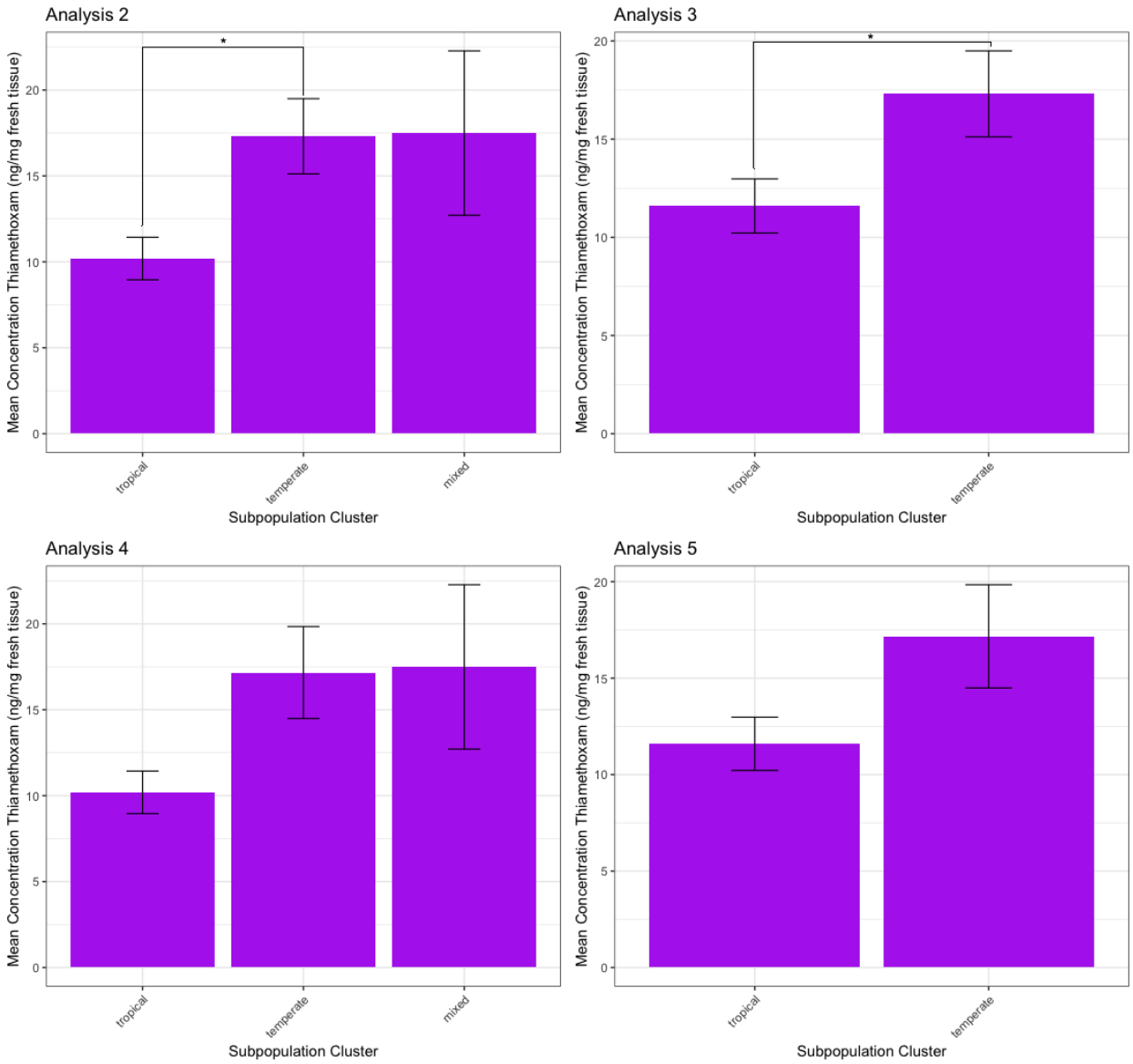

Figure 5. Subpopulation cluster analysis. P-values for the marked significant differences are as follows: Analysis 2: 0.0168; Analysis 3: 0.0178. Analysis 4's ANOVA yielded a p-value of 0.0519, and the tropical-temperate comparison in the subsequent Tukey's HSD gave a p-value of 0.0674. Similarly for Analysis 5, where ANOVA gave p = 0.0632 and the tropical-temperate comparison in Tukey's HSD gave a p-value of 0.0632.

3. Pollen screen

The piloted procedure for pollen shows efficacy in detecting small concentrations of thiamethoxam in the pollen, as shown in Figure 6. Because only one sample of B73 could be obtained, B73 was

once again left out of the statistical analysis.

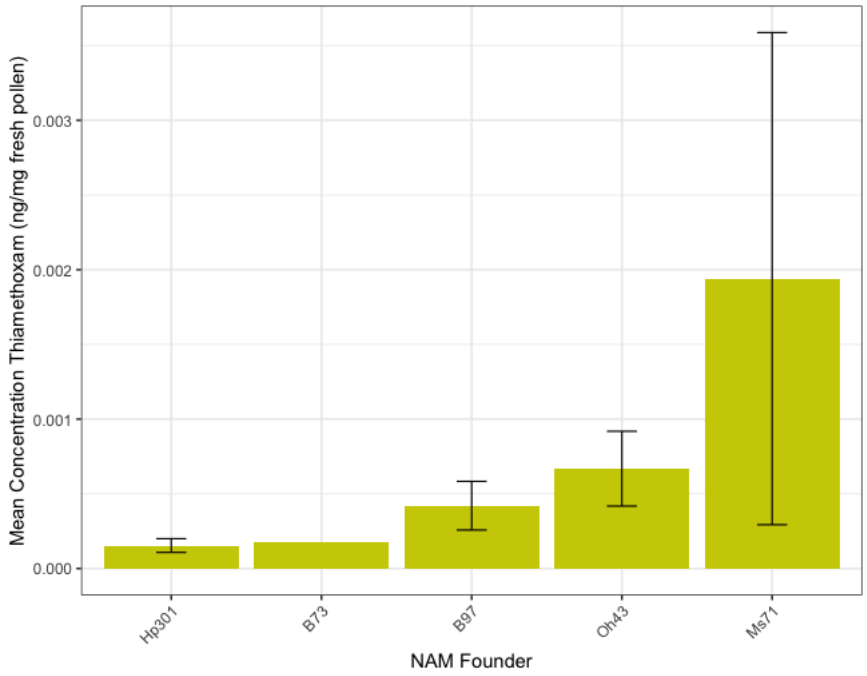

Figure 6. Mean concentration of thiamethoxam detected in fresh pollen samples. ANOVA on these results yielded a p-value of 0.764.

Percent AI translocated

I calculated a minimum percent AI translocated for the 8 inbred lines in the seedling replicate (Figure 7). This calculation only includes shoot tissue. There was no statistically significant difference among lines.

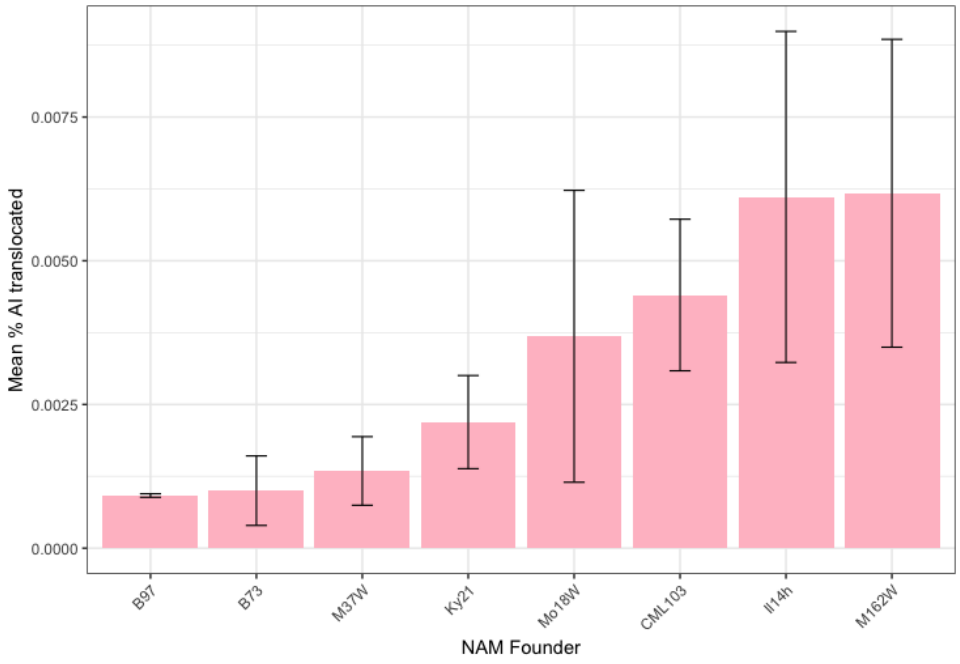

Figure 7. % AI translocated into shoot tissue after 16 days. ANOVA yielded a p-value of 0.0592.

Discussion

The most interesting outcome of these experiments is the observation that when sweetcorn and popcorn were included in the analysis, no matter how I defined the "tropical" and "temperate" groups, the tropical lines always exhibited significantly higher concentrations of thiamethoxam than their temperate counterparts. Additionally, when excluding sweet and popcorn, the temerpate-tropical comparisons returned p-values of 0.0674 and 0.0632. This result follows the trend that tropical lines were found to have lower concentration of thiamethoxam than temperate lines.

I also piloted a procedure for the detection of thiamethoxam in mature pollen. The low values of thiamethoxam detected suggest that the consideration of clothianidin as a metabolic product of thiamethoxam in planta is important to consider when analyzing the effect of thiamethoxam seed treatments on the concentration of insect-toxic compounds that are bioavailable in pollen. Nauen et al 2003 shows that 3 days after soil-drench thiamethoxam application, the concentration of clothianidin in cotton leaves was twice that of thiamethoxam. Based on this knowledge and my results

I would hypothesize that a similar breakdown occurs in maize, and that the observation of low levels of thiamethoxam in pollen is due to the conversion of thiamethoxam to clothianidin. I would expect that after the 70-100 days from treatment to anthesis, there would be very little remaining thiamethoxam in plant tissues, and that clothianidin would be the more important metabolite. While a hypothesis about the chemical fate of thiamethoxam after translocation into plant tissue is beyond the scope of this study, it would be interesting to look for all possible breakdown products in pollen to see what vestiges of the original AI remain at anthesis, and which make their way into the pollen.

However, in terms of just thiamethoxam, the result of almost 2 ng/g pollen in Ms71 is an important observation in terms of honeybee health. Laurino et al (2013) reported an LD50 of 4.40 ng/bee for thiamethoxam. Given that bees consume pollen as their main source of protein, it is within reasonable scope that a bee could consume enough pollen over the course of a day or days to be lethal or sublethal. Even without considering clothianidin at all, this result would imply that toxic concentrations of thiamethoxam, and presumably its metabolites, can make their way into mature pollen in maize.

Finally, I calculated a minimum measure of the percent thiamethoxam translocated into shoot tissue at 16 days for several genotypes. The calculated values are much smaller than the value reported in Alford & Krupke 2017, for two reasons. First, their analysis additionally included root and seed tissue. I therefore refer to the values I calculated as minimum measures, because by including root and seed tissue in the analysis, the percent AI translocated into all plant tissue will increase. Also important to consider is the conversion of thiamethoxam to clothianidin in planta. The analysis of Alford & Krupke is on the uptake of a direct clothianidin treatment. If thiamethoxam is in fact converted to clothianidin in maize tissue as it is in cotton tissue, then the analysis of Alford & Krupke differs from the analysis I would plan for thiamethoxam. The analysis of clothianidin uptake doesn't necessitate the analysis of thiamethoxam's breakdown into clothianidin. However, if thiamethoxam does break down to clothianidin in maize tissue, in order to account for how much thiamethoxam was originally taken up into seedlings from treatment,

it will be necessary to incorporate detection of clothianidin to understand truly how much of the original treatment is translocated. I would incorporate several stages of detection as well; Alford & Krupke measured clothianidin concentrations over a time course of 20 days post-planting. I would also consider adding the nitroguanidine moiety cleavage products detected by Sánchez-Hernández et al 2016 into this study in order to better understand how much initial AI was taken up.

The original intent of this study was to map the trait of thiamethoxam uptake. However, I observed no statistically significant difference in uptake between B73 and any other line. However, this could be due to several factors. On one hand, the exclusion of B73 from the data in the first screen due to the halved concentration of initial seed treatment and the lack of biological replicates of B73 in the pollen screen led to a dearth of data with which to examine the differences from other lines; I was only able to make the comparison between B73 and other lines in the smaller seedling replicate. Additionally, the variation among biological replicates was very large (up to and beyond an order of magnitude). I would take more replicates to ensure that such variation represents natural variation in the population; since there is such high variance among biological replicates. Another possible factor is the hypothesized conversion of thiamethoxam to clothianidin in planta. Without information about the concentration of clothianidin, it's impossible to know whether observed differences among lines are due to uptake of initial seed treatment or to conversion rates of thiamethoxam to clothianidin. As a future direction of this study I would repeat the screen with detection of both thiamethoxam and clothianidin, and use the presence of both compounds as a proxy for uptake as mentioned in the previous paragraph. With the incorporation of clothianidin as a compound of study, I hypothesize that significant differences between NAM lines and B73 would be found, and the trait would be able to be mapped with the NAM RIL as originally planned. My first hypothesis going into this project was that some lines take up more active ingredient from seed treatment than others. However, as mentioned above, an alternative hypothesis exists; that the difference in thiamethoxam concentration is due to a difference in conversion rate to clothianidin, rather than in uptake. Generating accurate percent AI translocation values along with detecting clothianidin in the LC-MS screens will provide the opportunity to truly compare the two hypothe-

ses. Additionally, a time course of thiamethoxam and clothianidin detection in plant tissues would allow comparison of the thiamethoxam to clothianidin ratio in inbred lines over time.

In conclusion, I found that there are significant differences between the concentration of thiamethoxam at 16 days post planting in tropical vs. temperate lines. This implies that there is some genetic difference in the uptake of seed treatment or the breakdown of thiamethoxam into clothianidin. I piloted a thiamethoxam extraction protocol for mature maize pollen, and calculated the minimum percent AI translocated for several genotypes. The results of this study highlight the importance of the thiamethoxam-to-clothianidin conversion in planta, an important phenomenon associated uniquely with thiamethoxam among the neonicotinoid pesticide family.

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