**Running title:**

**Effects of methyl jasmonate seed treatments on preference and performance of seed corn maggots.**

**Swayamjit Ray, Gen Chang Hsu, Noelle Pappous, Olivia Rooney and Jennifer S. Thaler**

**Abstract:**

Eliciting host plant resistance using jasmonates is a promising technique that has the potential to protect seeds and seedlings against multiple life-stages of herbivorous insect pests. Several hurdles exist for developing an effective technique including an application method that growers could use in the field, determining a dose that does not limit plant growth, and ensuring the plants are responsive in the abiotic conditions when the pest occurs. In this study, our goal was to test if treating corn seeds with the plant hormone methyl jasmonate would reduce the preference of ovipositing seed corn maggot adults (*Delia platura*) and the performance of seed corn maggot larvae feeding on seeds. We tested two methyl jasmonate application methods: soaking seeds in aqueous methyl jasmonate and coating seeds with a coumaryl-based matrix containing methyl jasmonate. We conducted dose response experiments in the laboratory using each application method to determine a concentration that induces resistance to the adult and larval flies with minimal reductions in seed germination and seedling growth. We found that seeds soaked with 0.2mM MeJA had the same germination rates and marginally lower early seedling growth compared to water-treated controls whereas 0.2 mM dry treatment did not affect seed germination or growth. In choice and no-choice bioassays with adult flies in the laboratory and field, we found reduced oviposition on both wet and dry treated seeds compared to controls. Larval performance was also lower in methyl jasmonate treated seeds. Lastly, the methyl-jasmonate induced resistance also occurred at the lower temperatures typical of the spring soil conditions when this fly is most damaging. In conclusion, MeJA application, especially using a coumaryl-based seed coating matrix, has the potential to deter adult fly oviposition and reduce maggot performance in spring temperature conditions with minor effects on seed germination and growth.

**Introduction:**

While the use of jasmonic acid and methyl jasmonate in foliar application and induction of host plant resistance to insect herbivory has been studied extensively (Howe and Jander, 2008), little is known about its success as a seed treatment. Exogenous foliar applications of jasmonic acid (JA) and its derivative methyl jasmonate (MeJA) have been shown to increase endogenous levels of JA in plants and subsequently increase plant resistance to insect herbivores feeding on leaves, roots, stems and flowers. In the last decade, MeJA and JA have also been investigated and shown to have potential as a seed treatment inducing resistance to insect herbivores in a range of plants, including tomato, cabbage and rice (Paudel et al., 2014; Strapasson et al., 2014; Bhavanam and Stout, 2021). Working in the leguminous crop, Andean Lupin (*Lupinus mutabilis*), Erazo-Garcia et al. found that MeJA treated seeds were less preferred by *D. platura* adult flies for oviposition and the larval performance was lower on MeJA treated seeds (Erazo-Garcia et al., 2021). We evaluated the potential for this technique in controlling the seed and seedling pest, *Delia platura*, in corn. Open questions remain in terms of establishing a jasmonate dose that provides resistance with minimum cost in terms of plant growth, developing an application technique that could be used by growers, and testing whether the plant-induced response occurs in the abiotic conditions of seed germination.

While many studies show the benefits of induced resistance in decreasing the preference and performance of herbivorous insects (Painter, 1951; Stout, 2013), jasmonate induced response can also come at a cost through reduced seed germination and seedling growth. Some of these costs may be the direct result of energetic investments in induction, while other costs may be indirect, arising from regulation of growth by defense signaling pathways (Züst and Agrawal, 2016). While energetic costs may be unavoidable, it may also be possible to activate resistance at a low enough level that it is not a stress with associated growth costs. For agricultural applications, it is important to determine a dose of MeJA that promotes resistance without a significant cost in terms of germination or early seedling growth which could affect stand formation or crop yield. Treatment of seeds with MeJA is done prior to planting while the seeds are dormant so that induction of host plant resistance occurs as the seeds become metabolically active during germination. Jasmonates have been shown to negatively affect the likelihood of germination by interacting with the abscisic acid pathway which can promote seed dormancy (Kraus and Stout, 2019; Bhavanam and Stout, 2021). However, recent studies show that it is possible to establish doses of MeJA that induce resistance without hampering seed germination or seedling growth. For example, Erazo-Garcia et al. (2021) showed that lupin seeds treated with 0.1 mM concentrations of MeJA induced resistance against *Delia platura* and did not affect seed germination or seedling growth (Erazo-Garcia et al., 2021). In rice, jasmonic acid application induced resistance to rice weevil but had negative effects on seedling emergence and growth early in the season, which did become smaller at the end of the season when yield was measured (Bhavanam and Stout, 2021).

Many studies on treating seeds with methyl jasmonate have been conducted using a method of applying the jasmonate by soaking the seed in an aqueous solution. The soaking method has been used with both methyl jasmonate and jasmonic acid, and which one provides better resistance depends on the host plant (Bhavanan and Stout 2021). While pre-emptive soaking of seeds with methyl jasmonate to induce plant resistance can be relevant in planting crops such as rice fields, many seeds cannot be planted when they are wet or after germination has begun. The use of piperonyl amides and coumaryl compounds as a basal matrix to dissolve lipophobic chemical pesticides is a common practice for coating corn seeds with systemic insecticides such as neonicotinoids and triazole fungicides (Wilson et al., 2015). The efficacy of the seed coating is primarily dependent on the lipophilicity (pKa value of 5.6 or less) of the pesticides that are used in conjunction with these seed coating matrices (Black et al., 2018; Yang et al., 2018). The pKa value of MeJA (mention pKa value here) made it a better fit to use it as seed coating in corn in conjunction with the seed coating matrix compared to JA (mention pKa value here). Therefore, we tested mixing MeJA with a coumaryl seed coating matrix, L650 (manufacturer?), to coat corn seeds.

While most studies of induced plant responses have been conducted at warm temperatures, the seeds of many crops are planted in the spring when soil temperatures are low. Several studies have shown that induction of plant resistance is temperature dependent, at least at higher temperatures (Havko et al., 2020b). While little research has been conducted on induction at cool temperatures, it may be lower due to an overall lower rate of plant metabolism (Tayyab et al., 2020; Repkina et al., 2021). For example, foliar treatment with JA has been shown to be temperature dependent in soybean where soybean aphids performed better on JA treated plants at 25°C compared to plants that were induced and grown at 17°C (Whalen and Harmon, 2015). However, little is known about how lower temperatures may affect induction of host plant resistance by jasmonates in seeds. Therefore, we also measured the effect of low temperature on seed germination, seedling growth and adult oviposition preference on seeds that were treated with MeJA.

The seed corn maggot (*Delia platura*) is a polyphagous below-ground pest with a diverse host range of more than 50 species (Gill et al., 2013). In the United States, corn is a major commodity crop and seed treatment with pesticides such as neonicotinoids is a common way to control early season belowground herbivores such *D. platura*. However, several recent studies have shown the devastating effect of neonicotinoids on non-target beneficial insects such as insect predators of herbivorous insects, bees, and several bird species (van der Sluijs et al., 2013; Main et al., 2018; Li et al., 2020; Grout Travis A. and Koenig). Therefore, it is imperative that we seek alternatives to replace the use of neonicotinoids as seed treatments to control insect herbivores of crop plants. One of the most damaging generations of seed corn maggots occurs in early spring after they emerge from diapause which coincides with planting season for corn in the temperate corn growing regions of United States. The mean soil temperatures in early spring in such regions can be as low as 15°C -20°C. The larvae of seed corn maggots feed on the cotyledons of the seeds during germination and the roots of emerging seedlings (Guerra et al., 2017).

In this study we explored the use of methyl jasmonate as a seed treatment to induce host plant resistance in corn to control seed corn maggot herbivory. Specifically, we 1) tested the effect of five different concentrations of MeJA on corn seed germination and seedling growth using a wet and dry application method;. 2) Using doses of the wet and dry application method that don’t limit germination, we conducted choice and no-choice oviposition preference assays with adult flies, and measured the performance of the seed corn maggot larvae. 3) We tested the effect of MeJA seed treatment on germination, seedling growth, and fly oviposition preference at cool temperatures.

**Results:**

**Wet methyl jasmonate seed treatment slowed germination and early seedling growth but germination and growth were not affected in dry treated seeds:**

We compared the rate of germination in corn seeds treated with 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM of aqueous methyl jasmonate to control water-soaked seeds. Seeds treated with 10mM methyl jasmonate showed less than 20% rate of germination, while seeds that were treated with 0.2mM, 0.4mM, 0.8mM and 1mM methyl jasmonate had no difference in total number of germinated seeds 7 days post treatment compared to controls (Fig 1a). However, there was a delay in germination with fewer seeds germinated at 3 days and 4 days post methyl jasmonate treatment with concentrations of 0.2mM, 0.4mM, 0.8mM and 1mM compared to water treated seeds (Fig 1a). The dry coating method did not result in differences in seed germination at two weeks compared to seeds with a control coating (Fig 1c).

The delay in the germination of water-soaked seeds treated with 0.2mM, 0.4mM, 0.8mM and 1mM of methyl jasmonate was reflected in reduced seedling height after 14 days (F3,20= 27.34, p<0.001; Fig 1b). Seedlings emerging from seeds treated with 0.2mM and 0.4mM methyl jasmonate showed the least amount of growth reduction (~12-15%) when compared to water treated seeds, so we used 0.2mM MeJA treated corn seeds to perform our subsequent bioassays. There was no difference in seedling growth using the dry treatment method (Fig 1d).

**Methyl jasmonate treated seeds were less preferred by adult flies:**

When given the choice to oviposit between water-treated and MeJA-treated corn seeds in two choice oviposition assays, the adult flies of seed corn maggots laid ~60% fewer eggs on 0.2mM MeJA treated seeds compared to water-soaked seeds (F1,14= 14.64, p<0.001; Fig 2a). We also performed this two-choice oviposition assay in the field setting where MeJA treated seeds had ~20% lower oviposition by adult flies compared to water-soaked seeds (F1,18 = 12.046, p=0.0027; Fig 2b). When the adult flies were exposed to either water-treated or MeJA-treated seeds in a no-choice assay in the lab, the average number of eggs laid on methyl jasmonate-treated seeds was half compared to water-treated seeds (χ2 = 90.5, *df* = 1, *P* < 0.001; Fig. 2e). Similarly, in a two-choice assay using dry coated seeds, we found that seeds dry-coated with 0.2mM or 0.4mM levels of MeJA had ~20% fewer eggs deposited on them compared to mock-treated seeds (Fig 2 c,d).

In stage-matched seeds, we did not find increased oviposition on control seeds, instead we found increased oviposition on the MeJA treated seeds (Fig 2f), indicating that the increased oviposition on the control seeds is not due to a longer window of availability for oviposition caused by differences in germination rate.

**Performance of seed corn maggot larvae was lower in methyl jasmonate treated seeds compared to water treated seeds:**

Thirty percent fewer seed corn maggot larvae successfully pupated when fed on MeJA treated seeds compared to water-soaked seeds (F1,8= 5.444, p=0.0479; Fig 3).

**Methyl jasmonate also reduced adult oviposition preference at cool temperatures**

The percentage of germination for corn seeds treated with MeJA was 5% lower compared to water-soaked seeds at 15 °C, a temperature in spring when corn is planted and the flies are active in the field (Fig 4a; F1,17 = 4.516, p-value = 0.0485). Three-week-old seedlings that emerged from seeds treated with MeJA-treated were also ~25% shorter than the water-soaked seeds (Fig 4b; F1,17 = 16.357, p-value < 0.0001). The deterrent effect of MeJA treatment on oviposition preference of the adult flies was maintained at the cool temperature. Seeds that were treated with MeJA had nearly 15% fewer eggs deposited on them compared to the number on water-treated seeds (Fig 4c; F1,14 = 6.041, p-value = 0.027).

**Methods:**

Plant material and insects: We used the corn hybrid variety 410 with a maturity time of 91-days that were obtained from Prairie Seeds (Illinois, USA) and were completely devoid of any seed treatment. Adult flies and larva of seed corn maggots were collected from the corn fields in Tompkins County in Upstate New York and were brought back to the lab. Adult flies were reared on 0.5% sucrose solution along with dry yeast extract powder and a dry powder diet consisting of …. as food source (Kim and Eckenrode, 1983). Organic Lima bean seeds were used to feed the larvae of seed corn maggots until they pupated. The flies that emerged from these pupae were released into the colony.

Methyl jasmonate seed treatment of corn seeds: Corn seeds were treated either by soaking them overnight in MeJA solution or MeJA was dry coated onto the seeds using the coumaryl-based seed coating matrix L-650. For the wet soaking method, two hundred corn seeds were soaked in 150ml of 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM MeJA solution overnight (for 14 hrs). The control seeds were soaked in water. The detergent Tween-20 was added to both the water-treated controls and MeJA solutions at the concentration of 45 parts per billion as a surfactant. For the dry treatment, corn seeds were coated with 1% (v/w) of coating matrix. For 100 grams of corn seeds, 1ml of coating matrix was used which was composed of 100ul of L650 and 900ul of water or water+MeJA. After treated with the coating matrix, the seeds were mixed in (get machine model and specifications from Alan) for (get time from Alan) mins. These seeds were then used for insect bioassays. The amount of MeJA needed to coat the seeds were determined by calculating the equivalent amount of MeJA that is absorbed by the corn seeds when soaked in a 0.2mM, 0.4mM or 0.8mM MeJA solution respectively overnight. For the corn variety we used, 1g of corn seed absorbed 0.2895 gram of water overnight. Therefore, in a 0.2mM MeJA solution, the corn seeds would absorb 12.98 micrograms of MeJA. Based on the density of MeJA (0.998g/mL), we used 13.00nl of MeJA per gram of corn seed to dry coat the seeds. For our experiments, we coated 1000 seeds (~220 grams of corn seeds) with 0.2mM, 0.4mM and 0.8mM equivalent amounts of MeJA and the amount of MeJA, L650 and water used to coat the seeds are summarized in Table 1. The number of seeds that germinated each day was measured for 7 days for seeds that were soaked in MeJA solution. The total number of seeds that germinated after 7 days was measured in MeJA dry coated. The height of seedlings emerging from both seeds that were soaked in MeJA and dry coated with MeJA were measured after 14 days of sowing.

Adult oviposition assays: For oviposition bioassays, 20 corn seeds that were treated with MeJA (overnight soaked or dry coated) or untreated control seeds were placed on sand in 8oz cups. The sand was kept moist by threading a cotton wick into the cup with sand that was wetted with water from a cup below it as demonstrated in the supplemental Figure S1. For two-choice oviposition assays in the lab, thirty male and female flies were selected from the lab colony that were the same age and were at least two weeks post eclosion. The flies were then released in 1X1X1 ft plastic cages with two cups containing corn seeds treated with MeJA or control untreated seeds. For two-choice assays performed in the field, 58 cm X 28 cm mesh cage was placed above the two cups with seeds to thirty flies in each cage. After 5 days, the cups with seeds were taken out and the number of eggs deposited by the flies in each cup were counted. A total of 16 replicates were set up for the two-choice assays in the lab. The growth chamber was set at 25C with a 14hr:10hr light:dark cycle for the lab oviposition assays. For oviposition assays that were performed at low temperatures, the growth temperature was set at 15C for the light cycle and 5C for the dark cycle (14hr:10hrs – light:dark cycle). For the field assays, ten replicates each were set up in the first week of July 2023 and then again in the first week of September 2023.

For the no-choice oviposition assays in the lab, a similar set-up was used as the two-choice assays, except the flies were offered only one type of seed at a time, either MeJA treated or water-soaked seeds. Ten cages were set up for each seed treatment and after 5 days, the cages that received the MeJA treated seeds, were given water treated seeds and vice versa. The order of the seed treatment presented was randomized, with five cages receiving water-soaked seeds and five cages receiving MeJA treated at the start of the assays. To count the number of eggs deposited by the flies in each cup, the contents of the cup was thoroughly mixed in 30% glycerol solution and then set aside at room temperature for 30 mins. Thereafter, the clear glycerol solution containing the eggs were decanted and sieved through a 1um sieve and the number of eggs were counted.

Synchronized seedling growth stage bioassay: Because we found delayed germination in the seeds treated with methyl jasmonate using the soaking method, we checked whether this could have caused the increase in oviposition on the control seeds. It is possible that the flies could only oviposit on seeds once they begin to germinate, essentially increasing the window of time available to oviposition in the bioassays. We tested this by germinating corn seeds soaked with 0.2mM MeJA two days prior to control water-soaked seeds to synchronize their stage of germination. The two-choice bioassays were set up as before in the growth chamber at 25C with thirty flies in each cage. A total of 12 replicates were set up for this bioassay.

Larval performance bioassay: We measured the performance of seed corn maggot larvae on corn seeds treated with aqueous MeJA by letting first instar seed corn maggot larva feed on corn seeds and measuring the percentage of larva that became pupa. Ten first instar seed corn maggot larva that were two days old were placed in 8-ounce cups with 10 MeJA treated seeds or control untreated seeds. The number larva that developed into pupa was counted after two weeks.

Statistical analyses: We used the Anova function in R version 4.3.1 (R core team, Massachusetts, USA) and used means separation with Tukey test at p <0.05 to examine the effect of seed treatment on oviposition preference by adult flies in the two-choice assays. The seed treatment was used as the fixed effect in these analyses. We also used the same statistical test with seed treatment as fixed effect to determine if larval performance was different in seed corn maggots that fed on MeJA treated seeds or untreated control seeds. To examine the effects of seed treatment on the oviposition performance in the no-choice assay, we fit a generalized linear mixed effects model (GLMM) with the number of eggs in each oviposition cup as the response, seed treatment and treatment order as the fixed effects, and cage as the random effect. We used a negative binomial distribution with a logit link function to account for overdispersion. The model was fitted via the glmmtmb() function in the R “glmmTMB” package (Brooks et al.). We checked the model assumptions using quantile residuals generated from the function “simulateResiduals()” in the R “DHARMa” package (Hartig, 2022). We used the likelihood ratio test to assess predictor significance using the “Anova()” function in the R “car” package (Fox and Weisberg, 2019). All analyses were performed in R version 4.3.1 (R Core Team 2023).

**Discussion:**

The efficacy of any elicitor-based strategy to control an insect pest depends on developing the key parameters that are contextually relevant for that particular plant species and the insect pest. As the use of jasmonates and jasmonic acid as elicitor-based seed treatment to manage insect pests gains momentum, our work shows the potential for MeJA to induce resistance against seed corn maggot herbivory in corn. Our work here brings clarity on three key considerations for using methyl jasmonate as seed treatment to combat seed corn maggot herbivory. First, we show that a concentration as low as 0.2mM MeJA can be an optimal dose to treat corn seeds by either soaking seeds overnight or dry coating them with coumaryl-based seed coating matrix to induce host plant resistance without significantly affecting seed germination. Second, we show that seeds that are soaked with 0.2mM MeJA solution or dry coated with an equivalent amount of MeJA with a seed coating matrix are both equally effective in deterring adult flies from ovipositing on treated seeds. Third, the induction of host plant resistance can deter adult flies from oviposition at temperatures as low as 5-15°C. This is especially significant in this system since adult flies of seed corn maggots emerge in late spring when the temperatures in temperate corn growing regions tend to be cool (Poveda lab, personal communication). Therefore, the use of MeJA as a seed treatment has the potential to be a viable method for corn growers.

In different plant species, the dose of MeJA affects the trade-off between growth and resistance. For example, rice seeds treated with 2.5mM methyl jasmonate induce resistance against rice weevil while maintaining growth, and tomato seeds treated with a 0.05 - 1 mM dose of methyl jasmonate suppress tomato fruit worm larval performance while maintaining growth and germination (Paudel et al., 2014). Worral et al. (2012) showed that performance of spider mites, *Manduca sexta* and *Myzus persicae* on tomato plants was reduced when seeds were treated with 3mM methyl jasmonate. While the root length was shorter in seedlings emerging from 3mM MeJA treated seeds compared to untreated controls, there was no long-term effect on plant height and fruit weight (Worrall et al., 2012). In our system, corn seeds treated with any concentration of MeJA between 0.2mM to 0.8 mM using the wet or dry method showed no difference in percent germination 5 days after sowing. The daily germination in the wet treatment showed some delays, but converged by day 5. This delay in germination may have caused the reduction in plant height seen at 14 days in the wet treatment; however, in the dry treatment we do not see any differences in plant height. Therefore, the costs of the dry treatment method appeared less than the aqueous method. While the bioassays in this study focused on the 0.2 and 0.4 mM MeJA treatment, the low costs of growth even at higher concentrations may allow for higher doses in the field. Taken together, it is clear that the dose of MeJA needed to treat seeds is unique to each plant species but that it is possible to find doses of MeJA that can induce resistance without incurring a high growth cost.

Prior research has shown that metabolic activity of the germinating seeds drives adult preference. Weston and Miller (1989) found that the *Delia platura* adults preferred to lay eggs on germinating lima bean seedlings over surrogate artificial seedlings, suggesting that the flies do not need visual cues from the germinating seeds but are attracted to other chemical stimulus from the germinating seeds (Weston and Miller, 1989). MeJA seed treatment is known to affect volatile emissions from plants emerging from treated seeds (Smart et al., 2013; Strapasson et al., 2014). Volatile compounds from MeJA treated lupin seeds deter ovipositionby *Delia platura* (Erazo-Garcia et al., 2021). Volatiles could play a role in oviposition decisions on corn seeds. Our results with stage-matched seeds show that the precise developmental stage in the days immediately after germination is not the determinant of where eggs are laid, also suggesting that it is chemosensory information from the MeJA treated corn seeds that causes reduced oviposition. Although larvae performance could have been affected by host volatiles, MeJA could also affect other seed and seedling traits. For example, in lupin, MeJA seed treatment induces expression of genes involved in jasmonate biosynthesis, including lipoxygenase and allene oxide synthase, as well as terpene synthesis and the antioxidant pathway in the embryonic axis (Erazo-Garcia et al., 2021). Methyl jasmonate may affect many plant traits that contribute to resistance to seed corn maggot.

We expected the effects of MeJA treatment on plant growth and induction to be temperature-dependent. Foliar induction of the jasmonate pathway can be enhanced at warmer temperatures (Havko et al., 2020). Tomato seedlings did not respond to wounding at temperatures below 20 °C (Green and Ryan, 1973). However, we found little work on the ability of plants to induce responses at cool temperatures (but see Whalen and Harmon, 2015), and no past work on seed induction. In our study, the seeds were held at a constant cool temperature for the duration of the assay. The effects of MeJA treatment on seed germination and seedling growth appeared to be stronger at the low temperature compared to our room temperature assays, although these experiments were conducted at separate times and so not directly comparable. It is possible that in a field assay, seasonal warming would ameliorate the germination and growth affects. In addition, recent studies show potential additional benefits of treating seeds with MeJA such as increased tolerance to cold in wheat (Repkina et al., 2021) and drought tolerance in corn and rice (Sheteiwy et al., 2018; Tayyab et al., 2020). Therefore, the costs and benefits of MeJA treatment are multifaceted and need to be assessed in field environmental conditions.

In conclusion, there is promise in the approach of developing jasmonate seed treatments that increase resistance to seed corn maggot adults and larvae with small or negligible effects on germination and growth in the lab. In our experiments, the MeJA dry seed treatment appears to be especially promising. Further experiments in the field will help validate the efficacy of this novel seed treatment approach to reducing pest damage.

**Acknowledgements:**

We would like to acknowledge Avni Bhalla, Michaela Fitzgerald, Natalie Brennan, Emily Cavanaugh, Laura Martinez, Jules Davis, Moshe Zaghouri, Arturo, Alexa, Chole Zhao, Masi, NY IPM (Alejandro, Ken, Jacob) and New York Ag & Markets for funding.

**Figure Legends:**

**Figure 1.** Germination rate and seedling height of corn seedlings post seed treatment with methyl jasmonate (MeJA). The seed germination rate of corn seeds soaked in 0.2-10mM MeJA solution and water (Mock) was measured for 7 days (a) and the percentage of germinated seeds dry-coated with MeJA was measured after 7 days (c). The height of seedlings emerging from both seeds treated with MeJA using the wet soaking method (b) and dry-coating method (d) was measured 14 days after sowing. One-way ANOVA was performed to measure significant difference among treatments at p=0.05. Means that are different from each other are denoted by different letters. Error bars indicate standard error of the mean.

**Figure 2.** Oviposition choice assays of adult flies of *Delia platura* on seeds treated with MeJA. The percentage of eggs deposited by adult flies of seed corn maggots in two-choice assays with water-soaked (mock) and 0.2mM MeJA soaked corn seeds were performed in the laboratory (a) and in the field (b). Two-choice oviposition assays were also performed with corn seeds treated dry coated with the seed coating matrix only (Mock) or 0.2mM MeJA equivalent (c) and 0.4mM MeJA equivalent (d). Total number of eggs deposited by adult flies on water-soaked (Mock) and 0.2mM MeJA soaked corn seeds were also measured in no-choice bioassays (e). The percentage of eggs deposited on seeds that were soaked in water or 0.2mM MeJA and were matched for growth stage was also measured (f). One-way ANOVA was performed to measure significant difference among treatments for the two-choice bioassays and the data from no-choice bioassay was fit into a generalized linear mixed model at p=0.05. Means that are different from each other are denoted by different letters. Error bars indicate standard error around the mean.

**Figure 3.** Larval performance of *Delia platura* on seeds treated with MeJA. The percentage of first instar seed corn maggot larva that matured to becoming pupa when fed on corn seeds soaked in water versus 0.2mM MeJA was measured after 14 days. One-way ANOVA was performed to measure significant difference among treatments. Means that are different from each other are denoted by different letters. Error bars indicate standard error around the mean.

**Figure 4.** Germination rate, seedling growth of corn and oviposition preference of *Delia platura* on corn seeds treated with MeJA at low temperature.The percentage germination of seeds (a) and height of seedlings (b) emerging from seeds soaked in water (Mock) or 0.2mM MeJA solution was measured 21 days after sowing in growth chambers at 15 °C daytime and 5 °C night temperature. Two-choice oviposition choice assays on seeds soaked in 0.2mM MeJA or water was also measured at these low temperatures (c). One-way ANOVA was performed to measure significant difference among treatments for the two-choice bioassays, seed germination rate and seedling height at p=0.05. Means that are different from each other are denoted by different letters. Error bars indicate standard error around the mean.

**Table 1: Calculation of the amount of MeJA used to coat corn seeds using L-650**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration of MeJA | Wt of MeJA (mg) | Vol of MeJA (uL) | Vol of L-650 (uL) | Vol of Water (uL) |
| 0mM (film coat control) | 0 | 0 | 220 | 1980 |
| 0.2mM | 220\*12.98 = 2855.6ug=2.855 | 2.86 | 220 | 1977.14 |
| 0.4mM | 220\*12.98\*2 = 5.71 | 5.72 | 220 | 1974.28 |
| 0.8mM | 220\*12.98\*4= 11.42 | 11.44 | 220 | 1968.56 |

**References:**

**Bhavanam S, Stout M** (2021) Seed treatment with jasmonic acid and methyl jasmonate induces resistance to insects but reduces plant growth and yield in rice, Oryza sativa. Front Plant Sci 12:

**Black BC, Cheng L, Donovan S, Taylor AG, Yang D** (2018) Relationships between compound lipophilicity on seed coat permeability and embryo uptake by soybean and corn. Seed Sci Res **28**: 229–235

**Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Machler M, Bolker BM** glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J **9**: 400

**Erazo-Garcia MP, Sotelo-Proaño AR, Ramirez-Villacis DX, Garcés-Carrera S, Leon-Reyes A** (2021) Methyl jasmonate-induced resistance to Delia platura (Diptera: Anthomyiidae) in Lupinus mutabilis. Pest Manag Sci **77**: 5382–5395

**Fox J, Weisberg S** (2019) An R Companion to Applied Regression. SAGE Publications

**Gill HK, Goyal G, Gillett-Kaufman JL** (2013) Seedcorn maggot, Delia platura (Meigen) (Insecta: Diptera: Anthomyiidae).

**Green TR, Ryan CA** (1973) Wound-induced Proteinase Inhibitor in Tomato Leaves: Some Effects of Light and Temperature on the Wound Response 1. Plant Physiol **51**: 19–21

**Grout Travis A.and Koenig PA and KJK and MSH** Neonicotinoid insecticides in New York state: economic benefits and risk to pollinators. **35**: 18–22

**Guerra PC, Keil CB, Stevenson PC, Mina D, Samaniego S, Peralta E, Mazon N, Chancellor TCB** (2017) Larval Performance and Adult Attraction of Delia platura (Diptera: Anthomyiidae) in a Native and an Introduced Crop. J Econ Entomol **110**: 186–191

**Hartig F** (2022) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html,

**Havko NE, Das MR, McClain AM, Kapali G, Sharkey TD, Howe GA** (2020a) Insect herbivory antagonizes leaf cooling responses to elevated temperature in tomato. Proceedings of the National Academy of Sciences **117**: 2211–2217

**Havko NE, Kapali G, Das MR, Howe GA** (2020b) Stimulation of Insect Herbivory by Elevated Temperature Outweighs Protection by the Jasmonate Pathway. Plants. doi: 10.3390/plants9020172

**Howe G, Jander G** (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol **59**: 41–66

**Kim TH, Eckenrode CJ** (1983) Establishment of a laboratory colony and mass rearing of Delia florilega (Diptera: Anthomyiidae). J Econ Entomol **76**: 1467–1469

**Kraus EC, Stout MJ** (2019) Seed treatment using methyl jasmonate induces resistance to rice water weevil but reduces plant growth in rice. PLoS One **14**: e0222800-

**Li Y, Miao R, Khanna M** (2020) Neonicotinoids and decline in bird biodiversity in the United States. Nat Sustain **3**: 1027–1035

**Main AR, Webb EB, Goyne KW, Mengel D** (2018) Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis. Ecological Applications **28**: 1232–1244

**Painter RH** (1951) Insect resistance in crop plants. Soil Sci 72:

**Paudel S, Rajotte EG, Felton GW** (2014) Benefits and costs of tomato seed treatment with plant defense elicitors for insect resistance. Arthropod Plant Interact **8**: 539–545

**Repkina N, Ignatenko A, Holoptseva E, MiszalskI Z, Kaszycki P, Talanova V** (2021) Exogenous methyl jasmonate improves cold tolerance with parallel induction of two cold-regulated (COR) genes expression in Triticum aestivum L. Plants. doi: 10.3390/plants10071421

**Sheteiwy MS, Gong D, Gao Y, Pan R, Hu J, Guan Y** (2018) Priming with methyl jasmonate alleviates polyethylene glycol-induced osmotic stress in rice seeds by regulating the seed metabolic profile. Environ Exp Bot **153**: 236–248

**van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP** (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr Opin Environ Sustain **5**: 293–305

**Smart LE, Martin JL, Limpalaër M, Bruce TJA, Pickett JA** (2013) Responses of Herbivore and Predatory Mites to Tomato Plants Exposed to Jasmonic Acid Seed Treatment. J Chem Ecol **39**: 1297–1300

**Stout MJ** (2013) Reevaluating the conceptual framework for applied research on host-plant resistance. Insect Sci **20**: 263–272

**Strapasson P, Pinto-Zevallos DM, Paudel S, Rajotte EG, Felton GW, Zarbin PHG** (2014) Enhancing Plant Resistance at the Seed Stage: Low Concentrations of Methyl Jasmonate Reduce the Performance of the Leaf Miner Tuta absoluta but do not Alter the Behavior of its Predator Chrysoperla externa. J Chem Ecol **40**: 1090–1098

**Tayyab N, Naz R, Yasmin H, Nosheen A, Keyani R, Sajjad M, Hassan MN, Roberts TH** (2020) Combined seed and foliar pre-treatments with exogenous methyl jasmonate and salicylic acid mitigate drought-induced stress in maize. PLoS One **15**: e0232269-

**Weston PA, Miller JR** (1989) Ovipositional Responses of Seedcorn Maggot, Delia platura (Diptera: Anthomyiidae), to Developmental Stages of Lima Bean. Ann Entomol Soc Am **82**: 387–392

**Whalen R, Harmon JP** (2015) Temperature alters the interaction between a herbivore and a resistant host plant. Arthropod Plant Interact **9**: 233–240

**Wilson RG, Orloff SB, Taylor AG** (2015) Evaluation of insecticides and application methods to protect onions from onion maggot, Delia antiqua, and seedcorn maggot, Delia platura, damage. Crop Protection **67**: 102–108

**Worrall D, Holroyd GH, Moore JP, Glowacz M, Croft P, Taylor JE, Paul ND, Roberts MR** (2012) Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. New Phytologist **193**: 770–778

**Yang D, Avelar SAG, Taylor AG** (2018) Systemic seed treatment uptake during imbibition by corn and soybean. Crop Sci **58**: 2063–2070

**Züst T, Agrawal AA** (2016) Mechanisms and evolution of plant resistance to aphids. Nat Plants. doi: 10.1038/nplants.2015.206