**Effects of methyl jasmonate seed treatments on adult oviposition preference and larval performance of seed corn maggots (*Delia platura*) in corn (*Zea mays*)**

**Running title (80 characters): Methyl jasmonate as seed treatments to combat seed corn maggot herbivory in corn**

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**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 (MeJA) 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 MeJA application methods: soaking seeds in aqueous MeJA and lab-scale, seed treatment using a commercial, film coating polymer mixed with MeJA. We conducted dose response experiments 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 conventional seed 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 conventional seed treatments compared to controls. Larval performance was also lower in MeJA treated seeds. Lastly, the MeJA induced resistance also occurred at the lower temperatures typical of the spring soil conditions when the fly is most damaging. In conclusion, MeJA application, conventional seed treatment technology, has the potential to deter adult fly oviposition and reduce maggot performance on a commercial scale in spring temperature conditions with minor effects on seed germination and growth.

**Introduction:**

While the use of jasmonic acid (JA) and methyl jasmonate (MeJA) 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 JA and its derivative 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 seed corn maggot (*Delia platura*)adults 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, *D. platura*, in corn. Open questions remain in terms of establishing a jasmonate dose that provides resistance with minimum costs 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.

The use of MeJA as a seed treatment can have a net positive effect on the plant growth and yield by reducing herbivory. Many studies have shown the benefits of induced resistance in decreasing the preference and performance of herbivorous insects (Painter, 1951; Stout, 2013), and increasing the dose can result in increased resistance. However, the benefits may level off at higher doses and eventually become disruptive and toxic to the plants. One of the negative side effects of jasmonate induced response can be through reduced seed germination which can delay 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 level that minimizes the 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 when the seeds are quiescent so that induction of host plant resistance occurs as the seeds become metabolically active after sowing during imbibition and 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 induce resistance against *D. platura* and do not affect seed germination or seedling growth (Erazo-Garcia et al., 2021). In rice, JA 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). The short-term negative effects on plant growth can therefore be offset in the long run with minimal or no effect on plant growth and crop yield when MeJA is used as seed treatments to combat insect herbivory.

Many studies on treating seeds with MeJA have been conducted using a method of applying the jasmonate by soaking the seeds in an aqueous solution. The soaking method has been used with both MeJA and JA, and which compound provides better resistance depends on the host plants (Bhavanan and Stout 2021). While preemptive soaking of seeds with MeJA to induce plant resistance can be relevant in planting crops such as rice fields, most field crop seeds are not hydrated and later dehydrated prior to sowing. Field corn seeds are commercially treated with the application of plant protectants for early season pest management. The plant protectants are mixed with a film coating polymer to achieve uniformity of application and adherence of seed treatment active ingredients to the seed surface, and commonly applied using rotary pan seed treatment technology (Afzal et al., 2020). The same commercial film coating polymer formulations applied with rotary pan technology can also be performed on a lab scale (Wilson et al. 2015).

There are many factors that determine if a chemical seed treatment can permeate through the seed coat and seed maternal covering layers and diffuse to the embryo. The primary physicochemical properties that determine the relative systemic uptake into seeds are molecular charge, lipophilicity, and molecular size, with molecular weight being < 500 (Yang et al., 2018b). Corn seeds were demonstrated to be permeable only to nonionic compounds, while ionic compounds are restricted by the pericarp-testa (Dias et al., 2014). Many species have this differential permeability to nonionic vs ionic compounds, termed selective seed coat permeability (Salanenka and Taylor, 2011). JA is an ionic compound and therefore would not be able to be taken up into corn seeds. In contrast, MeJA is a nonionic compound that can diffuse to the embryo based on its molecular charge. The second property is the lipophilicity measured by the partition coefficient of a compound between water and octanol, termed log Kow (Yang et al., 2018b). A similar log Kow of the organic compound and the seed coat permeability would have the greatest uptake potential of that compound. The log Kow of JA was 2.6 (<https://chemicalize.com>), while the optimum log Kow for uptake for the pericarp-testa of corn was 2.2 to 3.8 (Yang et al., 2018b). Collectively, the uptake of MeJA is in the optimal range for diffusion into 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 cool. Several studies have shown that induction of plant resistance is temperature dependent 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 perform 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 MeJA treated seeds.

The seed corn maggot (*D. 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 below-ground 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 maggot occurs in early spring after the adults emerge from diapause which coincides with planting season for corn in the temperate corn growing regions of the United States. The mean soil temperatures in early spring in such regions can be as low as 15-20°C. The larvae of seed corn maggot 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 MeJA as seed treatments to induce host plant resistance in corn to control seed corn maggot herbivory. Specifically, we 1) tested the effects of five different concentrations of MeJA on corn seed germination and seedling growth using a wet and a conventional seed treatment method, 2) conducted choice and no-choice oviposition preference assays with adult flies and measured the performance of the seed corn maggot larvae using the doses of the wet and conventional seed treatment methods that did not limit germination, and 3) tested the effects of MeJA seed treatment on germination, seedling growth, and adult oviposition preference at cool temperatures.

**Results:**

**Wet methyl jasmonate seed treatment slowed germination and early seedling growth but germination and growth were not affected by conventional seed treatments:**

We compared the rate of germination in corn seeds treated with 0.2 mM, 0.4 mM, 0.8 mM, 1m M and 10 mM of aqueous MeJA to control (water-soaked) seeds. Seeds treated with 10 mM MeJA showed less than 20% germination, whereas seeds treated with 0.2 mM, 0.4 mM, 0.8 mM and 1 mM MeJA had no difference in total germination at day 7 post treatment compared to controls (Fig. 1a). However, there was a delay in germination at day 3 and 4 post treatment with concentrations of 0.2 mM, 0.4m M, 0.8 mM and 1 mM compared to controls (Fig. 1a). Since we did not observe any difference in germination at day 7 after sowing with seeds soaked in MeJA for any concentration other than 10 mM MeJA, we measured germination in an endpoint assay at day 7 post sowing with seeds with conventional seed treatment with 0.2m M, 0.4 mM and 0.8 mM MeJA. The seeds treated with conventional seed treatment did not result in differences in seed germination at day 14 compared to seeds with a control coating (Fig. 1c).

The delay in the germination of water-soaked seeds treated with 0.2 mM, 0.4 mM, 0.8 mM and 1 mM of MeJA was reflected in reduced seedling height at day 14 (F3,20 = 27.34, p < 0.001; Fig. 1b). Seedlings emerging from seeds treated with 0.2 mM and 0.4 mM MeJA showed the least amount of growth reduction (~12-15%) when compared to water treated seeds, so we used 0.2 mM MeJA treated corn seeds to perform our subsequent bioassays. There was no difference in seedling growth using the conventional seed 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 adults flies laid ~60% fewer eggs on 0.2 mM 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 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). Similarly, in a two-choice assay using conventional seed treatment, we found that seeds that were treated with 0.2 mM or 0.4 mM levels of MeJA had ~20% fewer eggs deposited on them compared to water treated seeds (Fig. 2c and d). 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 MeJA treated seeds was lower compared to water-treated seeds (χ2 = 90.5, p < 0.001; Fig. 3a).

In our germination and growth bioassay, the water-soaked seeds germinated marginally faster than 0.2 mM MeJA soaked seeds, thus adding the possibility that earlier emergence may somehow interfere with oviposition preference by the adult flies in water-soaked seeds. Therefore, we also performed oviposition preference bioassays that were matched for developmental stage for these two treatments. In stage-matched seeds, we did not find increased oviposition on control seeds. Instead, we found increased oviposition on the MeJA treated seeds (Fig. 3b), 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 3c).

**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 = 0.0485). Three-week-old seedlings that emerged from seeds treated with MeJA were also ~25% shorter than the water-soaked seeds (Fig. 4b; F1,17 = 16.357, p < 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 = 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 this seed lot was not treated by the seed company. Adult flies and larvae of *D. platura* were collected from the corn fields in Tompkins County, 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 10 parts casein protein, 10 parts sucrose, 1 part brewer’s yeast and 1 part soy protein as food source (Kim and Eckenrode, 1983) (Rooney et al. 2024, in review . Organic Lima bean seeds were used to feed the larvae 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 by applying MeJA using lab-scale seed treatment equipment and a commercial film coating polymer, L-650. For the wet soaking method, two hundred corn seeds were soaked in 150 ml of 0.2 mM, 0.4 mM, 0.8 mM, 1 mM and 10 mM 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. Since soaking the corn seeds in 0.2 mM MeJA had a minimal effect on plant growth and no effect on germination rate, we used this concentration for our subsequent oviposition bioassays with the wet-soaking method. To simulate conventional seed treatment technology, we used a commercial, seed film coating polymer, L650 from Incotec, Urbandale, Iowa. For 100 grams of corn seeds, 1 ml of coating suspension was used which was composed of 100 ul of L650 and 900 ul of water or water MeJA. Seeds were treated in a Hege 11, seed treater (Wintersteiger, Salt Lake City, UT) for 0.5 mins, air dried overnight, and later used for insect bioassays. The amount of MeJA needed to coat the seeds was determined by calculating the equivalent amount of MeJA that is absorbed by the corn seeds when soaked in a 0.2 mM, 0.4 mM or 0.8 mM MeJA solution respectively overnight. For the corn variety we used, 1 g of corn seed absorbed 0.2895 gram of water overnight. Therefore, in a 0.2 mM MeJA solution, the corn seeds would absorb 12.98 micrograms of MeJA. Based on the density of MeJA (0.998 g/mL), we used 13.00 nl of MeJA per gram of corn seed to treat the seeds. For our experiments, we treated 1000 seeds (~220 grams of corn seeds) with 0.2 mM, 0.4 mM and 0.8 mM 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 seed treatment. The height of seedlings emerging from both seeds that were soaked in MeJA or treated 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 seed treatment) or untreated control seeds were placed on sand in 8-ounce 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 (see Supplemental Figure S1). For two-choice oviposition assays in the lab, 30 male and female flies were selected from the lab colony that were at the same age and were at least two weeks post eclosion. The flies were then released in 30 cm × 30 cm × 30 cm 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 × 28 cm mesh cage was placed above the two cups with seeds to thirty flies in each cage. After five days, the cups with seeds were taken out and the number of eggs deposited by the flies in each cup were counted and the percentage of eggs deposited on water-soaked or MeJA-soaked seeds was calculated for each cage. A total of 16 replicates were set up for the two-choice assays in the lab. For the field assays, 10 replicates each were set up in the first week of July 2023 and again in the first week of September 2023. The growth chamber was set at 25°C 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 15°C for the light cycle and 5°C for the dark cycle (14hr:10hrs light:dark cycle).

For the no-choice oviposition assays in the lab, a similar setup was used as the two-choice assays, except the flies were offered 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. Therefore, each cage containing thirty flies had the choice to oviposit on water-soaked seeds first and then on MeJA-soaked seeds or vice versa. The order of the –type presentation was randomized. The total number of eggs deposited in each cup was counted. To count the number of eggs deposited by the flies in each cup, the content 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 1 um sieve and the number of eggs were counted.

Synchronized seedling growth stage bioassay: Because we found delayed germination in the seeds treated with MeJA 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 0.2 mM 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 25°C with 30 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 larvae feed on corn seeds and measuring the percentage of larvae that pupated. Ten first instar seed corn maggot larvae that were two days old were placed in 8-ounce cups with 10 MeJA treated seeds or control untreated seeds. The number larvae that developed into pupae were counted after two weeks.

Statistical analyses:

We examined the effects of seed treatment on the percentage of germinated seeds and seedling height using a one-way ANOVA and performed the Tukey post-hoc test (α = 0.05) to compare between different MeJA concentrations. We examined the effects of seed treatment on oviposition preference by adult flies in the two-choice assays as well as the larval performance on MeJA treated and untreated seeds using a one-way ANOVA. To examine the effects of seed treatment on the oviposition preference in the no-choice assay, we fit a generalized linear mixed effects model with the number of eggs in each oviposition cup as the response, seed treatment and order in which each cage received either of the two treatments as the fixed effects, and cage as the random effect. A Poisson error distribution with a log link function was used in the model. The model was fitted via the glmmtmb() function in the R “glmmTMB” package (Brooks et al.). Predictor significance was assessed via the “Anova()” function in the R “car” package (Fox and Weisberg, 2019). All analyses were performed in R version 4.3.3 (R Core Team, 2024).

**Discussion:**

The efficacy of any elicitor-based strategy to control an insect pest depends on developing the key parameters that are contextually relevant for a specific 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 brings clarity on four key considerations for using MeJA as seed treatments to combat seed corn maggot herbivory. First, we show that a concentration as low as 0.2 mM MeJA can be an optimal dose to treat corn seeds by either soaking seeds overnight or by applying a conventional seed treatment with MeJA to induce host plant resistance without significantly affecting seed germination (Fig. 1a,c). Second, we show that seeds soaked with 0.2 mM MeJA solution or treated with a conventional seed treatment of an equivalent amount of MeJA with a seed coating matrix are both equally effective in deterring adult flies from ovipositing on treated seeds (Fig. 2 a-d). Third, the induction of host plant resistance can deter adult flies from oviposition at temperatures as low as 5-15°C (Fig. 4c). This is especially significant in this system since adult flies emerge in late spring when the temperatures in temperate corn growing regions tend to be cool (Poveda lab, personal communication). Fourth, an optimal dose of MeJA needs to be established for commercial varieties using conventional seed treatment application. Moreover, there may be varietal differences in dose response to MeJA. For example, a four-fold difference in seed uptake of a model nonionic compound was measured between two inbred lines (Yang et al., 2018a). Collectively, 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 2.5 mM MeJA induce resistance against rice weevil while maintaining growth, and tomato seeds treated with a 0.05 - 1 mM dose of MeJA 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 is reduced when seeds were treated with 3 mM MeJA. While the root length was shorter in seedlings emerging from 3 mM 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 0.2 mM to 0.8 mM using the wet or conventional seed treatment method showed no difference in percent germination 5 days after sowing. Although we did observe a delay in the daily germination in the wet treatment, the seedlings growth converged by day 5. This delay in germination may have caused the reduction in plant height seen at day 14 in the wet treatment. However, in the conventional seed treatments, we ddid not see any differences in plant height. Therefore, the costs of the conventional seed 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, the dose of MeJA needed to treat seeds is unique to each plant species, but 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 fly preference. Weston and Miller (1989) found that the adults prefer 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). Likewise, in corn most of the oviposition by *D. platura* happens before the seedlings have emerged from the soil. 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 *D. platura* (Erazo-Garcia et al., 2021). Volatiles may play a role in oviposition decisions on corn seeds. Our results with stage matched seeds suggest that the precise developmental stage in the days immediately after germination is not the determinant of where the eggs are laid. Rather, 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). MeJA 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, few studies have looked at the ability of plants and seeds to induce responses at cool temperatures (but see Whalen and Harmon, 2015). 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 therefore were not directly comparable. It is possible that in a field assay, seasonal warming would ameliorate the germination and growth effects. In addition, recent studies show potential additional benefits of treating seeds with MeJA such as increased cold tolerance 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 with respect to insect performance, longevity of treatment and long-term effects on growth and crop yield.

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 conventional seed treatment appears to be especially promising. Further experiments must be conducted in the field.

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**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 0.2-10 mM MeJA solution and water (control) was measured for 7 days (a) and the percentage of germinated seeds with conventional seed treatment 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 conventional seed treatment method (d) was measured 14 days after sowing. Means that are different from each other are denoted by different letters (Tukey test; *α* = 0.05). Error bars indicate standard errors around the means.

**Figure 2.** Oviposition choice assays of adult *Delia platura* on seeds treated with MeJA. The percentage of eggs deposited by adult flies in two-choice assays with water-soaked (control) and 0.2 mM 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 with MeJA using conventional seed treatment and the coating matrix only (control) or 0.2 mM MeJA equivalent (c) and 0.4 mM MeJA equivalent (d). Means that are different from each other are denoted by different letters (*α* = 0.05). Error bars indicate standard errors around the means.

**Figure 3.** (a)Total number of eggs deposited by adult flies on water-soaked (control) and 0.2 mM MeJA soaked corn seeds measured in no-choice bioassays. (b) The percentage of eggs deposited on seeds soaked in water or 0.2 mM MeJA and matched for growth stage was also measured in a two-choice oviposition assay. (c) Larval performance on seeds treated with MeJA. The percentage of first instar larvae that pupated when feeding on corn seeds soaked in water versus 0.2 mM MeJA was measured after 14 days. Means that are different from each other are denoted by different letters (*α* = 0.05). Error bars indicate standard errors around the means.

**Figure 4.** Germination rate, seedling growth of corn and oviposition preference of adult flies on corn seeds treated with MeJA at the low temperature.The percentage of germinated seeds (a) and height of seedlings (b) emerging from seeds soaked in water (control) or 0.2 mM MeJA solution was measured 21 days after sowing in growth chambers at 15°C daytime and 5°C nighttime temperature. Two-choice oviposition choice assays on seeds soaked in 0.2 mM MeJA or water was also measured at these low temperatures (c). Means that are different from each other are denoted by different letters (*α* = 0.05). Error bars indicate standard errors around the means.

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration of MeJA | Weight of MeJA (mg) | Volume of MeJA (ul) | Volume of L-650 (ul) | Volume of water (ul) |
| 0 mM (film coat control) | 0 | 0 | 220 | 1980 |
| 0.2 mM | 220\*12.98 = 2855.6 | 2.86 | 220 | 1977.14 |
| 0.4 mM | 220\*12.98\*2 = 5.71 | 5.72 | 220 | 1974.28 |
| 0.8 mM | 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

**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

**R Core Team** (2024). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

**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