Joint Research Report To:

OARDC Industry Small Grants &
Ohio Vegetable and Small Fruits Research and Development Program

Reducing insecticide use in cucurbits through in-furrow seed specific applications

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Background and Justification:
Researchers working on pesticide delivery systems continue to reduce the amount of pesticide in the environment by emphasizing precision placement, while maintaining or improving efficacy. With over 6,000 acres of pumpkin, zucchini, and cucumber grown in Ohio, cucurbits represent an economically important group of crops to test a novel precision in-furrow insecticide injector. Two critical factors supporting this project include in-row seed spacing up to 36 inches and a highly effective yet expensive systemic insecticide that provides early season insect control. This project was divided into four major phases beginning with the injector design then moving onto a series of field validation trials.

Phase 1: Design and construction of the Seed Specific In-furrow Insecticide Injector (SSIII)
Four agricultural engineers of the research group undertook the design and construction of the SSIII. The goal was to create an in-furrow applicator that meters out insecticide in discrete bands around each seed planted. This new applicator would eliminate the conventional practice of continuously applying insecticide in-furrow regardless of seed density, generating a significant savings of insecticide and cost to the grower. The injector operates according to the following description.

As the seed leaves the metering box on the planter it falls through a standard seed delivery tube. This tube is identical to any seed tube found on a conventional corn planter. Inside the seed tube is an optical sensor that detects the seed falling towards the soil surface. In a conventional planting system, this sensor would send a signal to the planter monitor to indicate that another seed has been planted. In this application, the sensor signal is directed to the injector controller, which initiates the insecticide application process. The injector begins delivering material (insecticide and water solution) as a stream directed at the bottom of the seed furrow before the seed actually reaches the soil surface. This is intentional so that the seed falls within the middle of the insecticide band.

Basic physics were employed to determine the "on time" of the injector and thus the amount of solution applied per seed. A target volume per seed application rate can be transferred electronically from the cab monitor to the row controller. The injector was calibrated for a given nozzle flow rate when operating at a steady system pressure of 10 pounds per square inch. By utilizing the calibration information, the row controller was quickly able to determine the injector "on time" required to deliver the exact amount of material per seed.

A solenoid valve was used to actually turn the flow of material on and off. Solenoid valves are not uncommon in agricultural applications, but this particular valve was miniaturized so that it could fit unobtrusively between the opening disks of the planter and quickly turn the flow of liquid on and off. At a mid-range application rate (5 milliliters / injection), the solenoid valve would be turned on for approximately 80 milliseconds to deliver the appropriate amount of material.

In summary, here is a verbal flow chart of how the system operates:

- Seed is dropped from the metering unit
- Seed passes into sensor window and sensor output is triggered
- Row controller notices the sensor has changed states and turns on the valve
- Row controller leaves the valve on for a predetermined amount of time
- Row controller turns the valve off and waits for the next seed to pass through

One aspect of this project not accounted for in the original design and construction was the complexity involved in controlling the length of the band regardless of ground speed. Currently the unit can inject a known volume of material from1-10 milliliters, but the length of this band in-furrow varies according to speed. For any given volume, the length of the band shortens at slower speeds and increases at faster speeds. This fact was known early in the design phase of the injector and is based solely on cost limitations associated with the type of components and electronics purchased. If additional funding becomes available, the ability to control the length of the band regardless of ground speed will be addressed.

Phase 2: Evaluation of SSIII band synchronicity with seed
Laboratory tests confirm that seed dropped through the seed tube triggered a burst injection of material. It was paramount to the success of the entire project that this injection band be synchronized with the seed drop under field conditions. The remaining phases of this project were conducted at the OARDC West Agricultural Research Station in South Charleston, Ohio.

Pumpkin, zucchini, and cucumber seeds were tested in this phase. Because these seeds vary considerably in size and weight, each type was synchronized and evaluated at planting speeds of 1.4, 2.0, and 2.8 mph. There were four replications of each treatment and each plot was 150 feet long. All seeds regardless of type were dropped at a spacing of 12-14 inches.

To determine the synchronicity between the injected bands with the seed, each variety was "planted" on the soil surface and "banded" using only water to determine how accurately the band overlaid the seed (Table 1). The “band” is actually a single stream of liquid directed at the bottom of the seed furrow. To plant on the soil surface, the planter’s press wheels were removed and the planter depth adjusted to drop seeds in a 0.5 inch shallow furrow. The tractor speed was set at the beginning and maintained throughout each plot. The seedbox and SSIII were engaged at the beginning of each plot.

There were 100 seed / band events recorded per plot. Only singulated seeds were counted, any double seed drops were excluded from the data. If seed was planted, e.g., dropped within the water band it was considered "in the band". If seed was planted, e.g., dropped within 2 inches of the band (front or back) it was considered a bounce, meaning the seed initially landed in the band, then bounced out. If a seed landed farther than 2 inches from the water band it was considered a miss. Out of 3600 total seed and band overlaps, only 69 cucumber, 42 zucchini, and 30 pumpkin seeds were recorded as misses. For each trial, the injection band contained 3 milliliters of water. The water bands were 4" long at 1.4 mph, 5" long at 2 mph, and 6" long at 2.8 mph. The percent synchronization between the seed and the water band or band plus bounce were not significantly different at the three speeds or by seed type. Combining all cucurbit data showed a significantly higher percentage of seeds banded at the slowest speed compared to the fastest speed.

Table 1. Seed and water band synchronization based on the means of four 100 seed count replicates. Treatments followed by same letter in column are not significantly different from each other.

	% Water Band Synchronization with Seed							
Seed Type	**Pumpkin**		**Zucchini**		**Cucumber**		**All Cucurbits**	
Planting speed	**Band**	**Band + Bounce**	**Band**	**Band + Bounce**	**Band**	**Band + Bounce**	**Band**	**Band + Bounce**
1.4 mph	97.0 a	98.3 a	96.0 a	98.3 a	91.8 a	95.0 a	94.9 a	97.2 a
2.0 mph	94.0 a	97.3 a	97.3 a	98.0 a	93.8 a	94.0 a	95.0 a	96.4 ab
2.8 mph	93.8 a	97.0 a	89.5 b	93.3 a	92.5 a	93.8 a	91.9 b	94.7 b

Phase 3: Bioassay studies with striped cucumber beetle on treated cucurbit leaf tissue
The previous study estimated the percent synchronicity between seeds and in-furrow bands produced by the SSIII at different speeds. Since these studies were performed on top of the ground, it was necessary to reproduce the study at a normal planting depth beneath the soil. Because the band and seed would be buried, visual confirmation of the overlap was impossible. It was necessary to develop another way to determine if the in-furrow bands were still synchronized with the seed drop.

The material used for this bioassay study was Admire 4F (imidacloprid), a systemic insecticide. Three different rates were tested for efficacy, a low label rate using SSIII (16 oz/A), a high label rate using SSIII (24 oz/A), a standard continuous in-furrow 24 oz/A rate, and a water only check. In addition to the high and low rates, row spacing was factored in as well. For example, pumpkin row spacing at the high rate was calculated at 15' and for the low rate was calculated at 5'. This same bracketing technique was used on zucchini at row spacing of 6' and 4' at the high and low rate, and cucumber at row spacing of 6' and 3' for the high and low rate. These treatments effectively bracketed the highest and lowest rates growers would reasonably use on these crops. Because row spacing and amount of carrier (gallons per acre) can greatly affect the concentration of the active ingredient, parts per million (PPM) equivalents have been calculated for each treatment for comparison. There were four replicates of each treatment, arranged randomly in the field for each crop.

Seeds for all bioassay studies were planted at 2 mph, resulting in a 5" band. The pumpkin plots were 150' long and seeded on June 10th at a density of 16" and thinned to every 32". Both the cucumber and zucchini plots were 75' long and seeded on June 16th at 12" and 15" respectively and were not thinned. Development stages were determined when 50% or more of the seedlings were in that stage.

Bioassays were conducted on pumpkin, zucchini, and cucumber seedlings at cotyledon, first true leaf, and second true leaf stages. Only zucchini seedlings were bioassayed at the 5th leaf stage. As each seedling progressed from cotyledon to first leaf, etc., the most recent developmental stage was clipped from the plant and placed in a clear 5 ounce plastic container. The dissected plant was then destroyed to prevent further sampling. The plant clippings were set inside a portable cooler to prevent wilting from the sun. Within 30 minutes, the freshly clipped tissue samples were transported inside a nearby building to begin the bioassay with the addition of one live striped cucumber beetle (StrCB) per container.

The StrCB were collected at the Western Agricultural Research Station from three areas approximately 30' x 100' seeded in early May with a mixture of pumpkin and squash to serve as cucumber beetle reservoirs for the bioassay experiments. No pesticides of any kind were used in these plantings. Striped cucumber beetles were aspirated off of foliage and out of flowers in the morning and placed 10 per vial. Once enough beetles had been collected to supply one bioassay experiment, they were placed in a refrigerator at 45° F to slow their physical activity. The beetles were used in bioassay experiments within a three hour period of being field collected.

The beetles were removed from the refrigerator and placed individually into each dish that already contained a recently cut seedling stage, then sealed with the lid. The containers were stored at room temperature, approximately 75° F for the duration of the bioassay. There were five bioassay tissue samples taken from each plot for a total of 90 samples for each seedling stage. Beetle mortality was recorded once every 24 hour period, for a duration of 72 hours. Seeds treated with Admire by either SSIII or continuous application were expected to uptake the nearby systemic insecticide via the roots, leading to increased StrCB mortality compared to seeds banded with plain water.

In each bioassay dish, dead StrCB were easily discernable from live StrCB. In 9 out of 10 bioassay trials, a portion of the StrCB that appeared dead in one inspection seemed to revive at a later inspection. Most of the revived beetles were only able to lie on their backs and slowly move their legs in a morbid state. Although the beetles were not absolutely dead, because they could not move, feed, or mate, they were categorized as functionally dead for the purposes of this study. Tukey's HSD was used to separate the percent mean mortalities between the treatments (Tables 2, 3, 4).

The concentration of Admire in each treatment (high, continuous flow, low) varied based on row width (which affects rate per 1000 feet of row) and the amount of carrier (water) per injection. Admire concentrations in the treatments ranged from 1070 to 7816 parts per million (Table 5). All ten bioassay trials showed significant differences between the water only check and other three treatments. This is an indication the treatments were successfully banded over the seed at a range of concentrations effective against the StrCB. Mortality at the "high" and "continuous flow" treatment levels was consistently higher than that of the "low" treatment. Seven of the ten bioassay trials showed increasing mortality over time. Four of those seven bioassays showed significantly higher mortality over time. The three remaining bioassays showed decreased mortality in the 48-72 hour bioassays, an artifact of the revived but morbid beetles.

Table 2. Mean differences in mortality of striped cucumber beetles bioassayed with cucumber plant stages using Tukey's HSD. Treatments followed by same letter in column are not significantly different from each other.

Stage	Bioassay Length	% Mean Mortality	P-value	Treatments	% Mean Mortality	P-value
cotyledon	18 hrs	72.5 A	0.979	Check	16.7 A	< 0.0001
	48 hrs	73.8 A		Low	86.7 B	
	72 hrs	73.8 A		High	90.0 B	
				Con't. Flow	100.0 B	
1st leaf	24hrs	53.8 A	0.045	Check	23.3 A	< 0.0001
	48 hrs	65.0 A B		Low	66.7 B	
	72 hrs	75.0 B		High	78.3 B	
				Con't. Flow	90.0 B	
2nd leaf	24 hrs	48.8 A	0.204	Check	6.7 A	< 0.0001
	48 hrs	57.5 A		Low	58.3 B	
	72 hrs	60.0 A		High	66.7 B	
				Con't. Flow	90.0 C	

Table 3. Mean differences in mortality of striped cucumber beetles bioassayed with pumpkin plant stages using Tukey's HSD. Treatments followed by same letter in column are not significantly different from each other.

Stage	Bioassay Length	% Mean Mortality	P-value	Treatments	% Mean Mortality	P-value
cotyledon	24 hrs	35.0 A	0.433	Check	1.7 A	< 0.0001
	72 hrs	37.5 A		Low	41.7 B	
	48 hrs	42.5 A		Con't. Flow	41.7 B	
				High	68.3 C	
1st leaf	24 hrs	30.0 A	0.023	Check	0.0 A	< 0.0001
	48 hrs	36.9 A B		Low	25.0 B	
	72 hrs	48.1 B		Con't. Flow	48.8 C	
				High	79.6 D	
2nd leaf	24 hrs	6.3 A	0.048	Check	3.3 A	0.003
	48 hrs	16.3 A B		Low	6.7 A B	
	72 hrs	20.0 B		High	21.7 B C	
				Con't. Flow	25.0 C	

Table 4. Mean differences in mortality of striped cucumber beetles bioassayed with zucchini plant stages using Tukey's HSD. Treatments followed by same letter in column are not significantly different from each other.

Stage	Bioassay Length	% Mean Mortality		P-value	Treatments	% Mean Mortality		P-value
cotyledon	24 hrs	21.3	A	< 0.0001	Check	5.0	A	< 0.0001
	48 hrs	70.0	B		High	70.0	B	
	68 hrs	72.5	B		Con't. Flow	70.0	B	
					Low	73.3	B	
1st leaf	72 hrs	52.2	A	0.658	Check	0.0	A	< 0.0001
	18 hrs	55.0	A		Low	55.0	B	
	48 hrs	57.5	A		Con't. Flow	77.9	C	
					High	86.7	C	
2nd leaf	48 hrs	41.9	A	0.012	Check	5.0	A	< 0.0001
	72 hrs	45.6	A B		Low	53.3	B	
	24 hrs	61.3	B		High	58.3	B	
					Con't. Flow	81.7	C	
5th leaf	24 hrs	15.0	A	0.240	Check	3.3	A	0.017
	48 hrs	27.5	A		Con't. Flow	28.3	A B	
	72 hrs	27.5	A		High	30.0	B	
					Low	31.7	B	

Table 5. Usage rates, PPM, and cost per acre for Admire ($4.30 / oz.) based on a seed specific in-furrow insecticide injection (SSIII) rate of 3 milliliter compared to continuous flow application.

	Rate/A (oz)	Row Width (ft)	Admire / 5" band (mls)	Seed Spacing (ft)	Total H2O (mls)	Total Admire (mls)	PPM Soln	Cost / A
Pumpkin[1]	24	15	0.10184	2.7	3,227	109.5	7,816	$ 15.93
Cucumber[1]	24	6	0.04073	1	21,780	295.7	3,197	$ 43.00
Zucchini[1]	24	6	0.04073	1.25	17,424	236.6	3,197	$ 34.40
Pumpkin[2]	16	5	0.02263	2.7	9,680	73.0	1,788	$ 10.62
Cucumber[2]	16	3	0.01358	1	43,560	197.2	1,076	$ 28.67
Zucchini[2]	16	4	0.01810	1.25	26,136	157.7	1,433	$ 22.93
Pumpkin[3]	24	5	0.03395	2.7	65,192	709.8	2,571	$103.20
Cucumber[3]	24	5	0.03395	1	65,192	709.8	2,571	$103.20
Zucchini[3]	24	5	0.03395	1.25	65,192	709.8	2,571	$103.20

[1] SSIII high rate of Admire, [2] SSIII low rate of Admire, [3] Continuous flow rate of Admire

Phase 4: Harvest data

Yield data were taken from the plants remaining after the bioassay trials for both pumpkin and zucchini. The initial cucumber bioassay trial had extremely poor germination and emergence (ca. 20%), barely enough to supply plant tissue for the bioassays. Consequently, this trial was replanted after the bioassay data were taken. Germination of the second planting was still below normal at ca. 60%.

MarketMore 86 was the cucumber variety chosen for this study. All fresh market cucumbers greater than 5" were harvested on September 2nd and 14th from a 50' section of row per plot, with individual length and total plot weight taken for each treatment (Table 6).

The first, second, and combined harvest data for average plot weight were significantly different from the check. The high and continuous flow treatments had significantly more fruit than the check in the first, second, and combined harvest data trials.

Table 6. Cucumber yield data taken on September 2nd and 14th. Treatments followed by same letter in column are not significantly different from each other.

	Treatments	**Avg. Plot Wt. (KG)**		**Avg. No. Fruit / Plot**	
Harvest 1	CHECK	19.8	A	45.0	A
	Con't. Flow	43.9	B	97.0	B
	High	40.6	B	84.0	B
	Low	29.8	AB	63.5	AB
Harvest 2	CHECK	12.6	A	45.0	A
	Con't. Flow	24.6	B	91.5	B
	High	23.4	AB	87.5	B
	Low	17.1	AB	57.3	AB
All Harvests	CHECK	16.2	A	45.0	A
	Con't. Flow	34.2	B	94.3	B
	High	32.0	B	85.8	B
	Low	23.4	AB	60.4	A

Magic Lantern pumpkin was the variety chosen for this study. Pumpkin harvest began after 90% of fruit turned orange. The harvest was split over two days, September 15th and 16th. Every fruit from a centered 75' section of row per plot was clipped, weighed, and had its color recorded (Table 7). No stand counts were taken in the pumpkin trial because seedling stands were hand thinned to approximately 3' spacing in each plot. There were no significant differences between the average number of fruit per treatment, average treatment weight, or average weight per fruit between any of the treatments.

Table 7. Pumpkin yield data based on split harvest, September 15th and 16th. Treatments followed by same letter in column are not significantly different from each other.

Treatments	**Estimated Tons / A**	**Mean Fruit / Trt.**	**Mean Trt. Wt. (Kg)**	**Mean Wt. / Fruit (Kg)**
Low	29.9	41.8 a	234.0 a	5.6 a
CHECK	25.8	35.5 a	201.9 a	5.7 a
High	26.8	36.3 a	210.1 a	5.8 a
Con't. Flow	28.0	37.0 a	219.3 a	5.9 a

Spineless Beauty was the zucchini variety chosen for this study. There were 5 zucchini harvests between July 26th and August 13th. All zucchini's greater than 5" were harvested from a 50' section of row per plot, with individual length and total plot weight taken (Table 8). There were no statistical differences in seedling stand counts between treatments.

For the first, second, and third harvest, the average plot weights were significantly different from the check. The first and fifth harvest averaged a significantly higher number of fruit per plot compared to the check.

Table 8. Zucchini yield data based on five harvests between July 26th and August 13th. Treatments followed by same letter in column are not significantly different from each other.

	Treatment	**Avg. Plot Wt. (KG)**		**Avg. No. Fruit / Plot**	
Harvest 1	CHECK	0.7	A	2.3	A
	Con't. Flow	4.0	B	9.0	B
	High	3.5	B	7.8	AB
	Low	4.1	B	8.5	AB
Harvest 2	CHECK	1.4	A	6.5	A
	Con't. Flow	3.3	B	11.5	A
	High	3.8	B	12.0	A
	Low	3.3	B	13.8	A
Harvest 3	CHECK	12.2	A	22.5	A
	Con't. Flow	18.5	AB	34.0	A
	High	19.4	AB	31.8	A
	Low	23.5	B	37.0	A
Harvest 4	CHECK	6.1	A	20.3	A
	Con't. Flow	6.7	A	25.8	A
	High	6.5	A	22.0	A
	Low	6.0	A	21.0	A
Harvest 5	CHECK	7.9	A	21.8	A
	Con't. Flow	8.0	AB	27.0	AB
	High	9.0	AB	25.3	AB
	Low	9.4	AB	31.3	B
All Harvests	CHECK	5.7	A	14.7	A
	Con't. Flow	8.1	A	21.5	A
	High	8.4	A	19.8	A
	Low	9.3	A	22.3	A

Conclusions

The initial construction of the SSIII took nearly 40 hours to design and build (Figure 1). The electronic and spray system components needed to assemble the injector were readily available for purchase from local merchants or online businesses. Only the cab mounted controller box was custom built by the engineering team. The SSIII material cost is approximately $1000 for a single row unit and $300 for each additional row. The injector was designed to be adapted to any planter that utilizes a seed monitor.

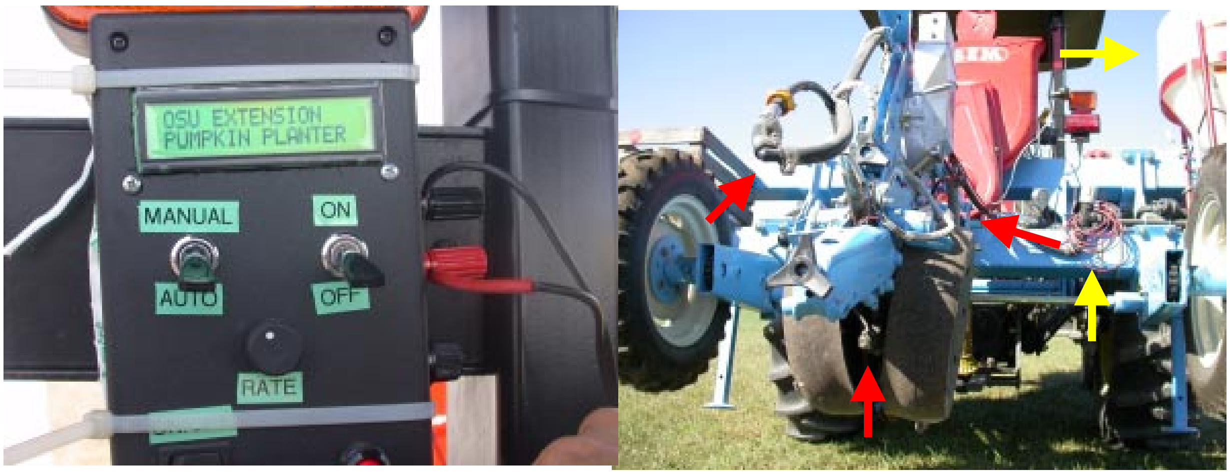

Figure 1. A picture of both the cab mounted controller box on the left and rear shot of planter and most of injector on the right. The tank and electric pump are visible on the right hand side of the planter (yellow arrows). Most of the plumbing, solenoid, and nozzle can also be seen (red arrows).

Trials to measure the accuracy of the injection band over the seed demonstrated the accuracy decreased slightly as ground speed increased and as the seed size decreased. Average coverage rates were 97.5% for pumpkins, 96.5% for zucchini, and 94.3% for cucumber.

To establish the efficacy of these insecticide bands over the seeds, bioassays were conducted with striped cucumber beetles to demonstrate uptake of the systemic insecticide by the seedlings at cotyledon, 1st and 2nd leaf stages, plus the 5th leaf stage for zucchini only. In all ten bioassay trials, StrCB mortality was significantly higher in the Admire treatments compared to the water only check

Although yields differences were observed between the four treatments of the three varieties, none were expected. This is because hand thinning stand populations in the pumpkins may have removed any differences in stand due to insect damage or disease. Cucumber stands were not manually thinned due to sporadic and lower than expected germination. Extremely low initial establishment in cucumbers was attributed to planting depth, not germination or treatment differences. The zucchini trial showed good emergence, was not hand thinned, and had no significant differences in stand count.

As a result of this research project economic savings for cucurbit growers could be quite substantial. Applying Admire with the SSIII at the high-labeled rate of 24 oz. per acre yielded a final cost per acre for pumpkin, zucchini, and cucumber at $15.93, $43.00, and $34.40 respectively. Admire applied continuously in-furrow at the same rate is $103 per acre (Table 5). Applying Admire with the SSIII at the low-labeled rate of 16 oz. per acre yielded a final cost per acre for pumpkin, zucchini, and cucumber at $10.62, $28.67, and $22.93 respectively. Admire applied continuously in-furrow at the same rate is $69 per acre.

Environmental benefits resulting from this research project are quite tangible. The SSIII reduced conventionally applied Admire in the field at the 24 oz. per acre rate to a range of 3.7 to 10 oz. per acre, without loss of efficacy. This translates to a 58.3 to 84.5 % Admire reduction. The SSIII also reduced conventionally applied Admire in the field at 16 oz. per acre to a range of 2.5 to 6.7 oz per acre without loss of efficacy. This translates to a 58.1 to 84.3 % Admire reduction.

In summary, there is overwhelming evidence that the SSIII can have immediate and substantial economic and environmental impacts on a wide range cucurbit production in Ohio. We expect to enhance the capability of the SSIII and verify many aspects of this research in 2005 trials.

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