Lygus Control Studies- 2025- on Alfalfa Produced for Seed

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Project Summary: Western U.S. alfalfa seed growers must control their key and direct crop-limiting pest, Lygus bug. Economic control of Lygus requires insecticide applications pre-bloom, during bloom, and post bloom which unfortunately can coincide with the timeframe during which managed and endemic pollinators are providing pollination service and are actively foraging and are provisioning their brood, rendering themselves and the next generation of these bees vulnerable to insecticide exposure. This limits the cohort of insecticides available for use during bloom. Hence the need for additional testing.

Objectives:

1. Test chemical alternatives for Lygus bug control without the use of chlorpyrifos.
2. Conduct baseline laboratory studies to determine the efficacy of the candidate insecticide Plianzolin (isocycloserum) on field captured Lygus bug adults under controlled conditions.

Progress by objective:

Objective 1: Test chemical alternatives for Lygus bug control without the use of chlorpyrifos.

Table 1 details multiple insecticides that could be considered by growers for use in potential pre-bloom programs prior to leafcutting bee or alkali bee emergence in late spring. Several of the insecticides could be used during bloom when bees are actively foraging. Beleaf 50 SG, Transform, Sefina, Mustang Maxx, and Lorsban Advanced are all registered under FIFRA Section 3 on alfalfa. Bifenture and Acephate 97 are registered on alfalfa grown for seed as a non-food/ non feed crop in Washington State and several other western US states under FIFRA Section 24C Special Local Need. PFR-97 20 WDG and BoteGHA are formulations that contain entomopathgenic fungi. These products are exempt from tolerance and legal to apply on alfalfa. **Plinazolin is not registered for use on alfalfa or alfalfa grown for seed**. Lorsban Advanced was applied as a long-term historical industry standard. Beleaf 50 WG and Sefina are the recommmeded insecticides for application during early and mid-bloom when bees are foraging. Transform is preferred by some growers late in the bloom cycle, but there has been an increase in the use of Transform by some growers, pre-

bloom. Mustang Maxx and Bifenture are synthetic pyrethroids. Lorsban Advanced and Acephate 97 are organophosphates. All 4 of these insecticides are highly toxic to bees and other beneficial arthropods and should not be applied when bees are foraging.

These plots were sprayed on May 28, 2025. The May 27 sweep sample was the pre-treatment sample 1 day before application. Insecticides were applied to 4 replicate plots of 12' by 20' per treatment using a CO_2 powered flat fan boom sprayer at the equivalent of 20 gallons per acre. Plots were sampled at 2, 5, 9, 13, 17, and 21 days after treatment on 5/30, 6/2, 6/6, 6/10, 6/13, and 6/17/2025, respectively. Sampling was accomplished by sweep netting three 180° sweeps per replicate plot on each sample date and counting the number of pest insects in the net including adult Lygus bugs, large and small Lygus nymphs, aphids. Alfalfa weevils and beneficial arthropods counted included spiders, big-eyed bugs, minute pirate bugs, nabids, lady bird beetles, and lace wing larvae captured within the net. Data were entered in MS Excel spreadsheets. Data were analyzed by analysis of variance and if treatment difference was significant (p<0.05), pairwise t-tests were completed between the untreated control and each respective insecticide treatment. There were no statistical differences in the abundance of alfalfa weevils and the beneficial insects and the numbers captured were consistently low among the samples collected, therefore the data are not shown for alfalfa weevil and beneficial arthropods.

The trade name, active ingredient, and rate per acre, of plots treated with insecticides on 5/28/2025

#	Trade name	active Ingredient	rate		plot #s
1	Untreated Control				103, 211, 301, 406
2	Beleaf 50 SG	flonicamid	2.8	oz/acre	111, 202, 305, 405
3	Transform	sulfoxaflor	2.25	oz/acre	110, 205, 304, 402
4	BoteGHA	*Beauveria bassiana*	3	qt/acre	106, 203, 307, 404
5	Sefina	afidopyropen	14	Fl oz/acre	102, 206, 311, 410
6.	Bifenture +	bifenthrin	8.5	Fl oz/acre	105, 210, 308, 407
	Mustang Maxx	zeta-cypermethrin	8.0	Fl oz/acre	
7.	Plinazolin	isocycloseram	1.6	FL oz/acre	107, 201, 310, 403
8.	Plinazolin	isocycloseram	2.0	Fl oz/ acre	101, 209, 302, 409
9.	PFR-97 20% WDG	*Isaria fumosorosea*	2	lbs /acre	109, 204, 306, 411
10.	Lorsban Advanced	Chlorpyrifos	32	Fl oz /acre	108, 207, 303, 408
11.	Acephate 97	acephate	1	lb /acre	104, 208, 309, 401

Results of the sweepnet samples for this pre-bloom are detailed in Table 2 through 5. Collected data were analyzed by ANOVA as a repeated measure among sample dates. If the *F*-test was passed by insect type on a given date, a pairwise *t*-test was completed between the specific insect abundance in the untreated plots vs the specific insect abundance in the insecticide-treated plots.

Table 2 details the data collected for adult Lygus bugs. Lygus bug adults are highly mobile and can be observed flitting throughout the canopy. We have run many years of insecticide trials in our WSU IAREC alfalfa research field. Among pests sampled, Lygus adults are typically the first group of pests to recover from insecticide treatment due to their high mobility and ability to move among the small plots. This proved to be true again in this 2025 trial. We were able to measure significant ($p<0.05$) differences in the abundance of adult Lygus bugs between most of the treatments compared to the untreated control for 2 and 5 days after treatment on May 30 and June 2, respectively. The abundance of Lygus bug dropped in our untreated control plots on 6/6, nine days after treatment and for the rest of the trial it was basically a wash.

Table 2. Analysis of variance results and the number of Lygus adults ± the std error of the mean captured per three 180° sweeps

Treatment	5/27/25	5/30/25	6/2/25	6/6/25	6/10/25	6/13/25	6/17/25
Mean Square *df*=10	24.30^{ns}	121.82**	56.20**	52.86^{ns}	77.60 ^{ns}	84.46^{ns}	33.61^{ns}
Error *df*=33	15.23	14.55	18.55	27.73	100.51	113.77	35.83
Untreated	8.75±2.02	19.25±3.58	15.75±4.50	5.00±1.47	18.50±6.38	20.00±5.74	21.25±4.77
Acephate 97	13.00±2.12	3.25±1.65**	5.00±1.08**	10.75±4.11	16.50±7.33	16.00±3.87	18.25±2.95
Beleaf 50 SG	13.50±1.71	8.75±0.85**	9.25±2.28*	13.50±1.04	17.25±6.06	19.00±7.22	14.75±1.03
Bifenture + Mustang	10.00±2.27	0.50±0.29**	1.50±0.50**	5.75±0.85	21.50±5.30	16.00±5.21	15.25±1.93
BoteGHA	12.25±1.65	15.25±1.31^{ns}	10.25±2.17	14.25±1.25	18.00±4.02	20.25±4.35	16.00±2.74
Lorsban Advanced	14.25±3.97	13.25±2.29*	7.75±1.81*	16.25±4.00	18.25±7.11	20.25±3.68	12.00±0.71
PFR-97	9.25±0.85	12.50±2.22*	8.00±1.00*	11.25±4.33	28.75±6.05	24.50±6.93	18.50±5.11
Plinazolin 1.6 oz	7.00±0.00	4.00±1.78**	4.00±1.08**	6.75±2.39	20.00±4.53	14.00±1.73	12.75±4.13
Plinazolin 2.0 oz	10.75±1.93	8.50±1.55**	8.75±2.59*	11.50±1.04	24.75±5.12	30.00±6.26	15.25±2.17
Sefina	11.50±0.50	8.25±0.48**	9.25±1.84*	13.00±3.29	18.25±7.18	18.25±7.18	11.00±0.41
Transform	7.50±1.44	9.75±2.46**	5.75±2.40**	9.25±0.85	12.00±3.63	15.25±3.15	14.50±2.50

For the *F*-Test ** is significant at $p<0.01$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly (p<0.01) lower than the untreated control.

Even with this breakdown in significant results shortly after insecticide treatment we did observe some interesting trends. As with our results from the past several years the Bifenture and Mustang Maxx tank mix exhibited high efficacy by highly significantly (*p*<0.01) reducing the abundance of Lygus adults for 2 and 5 days following treatment at 0.50 and 1.50 Lygus adults per three 180° sweeps. All the other treatments except entomopathogenic fungi BoteGHA significantly reduced the abundance of Lygus bug adults for 2 and 5 days following treatment. This was expected with the registered grower standards Acephate 97, Beleaf, Lorsban, Sefina, and Transform. Plinozalin as a candidate insecticide similarly reduced the abundance of Lygust bug adults at the 2 rates it was trialed at. Interestingly the entomopathogenic fungi PFR-97 reduced the abundance of Lygus adults as well.

Table 3 details our sweep net results for the number of large Lygus nymphs we captured in three 180° sweeps. We define large Lygus nymphs as being in the 3rd to 5th instars with identifiable wing pads in development. Our pretreatment sweeps averaged between about 2 to 4 large Lygus nymphs per three 180° sweeps. These counts jumped to an average of 12 nymphs per three 180° sweeps in the untreated control plots and the abundance of large Lygus nymphs in the untreated control plots fluctuated between 10 and 15 for each of the subsequent sampling dates up to 21 days after treatment. Initially, all the insecticide treatments significantly (*p*<0.01) reduced the abundance large Lygus nymphs compared to the abundance of large Lygus nymphs in the untreated control plots. Among the registered products the tank mix of Bifenture and Mustang Maxx, Acephate 97, Lorsban Advanced, and Transform treatment reduced the abundance of large Lygus nymphs. The candidate insecticide Plinozalin was extremely effective as well in providing very effective control of large Lygus nymphs for up to 3 weeks after treatment. The results with the entomopathogenic fungi-based insecticides varied, but we did observe a treatment effect that appeared to increase over time. The fact that the insecticides were maintaining a treatment effect for 21 days after treatment in the plots sampled on June 17 is impressive. Unfortunately, it was our plot quality that caused us to end sampling. Following this last sample date on June 17, the plots were swathed, and the hay was bailed, and the field was irrigated in preparation for a subsequent insecticide efficacy trial detailed below.

Table 3. Analysis of variance results and the number of large Lygus nymphs ± the std error of the mean captured per three 180° sweeps

Treatment	5/27/25	5/30/25	6/2/25	6/6/25	6/10/25	6/13/25	6/17/25
Mean Square *df*=10	3.60^{ns}	55.02**	59.57**	77.12**	79.11**	51.95**	49.25**
Error *df*=33	5.44	7.10	17.72	22.76	15.23	14.21	6.70
Untreated	3.25±1.65	12.00±2.65	10.75± 3.64	10.75±5.12	14.75±4.53	11.00±1.78	12.75±1.93
Acephate 97	4.00±1.41	0.50±0.29**	0**	0.25±0.25**	1.50±0.87**	0.50±0.50**	1.50±0.96**
Beleaf 50 SG	4.50±0.87	2.75±0.48**	6.50±3.40	8.25±0.48	5.25±1.89**	6.50±2.02	3.50±0.96**
Bifenture + Mustang	2.25±1.31	0**	2.25±2.25**	2.50±0.50**	0.50±0.50**	4.25±2.02*	2.00±0.71**
BoteGHA	3.00±0.71	6.00±2.34**	8.50±2.60	10.75±3.01	7.25±3.17*	8.25±3.66	2.50±0.96**
Lorsban Advanced	4.25±0.48	6.75±1.25**	1.25±0.75**	1.25±0.75**	0**	1.75±1.75**	0.75±0.48**
PFR-97	3.75±1.11	7.25±1.32*	10.50±2.06	8.00±3.70	7.75±1.49*	7.50±2.90	6.75±2.10**
Plinazolin 1.6 oz	5.50±1.26	0.50±0.29**	0.75±0.48**	0.50±0.50**	1.50±1.19**	0.50±0.29**	1.25±0.75**
Plinazolin 2.0 oz	4.25±0.95	1.50±0.87**	3.25±1.97*	0	0.50±0.50**	0.75±0.75**	1.00±0.41**
Sefina	2.50±1.32	2.50±0.65**	4.00±1.66*	8.75±3.35	3.75±1.03**	2.50±1.04**	4.50±2.10**
Transform	3.25±1.25	3.00±1.47**	3.00±0.58*	2.75±1.03**	2.25±1.31**	2.50±0.96**	4.00±1.29**

For the *F*-Test ** is significant at $p<0.01$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly ($p<0.01$) lower than the untreated control.

Table 4 details our sweep net results for the number of small Lygus nymphs we captured in three 180° sweeps. We define small nymphs from initial hatch through the second instar prior to the growth of wing pads. All the insecticides applied provided some significant level of reduction in the abundance of small Lygus nymphs compared to the untreated control. On the June 2 sample date 9 days after treatment Acephate 97 and the tank mix of Bifenture and Mustang Maxx had eradicated small Lygus nymphs entirely from the plots. Lorsban Advanced, both the 1.6 and 2.0 oz rates of Plinazolin , and Transform all exhibited counts of less than 1 small Lygus nymph compared to 13.5 small Lygus nymphs in the untreated control plots. There was a substantial Lygus hatch observed between June 13, 17 days after treatment and June 17, 21 days after treatment but the abundance of small Lygus bugs in all the

treatments were still significantly lower than in the untreated control plots. On June 17, 21 days after treatment we sampled small Lygus nymphs at 30.75 per three 180° sweeps in the untreated control plots. Plinazolin at the 1.6 oz and 2.0 rate had 6.25 and 7 small Lygus nymphs, respectively. The tank mix of Bifenture and Mustang Maxx exhibited 8 small Lygus nymphs per three 180° sweeps. All the rest of the treatments were beginning to break, but even the entomopathic fungi-based insecticides were still exhibiting a statistically significant (p<0.01) reduction in the abundance of small Lygus nymphs as compared to the abundance of small Lygus nymphs in the untreated control plots.

Table 4. Analysis of variance results and the number of small Lygus nymphs ± the std error of the mean captured per three 180° sweeps.

Treatment	5/27/25	5/30/25	6/2/25	6/6/25	6/10/25	6/13/25	6/17/25
Mean Square *df*=10	17.42^{ns}	77.19**	45.92**	70.82**	78.97*	66.55*	237.44**
Error *df*=33	15.24	26.22	9.86	13.54	29.11	28.70	74.14
Untreated	9.00±1.78	13.75±3.82	8.75±2.29	13.50±4.29	16.75±4.05	14.50±2.06	30.75±7.53
Acephate 97	11.75±1.03	0.25±0.25**	0**	1.00±1.00**	6.25±1.65*	5.50±1.50*	12.50±4.29**
Beleaf 50 SG	11.00±2.80	6.25±3.12**	7.00±0.82	6.50±2.53*	9.25±3.35	12.50±4.27	15.00±3.34*
Bifenture + Mustang	6.00±1.22	0**	0**	0.75±0.48**	1.25±0.95*	3.00±1.08*	8.00±7.34**
BoteGHA	10.50±2.63	7.00±4.74**	8.50±3.97	6.25±2.21*	5.50±3.28*	12.75±1.75	18.00±2.48*
Lorsban Advanced	8.25±0.75	3.75±1.03**	0.25±0.25**	0.50±0.50**	10.50±0.96	7.25±3.12	10.00±2.94**
PFR-0.497	12.50±3.01	9.00±2.55*	5.00±1.35	6.50±2.02**	10.50±2.63	11.00±0.82	15.25±3.12*
Plinazolin 1.6 oz	8.00±0.41	3.75±2.25**	0.50±0.29**	0.50±0.50**	1.00±0.58*	7.50±2.60	6.25±1.70**
Plinazolin 2.0 oz	12.75±2.17	3.75±1.65**	2.75±1.55*	0.25±0.25**	8.00±4.53*	8.50±4.80	7.00±3.42**
Sefina	10.00±1.58	5.75±2.06**	2.50±0.50**	2.25±1.03**	8.75±1.44*	15.00±2.58	25.75±4.29
Transform	8.75±2.10	3.00±2.38**	2.00±0.93**	0.75±0.75**	6.00±2.71*	14.75±1.75	14.25±2.50*

For the *F*-Test ** is significant at p<0.01; * is significant at p<0.05

For the means *t*-test * treatment mean is significantly (p<0.05) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly (p<0.01) lower than the untreated control.

Tabel 5 details the sweep net capture of aphids. Aphid abundance in these 2025 plots were slightly lower than in most prior years we've completed Lygus spray trials and populations never exceeded 50 aphids per three 180° sweeps. However, several of the insecticides provided very effective control of aphids. This included the tank mix of Bifenture and Mustang Maxx, Sefina, and Transform. Acephate 97 and Lorsban Advance initially provided significant control, but control began to break after about 2 weeks after treatment. Plinazolin did not provide aphid control. The application of the entomopathogenic insecticides provided some initial control of aphids, but the aphid populations in these plots recovered.

Table 4. Analysis of variance results and the number of aphids ± the std error of the mean captured per three 180° sweeps.

Table 5. Aphids per three 180° sweeps

Treatment	5/27/25	5/30/25	6/2/25	6/6/25	6/10/25	6/13/25	6/17/25
Mean Square *df*=10	128.54^{ns}	1129.65**	833.45**	1444.15**	1533.36**	1011.32*	294.26^{ns}
Error *df*=33	130.72	137.61	95.73	232.12	333.15	460.00	224.58
Untreated	23.50±3.77	49.25± 6.00	30.00±6.10	36.25±9.68	41.50±11.64	34.75±18.21	13.75±8.74
Acephate 97	30.00±5.11	3.25± 1.65**	1.50±0.87**	3.00±1.41**	17.00±4.76	23.25±4.80	14.00±9.72
Beleaf 50 SG	28.25±4.48	33.25±10.30*	23.50±3.30	27.75±4.64	30.75±12.46	28.75±10.62	25.25±8.46
Bifenture + Mustang	27.75±8.14	0**	0**	0**	2.50±1.66**	0*	2.00±1.22
BoteGHA	39.75±6.17	14.50± 7.92**	10.00±5.11**	21.00±9.55	26.50±8.21	38.25±14.58	15.50±6.76
Lorsban Advanced	30.00±2.61	0.75± 0.48**	0.25±0.25**	1.00±1.00**	21.75±13.65	19.25±15.01	24.50±10.06
PFR-97	25.00±8.91	16.50± 5.95**	25.00±9.05	28.00±11.80	37.25±11.75	42.50±15.18	20.75±5.07
Plinazolin 1.6 oz	26.50±3.30	14.00± 7.91**	17.25±9.50	33.50±16.50	48.50±12.45	33.50±9.13	22.75±6.51
Plinazolin 2.0 oz	38.75±5.57	36.50± 8.26^{ns}	40.75±3.79	54.75±3.94	63.00±6.79	39.75±5.93	27.75±10.94
Sefina	38.25±2.14	2.50± 1.05**	0**	0**	1.75±1.75**	1.25±0.95*	5.25±3.04
Transform	31.00±5.76	2.00± 1.41**	2.25±1.31**	0**	7.50±1.85*	4.25±3.06*	8.50±5.44

For the *F*-Test ** is significant at $p<0.01$; * is significant at $p<0.05$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly ($p<0.01$) lower than the untreated control.

Conclusions: Overall this was a very successful insecticide efficacy trial. The fact we were measuring treatment effects in the abundance of both large and small lygus at 21 days after treatment is heartening in that we have some very effective insecticides on Lygus bugs. We will conduct further studies with the candidate insecticide and investigate Plinazolin's registration via a 24C special local need as a potential acephate and chlorpyrifos replacement that would serve to mitigate insecticide resistance development by Lygus bugs to the synthetic pyrethroids.

Post bloom sprays 2025.

In mid-August a second insecticide efficacy trial was established following the same methodology described above in the pre-bloom trial in May and June. Plot sizes and sampling methods were identical. A pretreatment sample was completed on August 13, the day before the treatments were applied and the plots were sprayed on August 14, 2025. All sprays were applied in the equivalent of 20 gallons of water per acre. The treatments that were applied are detailed below in Table 6.

Table 6. The Trade name, active ingredient, rate per acre, and plot numbers of alfalfa plots treated with insecticides on 8/14/2025

1. Untreated Control				106, 211, 301, 410
2. Acephate 97	acephate	1	lb/acre	110, 204, 310, 409
+ Bifenture	bifenthrin	8.5	fl oz/ acre	
3. Transform	sulfoxaflor	2.25	oz/acre	104, 202, 305, 406
4. Sefina	afidopyropen	10	Fl oz/acre	102, 203, 306, 408
5. Sefina	afidopyropen	14	Fl oz/acre	109, 210, 303, 405
6. Bifenture +	bifenthrin	8.5	Fl oz/acre	103, 206, 302, 411
Mustang Maxx	zeta-cypermethrin	8.0	Fl oz/acre	
7. Plinozolin	isocycloseram	1.0	FL oz/acre	101, 207, 311, 404
8. Plinozolin	isocycloseram	1.6	Fl oz/ acre	111, 208, 308, 403
9. Plinolzolin	Isocycloserum	2.0	Fl o//acre	108, 209, 307, 401
10. Lorsban Advanced	Chlorpyrifos	32	Fl oz /acre	107, 201, 309, 407
11. Acephate 97	acephate			

With the exception of Sefina all of these insecticides are very toxic to bees and should not be applied during bloom when bees are actively foraging. We included the 2 rates of Sefina to determine if boosting the rate from 10 FL oz/ acre to 14 FL oz/acre increased the efficacy of Sefina to Lygus. Sefina is registered on alfalfa at 10 FL oz/ acre which is a rate more in line with a rate designed to control aphid pests. Sefina is registered on other crops for problematic pests like whiteflies at 14 FL oz/ acre and Sefina is registered under a Section 18 Emergency Exemption from Tolerance on strawberries in California specifically for Lygus bug control at 14 oz per acre. We have approached the registrant of Sefina, BASF to consider registering Sefina at the exaggerated 14 FL oz/ acre rate under 24C Special Local Need on alfalfa produced for seed as a non-food non-feed crop. BASF has requested additional efficacy data. For the past several years the tank mix of Bifenture and Mustang Maxx has provided very effective and durable control of large and small Lygus nymphs in our small research plots. We read in a report from Arizona cotton that a tank mix of Acephate 97 and Bifenture provided superior efficacy against Lygus in Arizona cotton fields. We included Lorsban Advanced, Acephate 97, and Transform as potential grower standards. **Plinozolin is not registered for use on alfalfa or alfalfa grown for seed.** In our pre-bloom efficacy trial in May and June, Plinozolin was an effective treatment and by applying Plinozolin in this trial at 1, 1.6, and 2 FL oz/ acre we are attempting to home in on a rate that is effective against Lygus on alfalfa produced for seed in support of a potential future Section 24C Special Local Need registration. In studies with honeybees Plinozolin was highly toxic. We anticipate that any 24C issued for Plinazolin on alfalfa grown for seed will have strict restrictions on applications during bloom when bees are actively foraging.

Following the applications that were made on 8/14/25 we sampled we sampled 1, 5, 8, 12, 15, 19, and 22 days after treatment (DAT) on 8/15, 8/19, 8/22, 8/26, 8/29, 9/2 and 9/5/2025, respectively. These research plots are small and Lygus adults are highly mobile and all treatment effects were washed out by 8/22/25 at 12 DAT. At 1 DAT on 8/15 all the treatments with the exception of Sefina at 14 oz significantly ($p<0.01$) reduced the number of adult Lygus (Table 7). By 8/19/25, five DAT only the Plinazolin treatment at 1 oz per acre significantly ($p<0.05$) reduced the abundance of adult Lygus in pairwise t-test against the untreated control. However, on the 8/29/25 sample date the probability result from the ANOVA was about $p=0.06$ with the Plinazolin treatments at 1.0 and 1.6 oz per acre being numerically superior and very close to being significant superior at controlling Lygus adults.

Table 7. Analysis of variance results and the number of Lygus adults ± the std error of the mean captured per three 180° sweeps

Treatment	8/13/25	8/15/25	8/19/25	8/22/25	8/26/25	8/29/26	9/2/2025
Mean Square *df*=10	12.24^{ns}	28.17**	27.05*	23.52^{ns}	13.04^{ns}	57.72^{ns}	79.67^{ns}
Error *df*=33	13.47	7.05	12.44	25.86	13.89	37.54	83.38
Untreated Control	8.50±2.55	10.50±1.71**	8.50±0.29	8.25±2.50	7.75±1.80	18.50±3.40	14.25±3.50
Acephate 97	10.75±1.11	1.75±1.44**	3.25±1.65*	6.25±2.75	8.75±1.84	13.00±3.72	26.25±4.64
Acephate 97+ Bifenture	12.00±2.27	2.50±0.96**	3.25±1.03*	6.00±2.34	6.00±0.91	11.25±3.06	16.25±2.89
Bifenture +Mustang Maxx	11.00±1.91	1.50±1.19**	4.75±2.21	5.25±1.93	7.25±1.44	15.25±3.20	24.75±3.75
Lorsban Advanced	9.50±1.89	2.75±1.44**	6.75±1.31	10.00±3.24	7.25±1.65	10.50±3.23	14.50±4.17
Plinozolin 1.0 oz	11.75±1.89	3.50±2.02**	2.50±0.87*	7.00±2.48	3.50±1.89	7.50±3.72	23.00±5.76
Plinozolin 1.6 oz	9.75±0.85	3.25±0.63**	6.50±2.50	9.00±2.27	7.00±2.80	7.00±3.03	15.00±5.64
Plinolzolin 2.0 oz	10.25±2.21	2.25±0.75**	3.75±1.80	5.25±2.06	4.75±1.93	11.00±3.19	19.28±5.10
Sefina 10 oz	11.50±2.72	4.25±1.93**	6.50±1.76	7.25±1.31	1.00±1.68	12.25±2.50	20.00±2.08
Sefina 14 oz	12.00±1.29	7.00±0.41	7.25±1.93	7.25±2.95	6.50±2.06	18.50±3.50	16.00±5.79
Transform	6.25± 0.25	3.50±1.04**	11.00±2.58	13.50±3.40	8.75±1.90	11.25±1.60	23.75±5.19

For the *F*-Test ** is significant at $p<0.01$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly ($p<0.01$) lower than the untreated control.

Table 8 details the abundance of large Lygus nymphs captured in the sweepnet samples. Every insecticide treatment significantly reduced the abundance of large Lygus nymphs through 8/19/2025 at 5 DAT. Treatment effect sputtered through the next several sample dates but we observed a resurgence in treatment effect on the 8/29/2025 sample date at 15 DAT. The abundance of large Lygus nymphs in the untreated control plots exceeded an average of 36 large Lygus nymphs per three 180° sweeps. Lorsban Advanced was a consistent pack leader in suppressing the abundance of large Lygus nymphs. Results with Plinazolin were encouraging and abundance of Large Lygus nymphs captured was consistently statistically or numerically lower than in the untreated control. Transform was somewhat of a disappointment in this trial in regards to providing large Lygus nymph suppression. Unfortunately, the abundance of large Lygus nymphs in plots treated with 10 oz of Sefina was somewhat and consistently lower than in the plots treated with 14 oz per acre of Sefina.

Table 8. Analysis of variance results and the number of large Lygus nymphs ± the std error of the mean captured per three 180° sweeps

Treatment	8/13/25	8/15/25	8/19/25	8/22/25	8/26/25	8/29/25	9/2/25
Mean Square *df*=10	22.52^{ns}	64.34**	165.75**	119.99*	288.41*	551.04**	368.80**
Error *df*=33	19.73	9.75	17.61	53.36	108.01	89.66	87.11
Untreated Control	9.25±0.25	15.25±1.03	22.75±3.92	15.75±3.04	17.25±9.00	36.53±8.66	27.00±4.71
Acephate 97	8.25±2.75	0.75±0.48**	0.50±0.50**	2.00±1.35*	1.50±0.65*	3.50±1.44**	3.25±2.63**
Acephate 97+ Bifenture	12.50±3.17	5.00±2.16**	4.00±1.35**	7.25±2.92	7.25±1.44	9.50±5.89**	15.25±4.40
Bifenture +Mustang Maxx	10.75±0.63	3.00±1.47**	2.75±1.25**	2.50±0.50*	4.75±2.02	5.00±3.03**	6.25±2.14**
Lorsban Advanced	13.25±2.95	4.50±2.72**	5.75±2.81**	2.25±0.85*	0*	0.75±0.75**	2.25±0.63**
Plinozolin 1.0 oz	10.75±1.25	5.25±1.89**	2.50±0.87**	2.75±1.60*	1.25±0.48*	6.00±2.48**	6.75±3.12**
Plinozolin 1.6 oz	9.00±0.91	5.00±0.91**	2.75±1.70**	2.75±1.70*	3.25±1.49	5.50±3.86**	4.00±3.37**
Plinolzolin 2.0 oz	8.50±1.19	1.25±0.63**	1.00±1.00**	2.25±0.85*	0.75±0.48*	6.50±3.23**	5.00±2.80**
Sefina 10 oz	16.25±3.94	1.25±1.25**	3.00±1.29**	6.00±5.67	18.00±7.73	6.00±3.49**	14.50±9.38
Sefina 14 oz	11.00±2.55	3.50±1.04**	11.50±2.73**	11.75±5.15	21.00±9.93	21.50±4.79**	28.25±2.78
Transform	10.25±1.25	7.00±1.96**	7.50±2.78**	15.75±7.82	19.50±6.70	29.00±7.63*	23.50±6.46

For the *F*-Test ** is significant at $p<0.01$, * is significant at $p<0.05$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly ($p<0.01$) lower than the untreated control.

As expected, the plots treated Acephate 97, Acephate 97 plus Bifenture, and Bifenture plus Mustang Maxx consistently reduced the abundance of large Lygus . These are all broad spectrum synthetic pyrethroid or organophosphate treatments. We observed no benefit from tank mixing Acephate 97 with Bifenture.

Table 8 details the abundance of small Lygus nymphs. Through an insecticide trial like the one we are detailing herein the small Lygus nymphs are dominated by the 1st and second instar nymphs prior to the development of wing pads in the later instars. Also as the trial progresses the presence of small nymphs in the plots is indicative of Lygus bug hatch in the plots.

Table 8. Analysis of variance results and the number of small Lygus nymphs ± the std error of the mean captured per three 180° sweeps

Treatment	8/13/25	8/15/25	8/19/25	8/22/25	8/26/25	8/29/25	9/2/25
Mean Square *df*=10	56.85^{ns}	28.17**	452.50**	707.41**	739.61	1034.05**	279.65*
Error *df*=33	67.45	7.05	61.45	67.64	112.23	324.90	128.50
Untreated Control	25.75±4.87	22.50±2.25	32.75±2.98	38.50±3.43	38.00±7.08	43.00±12.81	32.25±3.09
Acephate 97	18.50±2.72	0.50±0.29**	1.00±0.71	0.75±0.75**	1.75±1.44**	2.50± 0.96**	8.75±4.40*
Acephate 97+ Bifenture	17.75±1.18	2.75±1.25**	10.50±2.60	8.25±3.42**	7.00±3.37**	28.00±16.56	17.50±3.66
Bifenture +Mustang Maxx	24.50±8.76	3.25±2.14**	10.00±5.79	5.75±4.13**	8.75±5.43**	10.25± 8.02*	8.00±3.76*
Lorsban Advanced	23.00±3.94	4.00±3.08**	1.50±1.50	2.25±1.65**	2.25±0.85**	9.25± 5.14*	10.50±2.10*
Plinozolin 1 oz	20.75±4.44	6.75±2.66**	7.25±3.30	5.25±2.39**	3.75±2.46**	6.00± 3.67**	13.25±5.76*
Plinozolin 1.6 oz	19.00±3.34	5.50±1.19**	2.50±0.96	0**	7.50±6.20**	4.00± 2.48**	12.50±7.19*
Plinolzolin 2.0 oz	26.75±1.70	2.00±1.41**	2.25±1.93	0**	1.00±0.71**	21.75±10.77	10.25±1.11*
Sefina 10 oz	20.50±4.09	7.25±3.47**	10.75±5.39	18.25±9.55*	7.50±4.41**	15.75± 4.38*	15.50±9.72*
Sefina 14 oz	29.50±2.72	6.50±0.96**	28.25±7.00	30.00±5.02	30.75±7.95	19.50± 6.34	29.25±7.05
Transform	24.50±1.65	17.50±7.98	13.50±4.84	21.75±7.41*	31.00±9.34	51.75±12.97	21.75±6.45

For the *F*-Test ** is significant at $p<0.01$, * is significant at $p<0.05$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

For the means *t*-test ** treatment mean is highly significantly ($p<0.01$) lower than the untreated control.

We completed a final sweepnet sample on 9/5/2025 at 22 DAT. Adults were not counted due to them being so numerous and we had not calculated any statistical significance since the 8/19/2025 sample date 5 days after treatment. We observed a strong edge effect in replicate 4, plot#'s (401 through 411). Replicate 4 was bordered by alfalfa that had not been treated with insecticide all summer. We ran a second ANOVA in which we did gain statistical significance for the number of large Lygus nymphs by removing replicate 4 from the analysis (Table 9). There were obvious numerical differences among treatments for both large and small Lygus nymphs when all 4 replicates were analyzed and for small Lygus nymphs when replicate 4 was removed from the analysis, but the numerical differences were not statistically significant (Table 9).

Table 9. Analysis of variance results and the number of large and small Lygus nymphs ± the std error of the mean captured on 9/5/2025 at 22 days after treatment

	All 4 Replicates			Replicate 4 removed	
Treatment	Large nymphs	Small nymphs		Large nymphs	Small nymphs
Mean Square *df*=10	102.31^{ns}	167.35^{ns}	Mean Square *df*=10	153.65*	224.59^{ns}
Error *df*=33	93.95	141.33	Error *df*=22	48.60	154.36
Untreated Control	23.75±2.56	29.75±9.69		24.33±3.53	36.67± 9.60
Acephate 97	6.5±4.48	3.00±0.41		2.33±2.33*	3.00± 0.58
Acephate 97+ Bifenture	15.75±5.98	12.25±2.75		11.67±6.17*	10.67± 3.18
Bifenture +Mustang Maxx	11.75±5.66	13.75±9.59		10.67±7.86*	15.33±13.38
Lorsban Advanced	12.00±2.97	19.50±6.22		10.67±3.76*	16.67± 7.84
Plinozolin 1 oz	11.00±5.70	13.00±4.64		5.67±2.85*	13.00± 6.56
Plinozolin 1.6 oz	10.75±6.90	12.50±5.04		4.00±2.00*	8.00± 3.21
Plinolzolin 2.0 oz	9.25±5.36	13.00±4.30		4.00±1.53*	11.67± 5.78
Sefina 10 oz	14.50±4.50	12.00±7.15		10.67±3.33*	11.00±10.02
Sefina 14 oz	20.00±2.48	17.25±3.50		22.00±2.08	20.33± 2.33
Transform	17.75±4.46	15.50±5.24		14.33±4.06	12.00± 5.51

For the *F*-Test * is significant at $p<0.05$

For the means *t*-test * treatment mean is significantly ($p<0.05$) lower than the untreated control.

Conclusions: Overall this was a very successful

Conclusions: Overall we completed two successful insecticide efficacy trials on Lygus bugs in 2025. We observed some significant differences among insecticide treatments for up to 3 weeks following treatment. Results with the candidate insecticide Plinazolin were encouraging and further field trials will be completed in 2026.

Objective2. Conduct baseline laboratory studies to determine the efficacy of the candidate insecticide Plianzolin (isocycloserum) on field captured Lygus bug adults under controlled conditions.

Based on our promising results with the unregistered insecticide Plinazolin in our initial pre-bloom field trial we completed several controlled laboratory studies with Lygus bugs in June 2025. This serves as our baseline for the efficacy of an insecticide product prior to any wholesale use in the general alfalfa or alfalfa seed environment.

In completing our bioassays, we go out and collect Lygus bugs with sweep nets in our alfalfa seed field at WSU IAREC, in weedy habitats, and if possible, in alfalfa seed grower fields, typically in Touchet, Gardena, or Lowden, WA. We place the collected Lygus in a bug dorm and transport them to the North Building Entomology Laboratory at WSU IAREC. We then put a large handful of home garden green beans or purchase beans that were produced organically as a food and moisture source for the insects in the cage. We hold the Lygus overnight since we have observed some substantial mortality in this first 12 to 20 hours after the Lygus bugs are captured. It has been our experience that most Lygus adults after recovering capture and transport shock will live for approximately 5 to 7 days in captivity. The Lygus are then aspirated into plastic vials of cohorts of approximately 10 Lygus bugs per vial (Figure 1). When enough vials are filled with Lygus to complete the planned studies, the Lygus in the vials are anesthetized with a 2-second short burst of CO_2.

Figure 3. Aspirating Lygus into cohorts of 10 in plastic vials.

When anesthetized the Lygus are placed in a Petri dish lined with a paper beaker filter that's been wetted with 1.5 ml of tap water (Figure 2).

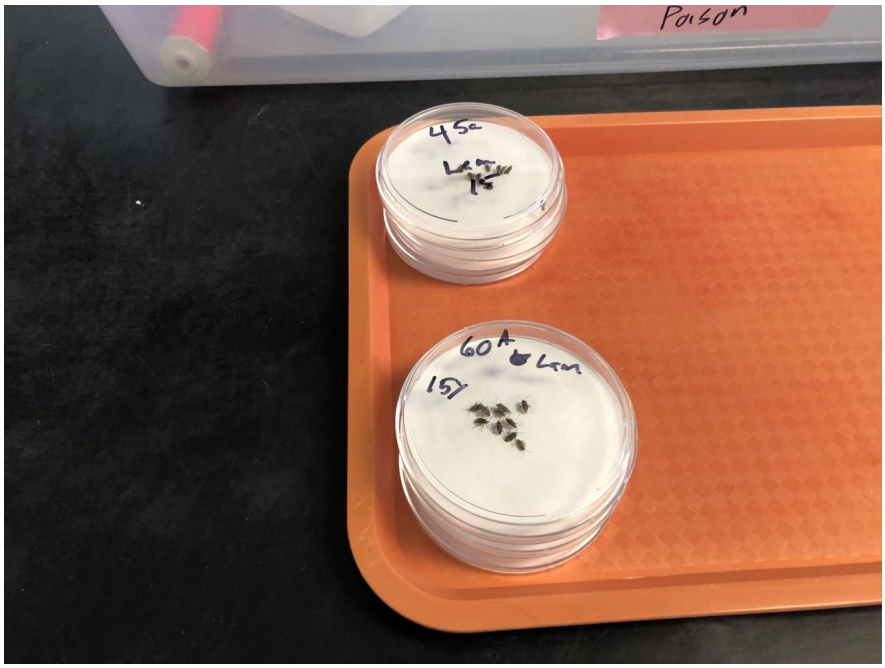

Figure 2. Lygus in Petri dish

The anesthetized Lygus are then treated with candidate insecticides, in this case plinazolin in serial dilutions in 2ml volume through our Potter precision spray tower. After insecticide

treatment a 2 cm piece of green bean is placed in the Petri dish with the treated Lygus for sustenance. The Lygus are then stored at ambient room temperature and are evaluated for mortality at 24 or 48 hours (or longer if possible) after treatment for most conventional insecticides. Pushing much beyond 72 hours after treatment often results in excessive mortality in our water control treatments.

In mid-June we started with our initial trials with Plinozolin using the methodology described above. In our initial trial we tested Plinozolin starting at a rate of 2.0 fl oz product per acre diluted the equivalent of 20 gallons of water carrier per acre. We calculated that this full 2.0 fl oz per acre rate equals approximately 80*u*l of Plinazolin per 100 ml water carrier for our bioassay tests. In our first test using this 80*u*l per ml concentration we completed serial dilutions at, 100%, 50%, 25%, 12.5%, and 0% (water control). After 24 hours 100% of the Lygus bugs were dead except in the 0% water controls, in which survivorship exceeded 90%. This initial test was completed on adult Lygus bugs collected from our alfalfa research plot at WSU IAREC. The following week we collected 4 populations and ran bioassays on them. One population was from our alfalfa research plot, the other 3 populations were collected near Touchet and Lowden, WA. One population was captured in a weedy mustard patch, another from pigweed in a newly seeded alfalfa seed field and the 3rd population was captured in an established 2nd year alfalfa seed field. In this study we tested Plinazolin in our bioassays at dilutions of 10%, 5%, 2.5%, 1.25%, 0.625% and 0% (water control) of the initial 0.80*u*l Plinazolin diluted in 100ml of water carrier. At 24 hrs after treatment the overwhelming majority of the treated Lygus adults were dead. At 48 hrs after treatment all the Plinazolin treated adults except 2 individuals in one bioassay Petri dish arena that had been treated at 0.625% were dead. And these 2 survivors were exhibiting distressed behaviors. Survivorship in the 0% water controls was greater than 90%.

So, on 24 June we collected another 3 populations and treated them on 25 June. Two of the populations were from the same pigweed patch and alfalfa seed field tested the prior week and the 3rd population was from another established alfalfa seed field near Lowden, WA. The dilutions tested on these populations were at 1%, 0.25%, 0.125%, 0.0625% and 0% water control, again based on the initial 100% dilution of 80*u*l of Plinzolin in 100ml of water carrier. Finally, some decent mortality dose-response results were observed. See table 10.

Table 10. Plinazolin efficacy against Lygus bugs in controlled laboratory bioassays. Data is shown as uncorrected percent mortality ± standard error at 24 and 48 hours after treatment.

Lygus Population	Plinazolin % Field Rate	% Mortility at 24 hr ± SE	% Mortality at 48 hr ± SE
AS-W	0%	0.97± 0.74	10.98±4.17
AS-W	0.0625%	5.56± 5.56	25.10±5.34
AS-W	0.125%	30.00±10.80	38.86±8.78
AS-W	0.25%	12.27± 4.83	80.91±5.54
AS-W	1%	88.18± 4.61	100
AS-B	0%	0	5.00± 5.00
AS-B	0.0625%	7.27±2.43	21.36± 6.57
AS-B	0.125%	0	52.50± 4.79
AS-B	0.25%	3.33±3.33	80.00±11.55
AS-B	1%	96.67±3.33	100
Pigweed	0%	10.00± 5.77	33.64±6.63
Pigweed	0.0625%	12.05± 7.36	56.14±6.26
Pigweed	0.125%	11.46± 4.55	58.86±5.05
Pigweed	0.25%	32.28±10.19	95.00±5.00
Pigweed	1%	100	100

On June 30, 2025 we collected adult Lygus bugs from a mixed stand of alfalfa forage and Timothy hay on the WSU IAREC campus and using our standard methodology described above we treated this population at 0%, 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, and 1.0% of a maximum (100% rate) of 2.0 oz in 10 gallons of water carrier per acre (80ul per 100 ml). The mortality in the water control (0%) was greater than we usually like to see, but we did have a fairly decent dose response (Table 11). These concentrations are so low that it does make it difficult to get completely accurate dose responses, but we do have some very encouraging results.

Table 11. Plinazolin efficacy against Lygus bugs in controlled laboratory bioassays. Data is shown as uncorrected percent mortality ± standard error at 24 and 48 hours after treatment.

Lygus	Plinazolin	% Mortility	% Mortality
XWSU IAREC	0%	8.90±3.72	22.16±6.12
WSU IAREC	0.03125%	11.52±5.14	19.27±1.20
WSU IAREC	0.0625%	5.00±2.89	25.36±2.70
WSU IAREC	0.125%	10.06±1.86	36.66±6.96
WSU IAREC	0.25%	10.10±5.89	43.57±8.35
WSU IAREC	0.5%	44.23±7.11	95.83±4.17
WSU IAREC	1.0%	88.64±8.60	100

Subsequently we analyzed the data detailed in Tables 10 and 11 to calculate the baseline dose response of field collected Plinazolin naïve Lygus bugs. The results are outrageous.

Population	LC_{50} (95% CI) ppb	LC_{90} (95% CI) ppb	Slope	X^2 (*df*)
Touchet Pigweed	198 (108-271)	746 (510-1820)	2.223±0.469	15.92 (14)
Lowden B Seed	405 (300-544)	1083 (741-2619)	2.995±0.503	18.27 (12)
Touchet W Seed	422 (423-496)	972 (745-1607)	3.54±0.649	11.80 (14)
Prosser Forage	641 (320-1020)	2379 (1489-7146)	2.25±0.342	73.33 (22)

Conclusions: After some effort we have finally quantified a rate range at which our methodology is effective. Very few insecticides that we've screened via our methodology have exhibited such a rapid knockdown of Lygus bug adults. Lygus bug adults as a general rule are tough insects to control. These data and additional bioassay data we plan to complete will give us a baseline assessment of the toxicity of Plinozalin to adult Lygus bugs in South Central Washington State prior to the insecticide's potential commercial release.