Title of Report
Renewal Progress Report for CDFA Agreement Number 16-0511-SA

Title of Project
Insecticide Resistance in the Glassy-winged Sharpshooter: Using Historical Use Patterns to Inform Future Management Strategies

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Reporting Period
The results reported here are from work conducted July 2016 to March 2017

Introduction
The CDFA PD-GWSS Area-wide Management Program relies on insect monitoring which triggers chemical control in citrus orchards and vineyards. This program, initiated in Riverside County in 2000 and expanded to Kern County the following year, was successful in keeping GWSS densities low from 2001-2008 (Figure 1). From 2009-2011, control was still adequate, but insect numbers increased. Despite continued insecticide usage, high densities of GWSS in 2012 and 2015 surpassed the 2001 density, while levels in 2013-14 nearly attained the 2001 level (Figure 1). It is important to note that the GWSS densities in the last 4 years have occurred while under chemical management, whereas the 2001 densities occurred prior to the widespread use of insecticides. Concomitant with large GWSS densities has been a resurgence of PD infection vines. While levels of PD in the General Beale region of Kern County were nearly undetectable from 2002-2009, they have increased in the last 5 years; the number of infected vines has increased in nearly all vineyards surveyed (Haviland 2015).

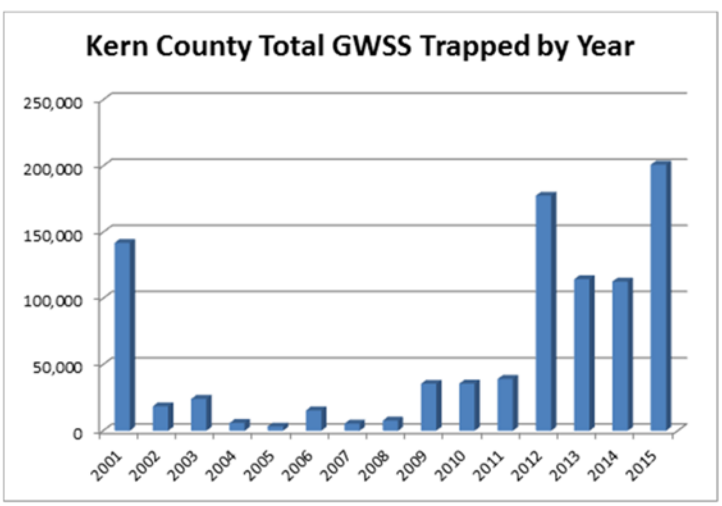


Figure 1. Total number of GWSS caught on CDFA traps in Kern Co. from 2001 – 2015. (From Haviland 2015)

Due to a number of factors, the systemic neonicotinoid insecticide imidacloprid has been used preferentially for GWSS suppression. Positive attributes of imidacloprid include systemic activity, persistence in treated plants, and selectivity for xylem and phloem feeding insects. Although data on the frequency of imidacloprid use since

2000 has not been compiled for the areawide program, it is generally believed that it has been used to a greater extent than other insecticides. In addition, citrus growers have used imidacloprid extensively for control of red scale and other citrus pests (Grafton-Cardwell et al. 2008) and grape growers have relied upon imidacloprid for vine mealybug control (Daane et al. 2006). With the selection pressure that has resulted from the combined use of imidacloprid across citrus and grape acreages over the past 15 years, there is reason to believe that the resurgence of GWSS is related to imidacloprid resistance.

With this background information, we initiated a pilot study to evaluate insecticide susceptibility of GWSS to a number of insecticides (Table 1). In this study, we collected GWSS on three dates in July and August, 2015 in organic citrus groves in the Edison area, then shifted to the General Beale Road area for three more dates in September and October Insects were subjected to a systemic uptake bioassay and a foliar insecticide bioassay adapted from Prabhaker et al. (2006b). From these bioassays, LC_{50} (lethal concentration that kills 50% of the population) values were calculated and compared to LC_{50}s determined in 2001 and 2002 (Prabhaker et al. 2006a).

Table 1. Insecticides tested in adult *H. vitripennis* bioassays in 2015.

Insecticide Class	Active Ingredient	Product	Application	Manufacturer
Neonicotinoid	Imidacloprid	Admire® Pro	soil	Bayer
	Thiamethoxam	Platinum® 75 SG	soil	Syngenta
	Acetamiprid	Assail® 70 WP	foliar	United Phosphorus
Butenolide	Flupyradifurone	Sivanto™ 200 SL	foliar	Bayer
Pyrethroid	Bifenthrin	Capture® 2 EC	foliar	FMC
	Fenpropathrin	Danitol® 2.4 EC	foliar	Valent
Organophosphorus	Chlorpyrifos	Lorsban® 4E	foliar	Dow
	Dimethoate	Dimethoate® 2.67 EC	foliar	Loveland

The data showed that GWSS tested in 2015 were less susceptible to the tested compounds than they were in 2001 and 2002. For the neonicotinoids, the LC_{50} values for thiamethoxam, imidacloprid, and acetamiprid were up to 1.78, 57.31, and 130 times, respectively, higher in 2015. Even larger differences existed for the pyrethroids bifenthrin (5066 times higher), and fenpropathrin (101 times higher) and the organophosphates chlorpyrifos (22190 times higher) and dimethoate (2150 times higher). We believe that the extraordinary differences in the pyrethroids and the organophosphates may be the result of different research protocols used in the 2001/2002 studies and the 2015 studies. In the earlier work, we used a petri dish assay which enclosed the treated leaves and insects, probably contributing to fumigation action and extremely the low LC_{50} values. In 2015, we used a screened clip cage which eliminated or greatly reduced the fumigation action of the insecticides. Even so, the data from all studies indicated that GWSS was less susceptible to most of the insecticides being used than it was 14 years ago. Similar results were obtained using topical bioassays for imidacloprid, bifenthrin, and fenpropathrin (Redak et al. 2015).

Of particular interest in our study was the fact that there was variation in the relative toxicities at different times and locations throughout the 2015 season (Perring et al. 2015). The LC_{50}s for imidacloprid increased 79-fold range from the first bioassay of the season to the last (Figure 2). However, bioassays for thiamethoxam showed a more modest range of responses that varied 26-fold between highest and lowest LC_{50}s. A third neonicotinoid, acetamiprid, was tested only one time from the Edison location and two times from the General Beale Road location, but also showed the same pattern of increasing LC_{50}s from General Beale Road as the season progressed. The two pyrethroids, bifenthrin and fenpropathrin, were equivalent to one another, but higher LC_{50}s occurred on the later sampling. The two organophosphate compounds were inconsistent in their responses, with low to high LC_{50}s. The recently registered butenolide insecticide flupyradifurone was tested only on the first and last dates, but also maintained the pattern of being less toxic against General Beale Road sharpshooters later in the season (Perring et al. 2015).

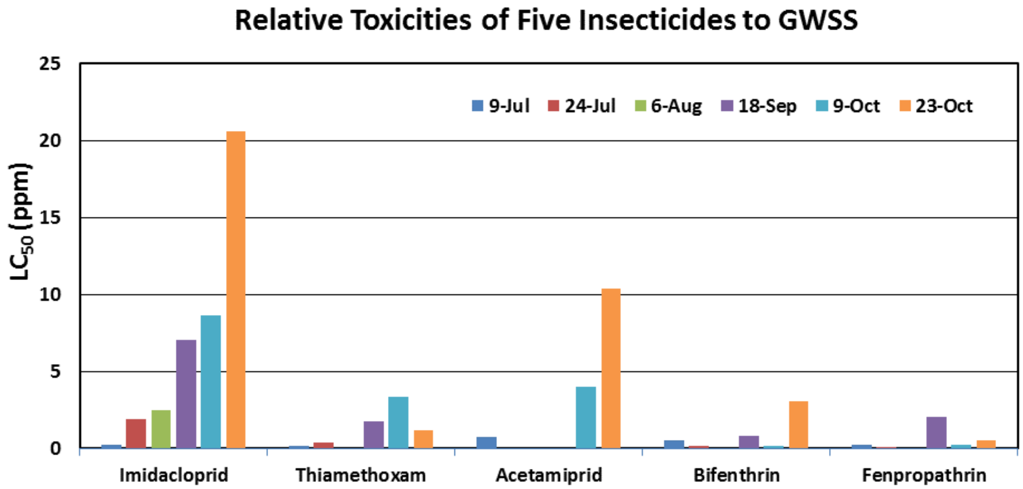


Figure 2. LC_{50}s for five insecticides tested over six dates between July 9 and October 23 in 2015. The first three columns of each series represent GWSS adults collected from an organic citrus field in the Edison area, whereas the second three columns represent collections from the General Beale Road area. Only three collection dates were tested against acetamiprid, and only five collections dates were tested against bifenthrin and fenpropathrin; all six collection dates were tested against imidacloprid and thiamethoxam. (From Perring et al. 2015)

Our previous work showed that GWSS was less susceptible to commonly used insecticides than it was in 2001-2002. Furthermore, the levels of susceptibility were geographically variable and dramatically declined over the course of the 2015 growing season (July-October). It is reasonable to think that consistent usage of materials over time would lead to resistance, and this is the most parsimonious explanation for the reduced toxicities measured in 2015 compared to 2001/2002 data. However, the variation in toxicity within the 2015 season also was related to location (organic vs. conventional) and time (higher LC_{50}s later in the season). These data suggest that factors like insecticide usage in a local context may be important determinants for how effective certain insecticides are in certain areas. Understanding these dynamics will lead to more informed selection of materials in the future.

Objectives

1. Conduct laboratory bioassays on field-collected *H. vitripennis* from Kern County to document the levels of resistance at the beginning of the 2016 and 2017 field seasons, and to document changes in susceptibility as each season progresses.
2. Document differences in insecticide susceptibility in GWSS collected from organic vs. non-organic vineyards (grapes) and/or orchards (citrus) and from different locations in Kern County.
3. Obtain and organize historic GWSS densities and treatment records (locations, chemicals used, and timing of applications) into a Geographic Information System for use in statistical analyses.
4. Determine the relationship between insecticide susceptibility of different GWSS populations and treatment history in the same geographic location and use relationships to inform future insecticide management strategies.

Activities and Accomplishments

Objective 1

Insecticide bioassays were conducted on *H. vitripennis* adults collected in table grapes on 26 July and 16 August, and in navel oranges on 4 October. Over 900 adults were obtained on 26 July, sufficient for testing six insecticides (Table 2) at five concentrations per insecticide plus an untreated control. Five replications of each insecticide concentration were used. Upon returning to the same vineyard on 16 August, only 300 adults were collected that provided enough insects for the testing of imidacloprid and thiamethoxam only. The 600+ adults collected on 4 October were highly dispersed in navel oranges and required sampling from numerous trees to collect enough insects for complete tests of four insecticides.

Bioassay procedures included a systemic uptake bioassay and leaf dip bioassay (Prabhaker et al. 2006a) that were used according to whether an insecticide was soil or foliar applied, respectively (Table 2). Five adults per clip cage were confined to treated citrus leaves for 24 h and then evaluated for mortality. The dose/mortality data were subjected to probit analysis to yield LC_{50}s and accompanying statistics for evaluating relative toxicities of the six insecticides.

Table 2. Insecticides tested in adult *H. vitripennis* bioassays in 2016.

Insecticide Class	Active Ingredient	Product	Application	Manufacturer
Neonicotinoid	Imidacloprid	Admire® Pro	soil	Bayer
	Thiamethoxam	Platinum® 75 SG	soil	Syngenta
	Acetamiprid	Assail® 70 WP	foliar	United Phosphorus
Pyrethroid	Bifenthrin	Capture® 2 EC	foliar	FMC
	Fenpropathrin	Danitol® 2.4 EC	foliar	Valent
Organophosphorus	Chlorpyrifos	Lorsban® 4E	foliar	Dow

Among the three neonicotinoid insecticides, LC_{50}s were highest for imidacloprid in Tests 1 and 3 in comparison to acetamiprid or thiamethoxam, but abnormally low in Test 2 relative to thiamethoxam (Table 3). The pyrethroid insecticides bifenthrin and fenpropathrin were similarly toxic to GWSS in Test 1 of 2016 as they had been in the 2015 bioassays. A second bioassay conducted with bifenthrin showed only a 2.2-fold difference in LC_{50}s between the July and October samples. The relative toxicity of chlorpyrifos (LC_{50}=11.49) to GWSS in Test 1 was considerably lower than for the other five insecticides, but it may be that the leaf-dip bioassay does not conform well to the toxicity profile of chlorpyrifos. Probit analyses on data from two chlorpyrifos bioassays in 2015 failed to yield an LC_{50} value, an indication of the mortality data not fitting the probit model. Variation in mortality data from field-collected insects is not unusual and is an important reason why multiple tests are required for confident interpretation of the results. Prior exposures of insects collected in the field to various insecticides are usually unknown, but could influence test results if residues are present on leaves or if contact by spray drift has occurred. Movement among crops and fields is facilitated by the strong flying capabilities of *H. vitripennis* and by the demand for higher amino acid content of xylem fluid that varies among host plants (Bi et al. 2007)

Table 3. Probit statistics for insecticides tested against *H. vitripennis* adults on three dates from July to October 2016.

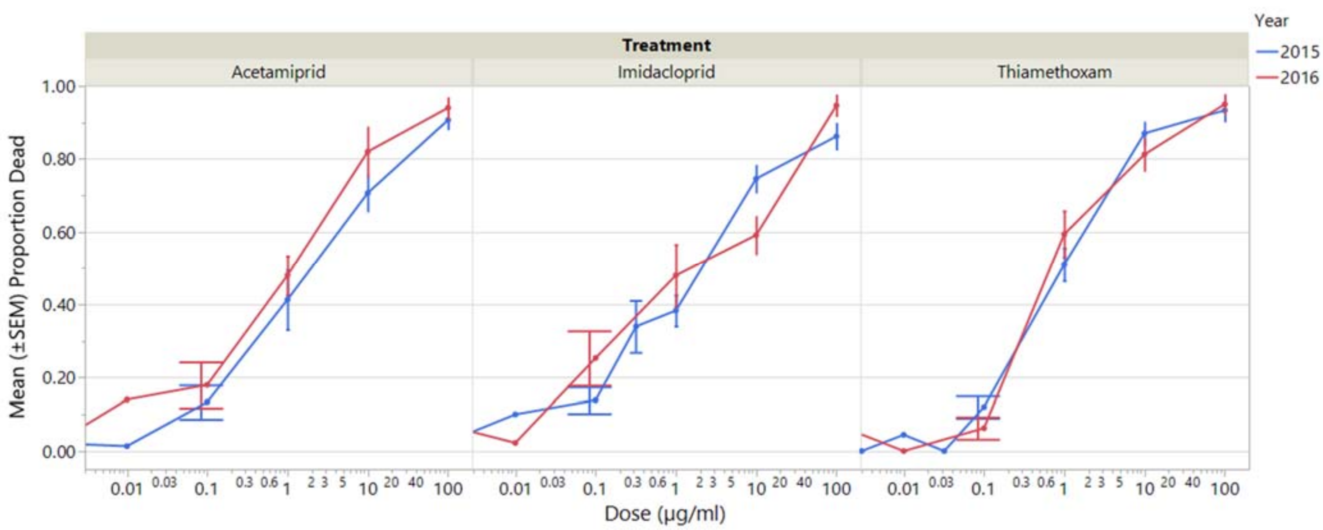
Location and Date	Compound	LC_{50} (µg/ml)	95% C.I.	Slope (± SE)	χ^2	df
July 26-28 Table Grapes (Test 1)	Imidacloprid	3.99	2.11 – 7.83	1.18 (0.19)	17.2	23
	Acetamiprid	1.76	0.66 – 5.15	0.59 (0.10)	15.6	23
	Thiamethoxam	0.53	0.32 – 0.84	2.45 (0.51)	10.2	22
	Bifenthrin	0.70	0.38 – 1.28	1.30 (0.20)	16.0	23
	Fenpropathrin	0.59	0.29 – 1.19	1.00 (0.15)	14.6	23
	Chlorpyrifos	11.49	2.05 – 357.83	0.44 (0.09)	37.6	23
Gen. Beale Rd Aug 16-17 Table Grapes (Test 2)	Imidacloprid	0.04	0 – 0.19	0.53 (0.16)	12.5	18
	Thiamethoxam	2.87	1.02 – 7.88	0.66 (0.13)	13.2	18

Gen. Beale Rd October 4-5 Navel Oranges (Test 3)	Imidacloprid	7.26	2.81 – 24.83	0.62 (0.11)	18.9	23
	Acetamiprid	0.40	0.16 – 1.02	0.97 (0.14)	32.1	23
	Thiamethoxam	1.21	0.68 – 2.09	1.34 (0.21)	20.4	22
	Bifenthrin	1.54	0.68 – 3.65	0.97 (0.14)	27.0	23

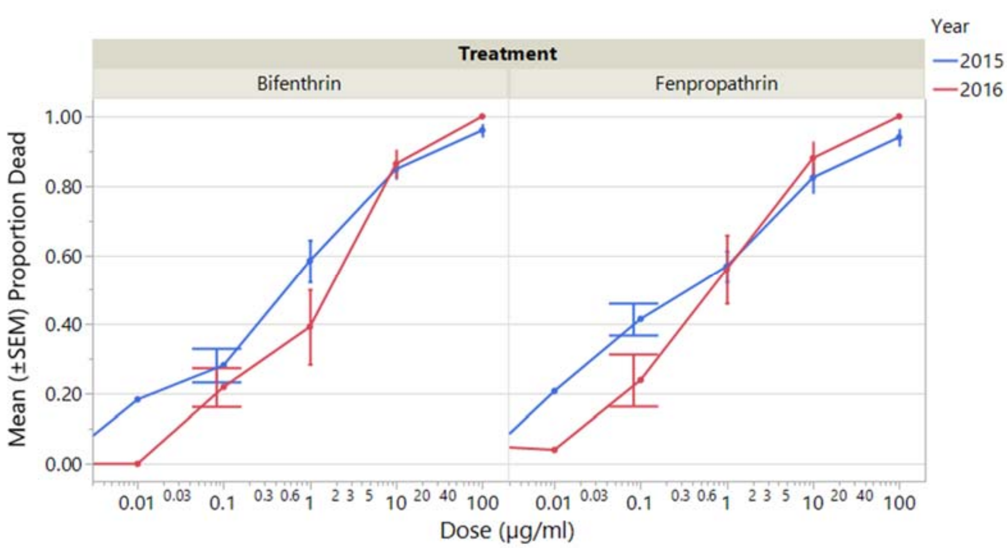
The drop in susceptibility to imidacloprid observed at the end of the 2015 season (Perring et al. 2015) raised real concerns that resistance to imidacloprid was present in *H. vitripennis* populations in the General Beale Road vicinity of Kern County. Not only did LC_{50}s for imidacloprid trend progressively upward through the 2015 season, a substantial decrease in susceptibility to acetamiprid also was observed on the last test date of 2015. However, a comparison of composite mortality curves from the 2015 and 2016 seasons for all three neonicotinoid insecticides indicate relatively little difference in mortalities at various concentrations of each insecticide (Fig. 3A). The only consistent difference (although not statistically) in mortality curves was for acetamiprid, to which *H. vitripennis* test insects in 2016 were actually slightly more susceptible than those tested in 2015. Relative differences in susceptibility to either imidacloprid or thiamethoxam varied inconsistently by concentration between years. Comparison of 2015-2016 mortality curves for the pyrethroids revealed a similar pattern for each compound (Fig. 3B). Higher mortalities were observed at lower concentrations in 2015, but then crossed over at either 10 µg/ml for bifenthrin or 1 µg/ml for fenpropathrin.

Fig. 3. Composite mortality curves for (A) three neonicotinoid insecticides and (B) two pyrethroids for 2015 and 2016.

A.



B.



Comparison of mortality curves for all five insecticides (Fig. 3) is rather tenuous due to the fewer number of bioassays conducted in 2016 relative to 2015. Nevertheless, identification of patterns of change to insecticide treatments in a particular population can only occur by gathering enough data points that reveal a trend or lack thereof. The related issue of what happened to *H. vitripennis* numbers in 2016 compared to previous years is one that should be addressed in the context of the pesticide use history in the General Beale Road area since 2001 and how it has affected annual variation in population densities. Has heavy insecticide use since 2001 caused resistance that has contributed to higher population densities over the last 4-7 years, or has pesticide use slackened in recent years to allow a resurgence of *H. vitripennis*? This question will be addressed as we begin to gather historical pesticide use records into a GIS platform that will enable us to relate spatial and temporal variation in pesticide use with present pesticide susceptibility.

Objective 2
The low number of GWSS in 2016 prevented robust collections of GWSS in organic and non-organic vineyards and citrus orchards. Thus we will approach this objective in 2017 with hopes of finding more insects.

Objectives 3 and 4
We have received crop coverage data from Kern County and have incorporated these coverages into a GIS, which will serve as our base layer. We are working on organizing all of the treatment records since 2001 and, using pesticide use records and assessor parcel numbers, we are creating GIS attribute layers that contain specific insecticides and the timing of those insecticide on vineyards and citrus orchards. At the same time, we are working with CDFA to obtain GWSS data from the last 16 years in the Kern County, Zone 3. This is the region that has had the highest GWSS densities and received the highest number of pesticide applications since 2001. There are thousands of traps, and our current efforts are aimed at cleaning up the trap data, eliminating duplications, and trying to get the data into unique attribute layers. Our goal is to see if we can document an impact of specific insecticide treatments on the numbers and locations of GWSS at various times post-treatment. Patterns that develop over repeated usage of the same materials could be an indication of reduced susceptibility to the insecticides. Furthermore, this study will inform us of repeated use of specific insecticides in certain fields, which may be related to levels of susceptibility that will be determined in our 2017 bioassays.

Publications and Presentations

Publications

Perring, T.M., Prabhaker, N., Castle, S., Haviland, D., Stone-Smith, B. 2016. Monitoring for insecticide resistance in the glassy-winged sharpshooter Homalodisca vitripennis (Germar) (Hemiptera: Cicadellidae) in California. Pp. 221-229 In T. Esser (Ed.) Proceedings, 2016 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Presentations

Perring, T.M., Prabhaker, N., Castle, S., Haviland, D., Stone-Smith, B. 2016. Insecticide resistance in GWSS: Using Historical Use Patterns for Future Management. Oral presentation (plenary session). 2016 Pierce's Disease Research Symposium, Sacramento, CA. 13 December, 2016.

Perring, T.M., Prabhaker, N., Castle, S., Haviland, D., Stone-Smith, B. 2016. Monitoring for GWSS Insecticide Resistance in California. Poster Presentation. 2016 Pierce's Disease Research Symposium, Sacramento, CA. 13 December, 2016.

Research Relevance Statement

Studies conducted in 2015 showed that GWSS were less susceptible to insecticides commonly being used to control it than they were in 2001 and 2002. Of particular concern was that the LC_{50} values for imidacloprid, bifenthrin, and fenpropathrin increased as the 2015 growing season progressed. Subsequent bioassays conducted in 2016 found the susceptibility to imidacloprid was well within the range, or lower than LC_{50}s observed in 2015. Similarly, LC_{50}s recorded for two other neonicotinoids, acetamiprid and thiamethoxam, and for two pyrethroids, bifenthrin and fenpropathrin, fell within the range of LC_{50}s for each compound observed in 2015. Thus there appeared to be no loss of efficacy due to insecticide resistance. Further monitoring conducted over the next few years should provide a more thorough evaluation of whether resistance to imidacloprid is occurring, and if so, where. Historical analyses of pesticide use patterns in relation to *H. vitripennis* yellow-sticky trap catches will provide essential information for understanding the basis of *H. vitripennis* resurgence in Kern County.

Layperson Summary

Insecticides have been a key component of the management program for Pierce's Disease, effectively reducing GWSS numbers. However, from 2012 through 2014 high population levels were present and densities in 2015 exceeded those in 2001, when the program began. In 2015, we documented lower susceptibilities to commonly used insecticides in Kern County populations of GWSS, with declining susceptibility as the season progressed. This suggested that treatment practices in the vicinity of the collection sites may have contributed to the lack of control. However, no further reduction in susceptibility was observed in the 2016 season, although fewer tests were conducted due to a decline in population densities compared to the previous year. Whether reduced GWSS numbers in 2016 were due to more aggressive insecticide applications or to natural variation is key to understanding the role that regional control programs play in GWSS management. In addition to continuing to monitor for resistance to insecticides, this project will explore the relationship between historical insecticide treatment records and current levels of susceptibility, informing how we effectively use insecticides in the future.

Status of Funds

This is a two year project that was initiated in July 2016. Funding expenditures are appropriate for the current place in the grant cycle.

Summary and Status of Intellectual Property

Aside from the published proceedings and the presentation at the CDFA PD conference, no intellectual property was produced as a result of this research project.

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