ABSTRACT

EFFECTS OF AN ANTHRANILIC DIAMIDE INSECTICIDE ON EARLY SEASON PEST CONTROL, QUALITY AND YIELD OF HEAD LETTUCE IN THE SALINAS VALLEY

The head lettuce (Lactuca sativa) industry valued at $962 million in 2014 is an integral component of California agriculture, with the Salinas Valley being one of the major production areas. A major concern for lettuce producers is how to effectively manage insecticides, such as the second generation anthranilic diamide (Group 28) cyantraniliprole marketed as Cyazypyr®, without creating resistance issues. This study examined insect control programs in lettuce using Cyazypyr® and a typical grower’s standard program to determine their impact on crop vigor, insect control and yield. A secondary objective was to evaluate the use of Normalized Difference Vegetation Index (NDVI) using the Trimble GreenSeeker®, as a quantitative method to measure crop vigor in comparison to existing visual vigor evaluation methods. Field trials were conducted in 2014 and 2015. Each experiment comprised of a randomized complete block design with four replications of an untreated check and Cyazypyr’s® different methods of application. Overall, there was a positive correlation between 0-200 visual vigor evaluations and NDVI ratings, with Greenseeker® NDVI readings of values of at least 0.75 providing a very strong (r²>0.8) indicator of crop vigor at the post thinning stage. The Verimark® alone soil surface band treatment provided the best control of Pea Leafminer. There were no significant marketable yield differences among treatments. However, lettuce treated with two foliar applications of Exirel® had the lowest number of culls, and therefore be the recommended application for the head lettuce grown on the clay and clay loam soils used in this study.

Kylie Baker
December 2016
EFFECTS OF AN ANTHRANILIC DIAMIDE INSECTICIDE ON EARLY SEASON PEST CONTROL, QUALITY AND YIELD OF HEAD LETTUCE IN THE SALINAS VALLEY

by

Kylie Baker

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Science in the Jordan College of Agricultural Sciences and Technology California State University, Fresno December 2016
APPROVED

For the Department of Plant Science:

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INTRODUCTION

Problem and Its Significance

The Salinas Valley of California’s Central Coast is synonymous with lettuce (*Lactuca sp.*) production, and for good reason. According to the California Department of Food and Agriculture (CDFA), 2014 lettuce production in California amounted to $2.032 billion (CDFA, 2016). Approximately 47% of that revenue was generated specifically from head lettuce production, amounting to $962 million (CDFA, 2016). The major production areas for iceberg (crisp head) lettuce in California are the Central Coast, the Southern Coast, and the Central Valley (Turini et al., 2011). Of those areas, head lettuce production is the highest in Monterey County, with 44,208 harvested acres in 2014 (Monterey County, 2014). With lettuce production ranking number six on California’s top ten valued commodities list for 2014, it is obvious that the head lettuce (*Lactuca sativa*) industry of Salinas Valley is an integral component of California’s agriculture.

To ensure the future of head lettuce production in the Salinas Valley for generations to come, and to continue to produce desirable yields, there is a need for new methods of improving crop protection, quality, and yield, while using sustainable and safe inputs. Among the many challenging problems facing lettuce growers in the Salinas Valley, a major concern is how to effectively manage insecticide use without creating resistance issues. Along with resistance issues, there are multiple looming restrictions for a number of important insecticides like dimethoate, endosulfan and diazinon, which may be followed by limitations of pyrethroids and other older products (Palumbo, 2011). Hence, there is a need for new chemistry to help maintain insecticide susceptibility in pest populations, and
also to explore the possible use patterns of insecticides that are safe and effective against common pests of lettuce (Natwick, 2011).

Two noticeable advantages of utilizing new modes of action are that new modes of action can serve as contenders for insecticide rotation programs, and can be accompanied by integrated pest management programs (Tiwari and Stelinski, 2012). In recent years, a new class of insecticides has been recognized, the anthranilic diamides (Group 28), which provide exceptional insect control by a unique mode of action, the ryanodine receptor (Cordova et al., 2006). Anthranilic diamide insecticides work by vigorously activating the ryanodine receptor, releasing stored calcium from the sarcoplasmic reticulum, which then triggers diminished regulation of muscle function (Cordova et al., 2006).

Of the Group 28 (diamide) insecticides, one product- cyantraniliprole, is now on the market and in the hands of the end user. Cyantraniliprole is a second generation anthranilic diamide insecticide that has recently been registered in California by DuPont Crop Protection™ under the trade name Cyazypyr®. One of the noteworthy aspects of Cyazypyr® is that it is a reduced risk insecticide, and has very low toxicity to vertebrates and non-target organisms (Tiwari and Stelinski, 2012). Cyantraniliprole, hereafter mentioned as Cyazypyr®, has proven to be proficient in controlling numerous Homoptera, Coleoptera, Diptera, Lepidoptera and Thysanoptera species (Teixeira and Andaloro, 2013). Although pest problems can vary depending on the growing region and time of year, the key pests of lettuce in California are leafminers, caterpillars, thrips, aphids and whiteflies (Turini et al., 2011), thus creating a unique fit for Cyazypyr® in the Salinas Valley head lettuce industry.

Within the crop protection industry, it is known that vigor indices can be valuable indicators of crop growth potential (Guthrie et al., 1993). Currently in
agricultural research field trials, a 0-200 visual vigor index scale is typically used to indicate crop vigor based on the untreated check (100 of the 0-200 scale). One other vigor index scale that is common is the 1-5 visual index scale, where a 3 value indicates the untreated check. Both of these vigor scales are completely qualitative scales even though they are represented by numerical values, and values on either scale can vary significantly depending on the person who is evaluating the plots in the trial. This can pose great difficulties in field trials where different individuals may be taking vigor evaluations for the same trial over a period of time. The fact that these two vigor scales are qualitative also makes it difficult to compare vigor ratings between trials if different individuals are involved with the trials. There is therefore a need for a simple, standardized way to measure crop vigor in the field and document the differences that can be observed visually in a quantitative fashion. This is especially important for a grower or person conducting field trials desirous of creating favorable conditions for vigorous crop growth as well as translating that vigorous growth into a more desirable yield (Guthrie et al., 1993).

One tool that could be used to achieve the goal of standardizing crop vigor evaluation methods is the Trimble GreenSeeker®, which uses Normalized Difference Vegetation Index (NDVI). The GreenSeeker® works by measuring the amount of red (visible) and infrared light that is reflected from the plant (Trimble, 2014). The NDVI value gives us an indirect measure of crop vigor in that a higher NDVI value indicates a larger amount of infrared light reflected by the plant and a more vigorous plant and a lower NDVI value indicates less infrared light reflected by the plant and a less vigorous plant. The GreenSeeker® and NDVI values may be a viable solution to replace the current unstandardized qualitative crop vigor
evaluation methods, and should therefore be assessed for its applicability to lettuce industry.

The problems facing California lettuce producers are complex, as growers have a high value crop to produce with continued limitations on options for pest control. New insecticide chemistries and their best use patterns need to be explored, as well as finding quantitative ways to measure crop vigor and health.
LITERATURE REVIEW

Group 28 (Diamide) Insecticides

During the mid-20th century, there were many discoveries in insecticide research that led to the capability to control almost any pest (Casida, 2015). This era was short lived due to methylcarbamates, organophosphates and chlorinated hydrocarbons losing their effectiveness on a selection of resistant pest strains or being banned due to toxicity issues (Casida, 2015).

With the discovery and development of diamide insecticides in the past 15 years, a new class of insecticides has been established that work by activating the ryanodine receptor (Lahm et al., 2005). Although this is a new mode of action for modern day synthetic insecticides, the ryanodine receptor has been of interest to agricultural insecticide researchers for some time. Ryanodine receptor channels get their name from a plant metabolite called ryanodine, which is a natural insecticide that affects calcium release by fixing the channels in a partly opened state (Lahm et al., 2005). In the 1940’s, the powdered stem wood of Ryania speciosa was used in integrated pest management programs as a botanical insecticide to control various lepidoptera larvae on corn and other crops (Casida, 2015). The development of Ryania speciosa came to a halt in the 1990s due to the intricacy of the structures involved, and because reconstructive chemistry did not improve the insecticidal activity (Casida, 2015). Nonetheless, this research did provide a basis for the development of modern day diamide insecticides. The modern class of diamide insecticides (Group 28), work by binding to the ryanodine receptors and triggering an uncontrolled release of calcium stores. Symptoms of control in pests start with feeding cessation, leading to lethargy, paralysis, and eventually death (Lahm et al., 2005).
The Insecticide Resistance Action Committee (IRAC) Group 28 (diamide) mode of action insecticides currently includes three different active ingredients; chlorantraniliprole, cyantraniliprole, and flubendiamide (IRAC, 2016). Although the mode of action (ryanodine receptor activation) is the same in all three active ingredients, chlorantraniliprole and cyantraniliprole belong to the anthranilic diamides sub group and flubendiamide belongs to the phthalic diamide sub group. The diamide class of insecticides have already acquired a large market share, and it is estimated that their global gross revenue will be more than $2 billion by 2020 (Teixeira and Andaloro, 2013). Lack of insecticide resistance management programs and education globally has led to large amounts of insecticide resistance in the past with other chemistries. This is an issue that diamide manufacturers and the industry wanted to combat early, so in 2008 the IRAC International Diamide Working Group was created in hopes to delay or prevent resistance (Teixeira and Andaloro, 2013). The IRAC Diamide Working Group has now grown to also include universities, government, consultants and the distribution channels along with manufacturing companies (Teixeira and Andaloro, 2013).

Anthranilic diamides have gained notoriety in the marketplace and set themselves apart from other chemistries because of their favorable ecological, biological and toxicological attributes (Teixeira and Andaloro, 2013). Finding the best methods to use and apply these new insecticides is part of the learning process as well, and can be critical to their success once they are in the hands of the end user. “Although most of the newly developed products that growers use are very effective against the key lettuce insect pests, they tend to be very expensive. Thus, it is critical that growers continue to explore how to use newer products more cost-effectively” (Palumbo, 2014). Through many years of research, the anthranilic
diamides have proven to offer a distinctive substitute for existing pest management approaches (Cordova et al., 2006). The development of diamide insecticides have helped to re-establish confidence that effective and safe pest control can still be achievable (Casida, 2015).

**Cyantraniliprole (Cyazypyr®)**

Early formulations of Cyazypyr® were developed by DuPont and granted United States patents in July 2007, years after the initial discovery of the compound (Hughes et al., 2004). As previously mentioned, Cyazypyr® is a second generation anthranilic diamide insecticide, the first generation being chlorantraniliprole (Rynaxypyr®- Altacor® and Coragen®). Cyazypyr® has two different methods of delivery, foliar applications as well as via root systemic uptake of the plant, making it usable for application directly to the soil via surface band, shank injection, drip irrigation, at plant hill drench (transplant water), and in furrow sprays (Teixeira and Andaloro, 2013). One other method of applying Cyazypyr® that is gaining popularity in vegetable crops is the tray drench application, where transplants are sprayed in the greenhouse before transplanting (Joseph, 2014). The foliar formulation of Cyazypyr® has been taken to market as Exirel®, while the soil applied formulation is being marketed as Verimark®. Exirel® and Verimark® were both registered for use in the United States in January 2014 (US EPA, 2014a; US EPA, 2014b). Verimark® was registered for use in California in May 2014, while Exirel® was registered in California in August 2014 (CA DPR, 2014a; CA DPR, 2014b).

Verimark® is classified as a United States Environmental Protection Agency (US EPA) category IV insecticide with no signal word. Exirel® is classified as an US EPA category III insecticide with a Caution signal word, due
to the fact that the product formulation is a suspoemulsion (oil in water emulsion) (US EPA, 2014b). The United States Environmental Protection Agency (US EPA) reduced risk committee evaluated Cyantraniliprole (Cyazypyr®) in 2012 and concluded that based on the ecotoxicity risk profiles and low mammalian toxicity, Cyazypyr® “poses less risk compared to registered alternatives” (US EPA, 2015).

In a 2010-2011 study conducted in Holtville, California by Eric Natwick with University of California Cooperative Extension- Imperial County, Cyazypyr® along with other insecticides were evaluated for control of Western Flower Thrips (Frankliniella occidentalis) in Romaine lettuce (Natwick, 2011). Yield evaluations were also conducted for number of marketable hearts, total number of hearts, percent of marketable hearts and kilograms of marketable hearts. This study showed that “Cyazypyr® applied as multiple foliar applications showed efficacy against thrips” (Natwick, 2011). Furthermore, all the treatments had significantly more Romaine hearts and higher percentages of marketable hearts than the untreated check.

In 2013-2014, Dr. John Palumbo with University of Arizona ran two head lettuce trials at the Yuma Valley Agriculture Center in Yuma, Arizona. The first study evaluated the efficacy of three foliar applications of Exirel® at 13 fluid ounces per acre compared to other diamides and insecticide standards for control of Beet Armyworm (Spodoptera exigua) and Cabbage Looper (Trichoplusia ni). This study showed that “the Exirel® treatment provided equivalent larval control to the other diamide insecticides (Coragen, Vetica, Voliam Xpress) as well to the industry standard treatments, Radiant and Proclaim + Pyrethroid (Palumbo, 2014). The second study evaluated the efficacy of a single shank application of Verimark® at 13.5 fluid ounces per acre compared to other diamides and
insecticide standards in controlling Leafminer (Liriomyza langei), Beet Armyworm (Spodoptera exigua) and Cabbage Looper (Trichoplusia ni). In this trial, there were two Verimark® treatments, one with Verimark® alone and one with Verimark® + Admire Pro. The results of this trial indicated that “overall the addition of Admire Pro with Verimark® did not improve Leafminer control over Verimark® applied alone, but both Verimark® soil treatments provided significantly better control than Coragen®+Admire Pro and Durivo” and that Verimark® alone provided Cabbage Looper and Beet Armyworm control that was equivalent to the other treatments. (Palumbo, 2014). Palumbo also states in his conclusions that “these results further validate previous studies that suggest Verimark® can provide excellent levels of cross-spectrum activity in head lettuce” (Palumbo, 2014).

Dr. Palumbo also ran a Green Peach Aphid efficacy trial in head lettuce in 2013 that included an Exirel® treatment with two foliar applications. The results from this trial showed that “all spray treatments significantly reduced the Green Peach Aphid numbers at each sample interval relative to the untreated check for 14 days” (Palumbo, 2013). On the majority of the evaluation dates, the data show that Exirel® performed similarly to Sivanto (Flupyradifurone -Bayer) and Torac (Tolfenpyrad -Nichino) in controlling Green Peach Aphid.

Even though all the field studies discussed above were conducted by investigators affiliated with US accredited universities or by independent cooperators and manufacturers, there is a lack of scientific peer reviewed literature on similar insecticide related field studies. Hence, in an attempt to partially fill that gap in the case of lettuce production in California, it is critical that scientists conduct collaborative publishable applied research with growers in the Salinas Valley.
Historical Crop Vigor Evaluation Methods

There is no one standard method of evaluating crop vigor. Along with different evaluation indexes, the definition of vigor varies greatly among crop species. This is partly due to the fact that in some crops, an indication of vegetative growth is desired while in others reproductive growth is preferred. There is an abundance of seed vigor definitions in the literature, but none for crop growth indices. Before determining the best way to evaluate crop vigor, it is important to clearly define what desirable aspects describe positive crop vigor in head lettuce.

According to the United States Department of Agriculture (USDA) lettuce grading standards, there are three grades for head lettuce, U.S. Fancy, U.S. No. 1 and U.S. No. 2 (USDA AMS, 1997). These standards are used consistently among growers and packers to determine harvest quality. The best USDA grade, U.S. Fancy, is defined as having the following basic requirements; fresh, green, not soft, and not burst, free from; decay, russet spotting, and doubles, free from injury by; tip burn, downy mildew, field freezing, and discoloration, not damaged by any other cause, fairly well trimmed (USDA AMS, 1997). There are also four USDA classes for head solidity (density); Hard, Firm, Fairly Firm and Soft. The most desirable class is Firm, meaning that the head is compact, but may yield slightly to moderate pressure. (USDA AMS, 1997). Firm heads are known to have the best storage life (Saltveit, 2014). Head density is critical in head lettuce, as soft heads are more prone to damage after harvest, and hard heads are more prone to develop physiological disorders during storage (Saltveit, 2014).

Unlike a succinct definition for seed vigor as “seed properties that determines potential for fast and uniform emergence, and development of seedlings under a wide range of field conditions” (AOSA, 2002), a comprehensive
search of the scientific literature revealed no concise definition for growth vigor in lettuce. However, based on the USDA grading standards for lettuce presented earlier and the following online definition of crop vigor as “a measure of the increase in plant growth or foliage volume through time after planting” (Biology Online, 2016), it was possible to formulate an applicable definition of crop vigor in head lettuce as follows: Vigorous head lettuce should adhere to all of the U.S. Fancy guidelines throughout the growing season including desirable head density, as well as show increased plant size or leaf count in comparison to less vigorous plants.

It is difficult to determine exactly what physiological functions within lettuce plants are attributed to the desirable qualities in head lettuce, possibly because these traits are addressed through seed selection. It is known however, that fertilizer requirements in head lettuce can greatly affect yield and quality. For example, an inadequate supply of Phosphorus (P) and Nitrogen (N) can limit photosynthesis and cell expansion causing leaf growth to be slower as well as a lower leaf area index (Marschner, 1995). Some studies show that there might be some quality and yield benefits to N and P fertilizer applications due to their role in modulating disease resistance (Marschner, 1995). Due to the importance of fertilizer requirements in head lettuce, a properly managed fertility management program along with a pest management program is critical to producing quality head lettuce.

Having now proposed a definition for crop vigor in head lettuce, it is now possible to further examine the different methods that are used to evaluate crop vigor in agricultural field trials. Four general necessities associated with a worthy vigor index are: (1) the evaluation is easy to conduct; (2) it reveals a balance between vegetative and reproductive growth; (3) it can be an early indicator for
management decisions; and, (4) it includes a basis for comparison (Silvertooth et al., 1996). These necessities for a worthy vigor index were developed for cotton management, but can justifiably be adopted in any other crop, including head lettuce.

Because there is no standard definition for crop vigor across all crop species, many different methods of evaluating this “vigor” have evolved over time. The most common methods of evaluating crop vigor in field trials is one of four index scales, 1-5, 1-9, 1-10 and 0-200. In a 2002 tomato cultivar study conducted by the University of Tennessee, plants were rated on a 1-5 scale, with 5 being the most vigorous rating (Coffey, 2002). The 1-5 scale was used again in a 1999 lettuce variety study from the University of Arizona (Zerkoune, 1999). In a 2008 tomato trial from Purdue, crop vigor was evaluated on a 1 (least vigorous) to 9 (most vigorous) scale (Maynard, 2008). The main issue with all of these different vigor scales, and using different vigor scales even within the same crop species is that it makes it very difficult to look at multiple studies and understand exactly what each scale means. In most of the studies that use crop vigor indices, the authors usually provide a definition for what the highest and lowest values mean, but they typically omit any description for the middle values, thereby leaving the intermediate assessments up for interpretation. The other problem with using all of these different scales is that the values may vary significantly depending on the person evaluating the study.

When discussing crop vigor evaluation methods, it is critical to identify the significance of measuring crop vigor. A vigor evaluation can signify the amount of growth that can be expected later in plant development (Silvertooth et al., 1996). The thought process is that if the plant has a better, more vigorous start in early plant stages that continues throughout the growing season, the end of season
yield should reflect that the crop was more vigorous, or it should take those more vigorous plants less time to reach harvest maturity. It is also important to develop a quantitative, non-destructive method for assessing crop vigor in head lettuce throughout the growing season.

**Normalized Difference Vegetation Index (NDVI) and the Trimble GreenSeeker®**

Along with new chemistries, new technologies are emerging that may have the potential to help quantify the possible crop vigor benefits of different products and application methods. There are new technologies that have been developed in recent years that are taking advantage of Normalized Difference Vegetation Index (NDVI) to determine crop vigor and fertilizer requirements. NVDI is expressed by the equation \( \text{NDVI} = (\text{NIR} - \text{RED})/ (\text{NIR} + \text{RED}) \), where NIR represents the near-infrared light and RED represents visible red light. Healthy, or vigorous vegetation absorbs a majority of the visible light that hits it while reflecting a large portion of the near-infrared light that it comes into contact with (Figure 1). Thinner, unhealthy or less vigorous vegetation reflects more of the visible light that hits it and less of the near-infrared light (Weier and Herring, 2000).

One company that has been a frontrunner in developing these technologies is Trimble, who manufactures the GreenSeeker® and GreenSeeker® Handheld devices. Commercially, the Trimble GreenSeeker® is being used in the field for many different crops to determine Nitrogen requirements. Along with the software program that the company sells, the GreenSeeker® allows the applicators to seamlessly apply varying rates of fertilizer as they travel through the field (Trimble, 2014). The device that was utilized in these thesis trials was the Trimble GreenSeeker® Handheld, a more compact version of the original device. The GreenSeeker® Handheld features an LCD screen, a high-quality optical sensor that
Figure 1. Illustration of how NDVI reading works on healthy vegetation (left) and unhealthy vegetation (right) Source: (Weier and Herring, 2000)

instantly measures plant vigor, and a hand grip with a pull trigger. The sensor works by emitting brief bursts of red (visible) and infrared light and then measuring the amount of each type of light that is reflected from the plant (Trimble, 2014). The sensor will continue to sample the area as long as the trigger remains pulled. The measured value is then displayed on the screen in terms of NDVI, which ranges from 0.00 to 0.99. The resolution and detection limit of the measurements taken by the GreenSeeker® is one hundredths. The strength of the detected light (closeness to 0.99) is a direct indicator of the health and vigor of the crop being sampled, therefore the higher the NDVI reading, the healthier and more vigorous the plant.

For over 20 years, The National Aeronautics and Space Administration (NASA) has been using satellites to gather NDVI data for various uses, including
using NDVI as an indicator of drought as well as monitoring major fluctuations in vegetation on Earth’s surface (Weier and Herring, 2000).

NDVI technology is now being used by a numerous individuals, companies, and countries to monitor crops in various ways, including handheld sensors, drones or unmanned aerial vehicles, and satellites. In a 2016 study conducted in South Korea, an unmanned aerial vehicle with NDVI sensing technology was used to evaluate rice paddies grown under different Nitrogen requirements. The researchers concluded that “remote sensing technology is a simple way to quantitatively obtain reliable two-dimensional remote sensing information on crop growth” (Jeong et al., 2016).

In a 4-year Australian canola variety study published in 2014, there was evidence that in season NDVI readings were well correlated with final grain yield. In that study, the authors suggested that canola breeders may use NDVI to quantitatively select for vigorous genotypes that are more likely to have higher grain yields (Cowley et al., 2014). In addition to the canola studies, there have been positive relationships found between NDVI values and grain yield in rice, wheat, maize, and soybean (Ma et al., 2001; Boken and Shaykewich, 2002; Basnyat et al., 2004; Gutierrez-Rodriguez et al., 2004; Teal et al., 2006; Holzapfel et al., 2009; Harrell et al., 2011).

One aspect of research to be considered is why different insecticide treatments may positively or negatively affect the NDVI readings. The early season plant protection provided by Cyazypyr® “helps produce stronger, resilient crops for more appealing, better results at harvest. By protecting tender vegetable plants…from many damaging and costly sucking and chewing pests, Verimark® helps foster stronger, healthier crops …for improved quality and yield at harvest (DuPont, 2016). These treatments may give the crop a chance to focus on
increasing nitrogen and chlorophyll content, which is what NDVI is indirectly measuring (higher NDVI readings mean greener, healthier plants).

The main gap in research when it comes to correlating crop vigor to end of season yield is that much of the research has been conducted on corn, soybean and grain crops (Trimble, 2014). The use of NDVI and remote sensing technologies is beginning to catch on in tree nuts, vines and vegetable crops, but there is not as much research readily available in vegetable crops, specifically head lettuce.
OBJECTIVES

1. This study examined insect control programs in lettuce using Cyazypyr® and a typical grower’s standard program to determine their impact on insect control and identify any additional benefits on yield and quality.

2. To evaluate the use of the Trimble GreenSeeker® Handheld device in the field to determine lettuce crop health and vigor by NDVI (Normalized Difference Vegetation Index).

3. Identify the benefit(s) of the Trimble GreenSeeker® Handheld device and define a standard procedure for using the device in lettuce production in the Salinas Valley.
MATERIALS AND METHODS

Two separate replicated field trials were conducted in 2014 and 2015 with head lettuce (*Lactuca sativa* L. cvs Bandelier & Regency) in Salinas, California. Both trials followed the same protocol and procedures outlined in Appendix A. Each trial was set up as a randomized complete block design (RCBD) with four replications. Each plot was 13.33 feet wide (two 80 inch, five line beds per plot) by 30 feet long. Care was taken to place the trials in locations of the grower’s fields that were uniform in topography and water retention. There were five treatments in each trial, including an untreated check (Table 1). Different application methods and combinations of Cyazypyr® formulations were used in these trials to determine what the best application method is to obtain the highest quality end product and provide early season pest control. The treatment list, rates and application timings in Table 1 was followed for both trials.

Table 1. Treatment List, Rates, and Application Timings for Both Trials

<table>
<thead>
<tr>
<th>TRT #</th>
<th>TREATMENT COMPONENT</th>
<th>FORMULATION</th>
<th>RATE</th>
<th>UNIT</th>
<th>TIMING</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>UNTRCHK, UNTREATED</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>ATPLANT, SOIL BAND APPLICATION AT PLANT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>FOLIAR, 14 DAYS AFTER PLANTING AND 14 DAYS LATER (broadcast)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>ATPLANT, SHANK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Verimark Soil Band at plant 13.5 fl oz/A + Admire 10.5 fl oz/A shank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&gt;VERIMARK (cyantraniliprole) (concentrated band)</td>
<td>SC 200.00 GL</td>
<td>13.50</td>
<td>FLOZ/A</td>
<td>01 ATPLANT</td>
</tr>
<tr>
<td>B</td>
<td>&gt;ADMIRE PRO (imidacloprid) (shank)</td>
<td>SC 4.60 LG</td>
<td>10.50</td>
<td>FLOZ/A</td>
<td>03 ATPLANT</td>
</tr>
<tr>
<td>2</td>
<td>Verimark Soil Band at plant 13.5 fl oz/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&gt;VERIMARK (cyantraniliprole) (concentrated band)</td>
<td>SC 200.00 GL</td>
<td>13.50</td>
<td>FLOZ/A</td>
<td>01 ATPLANT</td>
</tr>
<tr>
<td>3</td>
<td>Exirel foliar 14DAP fb Exirel foliar 14DA+Admire 10.5oz/A shank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&gt;EXIREL (cyantraniliprole) (foliar)</td>
<td>SE 100.00 GL</td>
<td>20.50</td>
<td>FLOZ/A</td>
<td>02 FOLIAR</td>
</tr>
<tr>
<td>B</td>
<td>&gt;EXIREL (cyantraniliprole) (foliar)</td>
<td>SE 100.00 GL</td>
<td>20.50</td>
<td>FLOZ/A</td>
<td>02 FOLIAR</td>
</tr>
<tr>
<td>C</td>
<td>&gt;ADMIRE PRO (imidacloprid) (shank)</td>
<td>SC 4.60 LG</td>
<td>10.50</td>
<td>FLOZ/A</td>
<td>03 ATPLANT</td>
</tr>
<tr>
<td>4</td>
<td>Admire Pro SC 10.5 fl oz/A shank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>ADMIRE PRO (imidacloprid) (shank)</td>
<td>SC 4.60 LG</td>
<td>10.50</td>
<td>FLOZ/A</td>
<td>03 ATPLANT</td>
</tr>
<tr>
<td>5</td>
<td>Untreated check</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>UNTREATED CHECK</td>
<td>NA</td>
<td>0.00</td>
<td>NA</td>
<td>00 UNTRCHK</td>
</tr>
</tbody>
</table>
The rates used for all products were the maximum label rates for head lettuce, as to assess the maximum potential benefits of each product. All cautions listed in the product labels and material safety data sheets (MSDS) were adhered to, including the use of personal protective equipment, application methods and requirements, reentry intervals, and pre-harvest intervals.

Irrigation and fertilizer application were performed by the grower as per his standard schedules for iceberg lettuce, and care was taken to ensure that all plots received nutrients and water at the same time. This ensured that fertilizer rate and water application did not confound the experiment and the research was focused on evaluating the performance of Cyazypyr® and its different formulations and application methods along with the grower’s standard program. The grower also had crews hand thin each trial at the appropriate timing to ensure 10-inch spacing between plants. It is important to note that the typical spacing within plants for head lettuce (thinning space) is usually 12 inches, but for small plot trials 10-inch spacing is used when taking trials to harvest to ensure that there are enough plants to harvest at the end of the season. The experimental plots were hand weeded.

**Trial 1 (2014)**

Trial 1 (2014) was conducted with grower J.S. Tamagni and Sons on the Machado ranch near the intersection of Blanco Road and Davis Road (36.66400°, -121.68161°). Trial one was planted on August 7, 2014. At plant applications were made at the same time as planting. The soil texture was clay, with 22% sand, 28% silt and 50% clay. The soil series was Clear Lake clay. The soil pH was 8.0, organic matter was 1.7% and the cation exchange rate (CEC) was 37.0 meq/100g. At planting, the air temperature was 63°F, relative humidity was 74% and wind speed was 3.5 miles per hour. The soil surface and subsurface was dry and the soil
temperature at 4 inches deep was 66°F. Bandelier variety iceberg lettuce was planted at 2.4-inch spacing and 164,000 seeds per acre.

All applications that occurred during planting were made by myself and my team using a tractor-pulled single bed planter that has been modified for small plot trials (see Appendix B). The single bed planter has the ability to apply the shank and soil band applications while planting the seeds using compressed carbon dioxide (CO₂) as the propellant for the applications (see Appendix B).

For the soil surface band applications at plant (treatments 1 and 2), 25% overage was included in the mix size to ensure that there would be enough product to charge the sprayer. The mix size for the at plant band applications was 5096.62 mL, split into two-3 liter bottles (Figure 2). Five TJ 4002E nozzles were used for the at plant band applications. The tractor was calibrated to deliver 29.35 gallons per acre at 40 PSI while traveling 2 miles per hour.

Figure 2. Treatment 1 (Verimark) at plant soil surface band- tank mixed for spraying
For the at plant shank applications (treatments 1, 3 and 4), 25% overage was included in the mix size to ensure that there would be enough product to charge the shanks. The mix size for the at plant shank applications was 4018.26 mL, split into two- 3 liter bottles (Figure 3). The tractor was calibrated to deliver 23.14 gallons per acre at 46 PSI while traveling 2 miles per hour.

![Image](image.png)

Figure 3. Treatment 1 (Admire Pro®) at plant shank- tank mixed for spraying

Treatment 3 included two foliar applications of Exirel®, one 14 days after planting and another 28 days after planting. The first foliar application of Exirel was on August 21, 2014. The sprayer used was a CO₂ powered backpack sprayer with 5 TJ 4002E nozzles directed at the seed lines (see Appendix B). The air temperature was 50°F, relative humidity was 71%, and wind speed was 1.2 miles per hour. The soil surface and subsurface was dry and the soil temperature at 4 inches deep was 72°F. The mix size was 3057.00 mL, split into two, 2-liter bottles.
The hand boom was calibrated to deliver 20.00 gallons per acre at 40 PSI. The crop stage at the first foliar application (August 21, 2014) was three true leaves.

The second foliar application of Exirel® for treatment 3 was applied on September 5, 2014. The sprayer used was a CO₂ powered backpack sprayer with 5 TJ 4002E nozzles directed at the seed lines. The air temperature was 53°F, relative humidity was 75%, and wind speed was 0.9 miles per hour. The soil surface was dry and the subsurface was moist. The soil temperature at 4 inches deep was 71°F. The mix size was 3057.00 mL, split into two, 2-liter bottles. The hand boom was calibrated to deliver 20.00 gallons per acre at 40 PSI. The crop stage at the second foliar application (September 5, 2014) was eight true leaves.

**Trial 2 (2015)**

The second trial (2015) was also conducted with J.S. Tamagni and Sons on their home ranch near the intersection of South Main Street and Foster Road. Trial two was planted on May 19, 2015, along with the “at plant” treatment applications outlined in Table 1. The soil texture was clay-loam, with 28% sand, 40% silt and 32% clay. The soil series was Salinas clay loam. The soil pH was 8.0, percent organic matter was 2.5 and the cation exchange rate (CEC) was 26.0 meg/100g. At planting, the soil temperature was 62°F, relative humidity was 57% and wind speed was 6.0 miles per hour. The soil surface and subsurface was moist and the soil temperature at 4 inches deep was 60°F. Regency variety iceberg lettuce was planted at 2.4-inch spacing and 164,000 seeds per acre.

All applications that occurred during planting were again made by myself and my team using the same tractor-pulled single bed planter that was used in Trial 1.
For the soil surface band applications at plant (treatments 1 and 2), 25% overage was included in the mix size to ensure that there would be enough product to charge the sprayer. The mix size for the at plant band applications was 5450.19 mL, split into two 3-liter bottles. Five TJ 4002E nozzles were used for the at plant band applications. The tractor was calibrated to deliver 31.37 gallons per acre at 40 PSI while traveling 2 miles per hour.

For the at plant shank applications (treatments 1, 3, and 4), 25% overage was included in the mix size to ensure that there would be enough product to charge the shanks. The mix size for the at plant shank applications was 3631.62 mL, split into two 3-liter bottles. The tractor was calibrated to deliver 20.90 gallons per acre at 40 PSI while traveling 2 miles per hour.

Treatment 3 included two foliar applications of Exirel®, one 14 days after planting and another 28 days after planting. The first foliar application of Exirel was on June 2, 2015. The sprayer used was a CO₂ powered backpack sprayer with 5 TJ 4002E nozzles directed at the seed lines. The air temperature was 65°F, relative humidity was 71%, and wind speed was 2.0 miles per hour. The soil surface and subsurface was dry and the soil temperature at 4 inches deep was 62°F. The mix size was 3057.00 mL, split into two, 2-liter bottles. The hand boom was calibrated to deliver 20.00 gallons per acre at 40 PSI. The crop stage at the first foliar application (June 2, 2015) was two true leaves.

The second foliar application of Exirel® for treatment 3 was applied on June 17, 2015. The sprayer used was a CO₂ powered backpack sprayer with 5 TJ 4002E nozzles directed at the seed lines. The soil temperature was 60°F, relative humidity was 69%, and wind speed was 5.0 miles per hour. The soil surface was dry and the subsurface was moist. The soil temperature at 4 inches deep was 70°F. The mix size was 3057.00 mL, split into two, 2-liter bottles. The hand boom was
calibrated to deliver 20.00 gallons per acre at 40 PSI. The crop stage at the second foliar application (June 17, 2015) was eight true leaves.

**Evaluations**

All evaluations discussed below were conducted for Trial 1 and Trial 2 using the same procedures with the exception of the harvest evaluations. All evaluations were conducted without knowledge of what treatment each plot received in order to prevent any bias in data outcome. The two exceptions to this were the crop response (phytototoxicity) and visual vigor evaluations. For these evaluation methods, the untreated plots were known in order to provide a basis for evaluation for all other plots.

**Crop Health Evaluations**

Caution was taken to ensure that the proper reentry interval had elapsed before entering the trials for all evaluations. Leaf counts were taken after the first true leaf emerged weekly until heads began to form. Ten plants were selected at random per plot for leaf count evaluations. For Trial 2 (2015), a 50 day after planting leaf count was planned to be conducted but upon arrival, the field was too wet from irrigation so the evaluation was not done. Crop destruct plant weights, with roots included, were taken after the first true leaf emerged, with follow up evaluations every other week until harvest. Ten plants were selected at random per plot for plant weight evaluations. To determine the effect of each treatment on emergence, plant stand counts were taken after seedling emergence, approximately 11 and 15 days after planting and 7-10 days post thinning. The number of seedlings that emerged per 20 foot of row was counted. Phytotoxicity ratings for potential crop response caused by the treatments were taken weekly starting 7 days after planting until harvest.
Crop Vigor/NDVI Evaluations

The GreenSeeker® was charged for four hours prior to use in the field. All weeds (dead or living) were manually removed from the trial before using the GreenSeeker® as to not hinder the NDVI reading for the crop. To ensure accuracy of the NDVI readings, the sensor was held thirty-six inches (36”) above the crop while the trigger was pulled. The sensor has an oval field of view, and the field of view for the sensor widens as the height of the sensor increases (Trimble, 2014). A base NDVI reading was taken to ensure that the GreenSeeker® can pick up the vigor differences in the field. This was done by first taking a measurement for a visibly less vigorous area of the field, and then take a reading for a visibly more vigorous area in the field. The measurement for the visibly more vigorous area was greater than the less vigorous area, showing that the GreenSeeker® would indeed be capable of reading the differences in vigor across the field. Starting with the untreated check, the “pull and walk” method was used to find a NDVI rating for two passes per plot (covering all five seed lines). The pull and walk method simply consists of pulling the trigger at the beginning of the first seed line in the plot and holding the trigger while walking to the end of the plot, then releasing the trigger. The NDVI value for each pass (2 passes per row, 2 rows per plot) was recorded and entered as subsample data to find the NDVI reading per plot. NDVI evaluations were taken every week starting after emergence until ten days before harvest.

Visual vigor evaluations were also taken on the same days as the NDVI evaluations. The industry standard, a 0-200 visual vigor index was used, with 100 being the Untreated Check to find the vigor rating per plot. Plots were visually compared to the Untreated Check using the before mentioned definition of lettuce crop vigor:
Vigorous head lettuce should adhere to all of the U.S. Fancy guidelines throughout the growing season including desirable head density, as well as show increased plant size or leaf count in comparison to less vigorous plants. Visual vigor evaluations were taken weekly starting 14 days after planting until harvest. Photo documentation was taken of the plots throughout the trials in order to capture any vigor differences between treatments as well as photos of the untreated check. During data analysis, the visual vigor ratings were compared to the NDVI readings to find any correlation or trends between the two methods to determine if using the GreenSeeker® is comparable to the visual vigor ratings and a reliable vigor rating tool.

Insect Control Evaluations

Insect evaluations were made at 7, 15, 22, 29 and 36 days after planting for the 2014 trial. For the 2015 trial, insect evaluations were conducted 7, 14, 21, 29, and 35 days after planting. Insect evaluations were made from the three middle seed lines of each bed in the plot, and five feet in from the ends of the plot. Ten plants were selected at random in each plot for insect evaluations, five plants from each bed in the plot. The number of insects per plant was recorded. The data presented is the mean count per plant from the four replications. Adults and nymphs/larvae were recorded separately. All pests present during each trial were recorded, including the anticipated pests: Pea Leafminer (2014 and 2015), Green Peach Aphid (2014), and Western Flower Thrips (2015). The percentage of plant damage by pea leaf miner due to stippling was recorded per plot. The number of pea leaf miner mines per five or ten plants per plot was also recorded.
Yield Evaluations

For Trial 1 (2014), harvest was made by a local, experienced commercial harvest crew in order to determine the acceptable harvest qualities. It was planned to again use a harvest crew for Trial 2 (2015), but unfortunately due to a shortage of labor, the harvest for Trial 2 (2015) was conducted by myself and my team.

For both trials, the yield in pounds per plot as well as pack out (number of boxes) per plot was recorded so that per acre yield could be calculated. Only US Fancy grade lettuce was harvested for both trials (Figure 4) (USDA AMS, 1997).

§51.2510 U.S. Fancy.
"U.S. Fancy" consists of heads of lettuce which meet the following requirements:
(a) Basic requirements:
   (1) Similar varietal characteristics;
   (2) Fresh;
   (3) Green;
   (4) Not soft;
   (5) Not burst;
(b) Free from:
   (1) Decay;
   (2) Russet spotting;
   (3) Doubles;
(c) Free from injury by:
   (1) Tipburn;
   (2) Downy mildew;
   (3) Field freezing;
   (4) Discoloration;
(d) Not damaged by any other cause.
(e) Each head shall be fairly well trimmed unless specified as closely trimmed.
(f) For tolerances see §51.2513.

Figure 4. US Fancy head lettuce requirements description

The number of unmarketable heads (culls) per plot at harvest was recorded by counting the number of heads that were left in the field after harvest for various reasons (disease, pest damage, size, etc.). Photo documentation of harvests was also recorded.
Data Analysis

All data was analyzed using FieldPro Biodata Management version 2015.09a (Heartland Technologies, Inc.). FieldPro has the ability to run a multitude of statistical tests through SAS, including data averages, ANOVA tables, F test, LSD, standard deviation, Duncan's Multiple Range Test (DMRT), Tukey’s HSD, and coefficient of variance. Data and statistical reports were exported from FieldPro to Excel spreadsheets and Word documents. Subsample data was entered where applicable (insect counts, NDVI readings, leaf counts, yield evaluations). Using FieldPro for these trials was crucial to capture all of the essential information about the trials, including application information, equipment, weather data, pest and crop species and stages, and treatment lists. FieldPro is currently being used by DuPont and their cooperators as the primary data management software, and I have been provided with extensive training on the use of the program in my role as the Western Region Data Specialist. Using FieldPro to capture data for this project ensured that all the integral information about the trials was recorded, analyzed, and easily accessible.

Statistical analysis for all evaluation parameters was performed using SAS Institute, Inc. 2000 by means of FieldPro export. A one-way ANOVA was conducted at $\alpha=0.05$ for all evaluation parameters for both trials. Where significant differences were found, the means were separated using Tukey’s (HSD) test. Regressions were conducted in Excel v. 2016 to determine the relationship between visual vigor ($y$) and NDVI ($x$).
RESULTS AND DISCUSSION

Crop Health

Leaf Counts

For Trial 1 (2014), leaf counts were taken 15, 22, 29, 36, and 50 Days After Planting (DAP) (Figure 5). At 15 DAP, the mean leaf count per plant did not show any differences among treatments according to Tukey’s HSD at α=0.05.

At 22 DAP there was a significant difference between treatments according to Tukey’s HSD at α=0.05, (F (4,12) =4.31, p = 0.0216). Verimark® + Admire Pro, Verimark® alone, and Admire Pro followed by (fb) Exirel® treatments had significantly more leaves per plant than the grower’s standard treatment (Admire Pro alone) and the Untreated Check. At 29 DAP, there was a significant difference
between treatments (F (4,12) = 5.99, p = 0.0069), as the grower’s standard
treatment (Admire Pro alone) had approximately 14% less leaves than the
Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments.
At 36 DAP, there was a significant difference between treatments (F (4,12)
= 16.52, p = <0.0001). The Verimark® + Admire Pro and Verimark® alone
treatments had significantly more leaves per plant than the grower’s standard
(Admire Pro alone) and Untreated Check at 36 DAP. At 50 DAP, there was a
significant difference between treatments (F (4,12) = 23.08, p = <0.0001).
Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments
had approximately 33% more leaves per plant than the grower’s standard
treatment (Admire Pro alone).

For Trial 2 (2015), leaf counts were taken 14, 21, 29, and 35 Days After
Planting (DAP) (Figure 6). Similar to Trial 1, at 14 DAP the mean leaf count per
plant did not show any differences among treatments according to Tukey’s HSD at
α = 0.05. At 21 DAP there was a significant difference between treatments
according to Tukey’s HSD at α = 0.05, (F (4,12) = 6.31, p = 0.0057). All four of the
treatments had significantly more leaves than the Untreated Check at 21 DAP. At
29 DAP, the mean leaf count per plant did not show any differences among
treatments according to Tukey’s HSD at α = 0.05. At 35 DAP, there was a
significant difference between treatments according to Tukey’s HSD at α = 0.05, (F
(4,12) = 5.01, p = 0.0131). The Verimark® + Admire Pro and Admire Pro fb
Exirel® treatments had significantly more leaves per plant than the grower’s
standard treatment (Admire Pro alone).
For both trials, on the majority of evaluation dates when there were significant differences in leaf count per plant among treatments, the treatments that included Cyazypyr® had higher leaf counts compared to the grower’s standard treatment (Admire Pro alone) and the Untreated Check. As reported by Portillo et al., (2014) for citrus in Florida, Cyazypyr® insecticides can be very effective, through their mode of action that impacts insect behavior by impairing muscle function, in controlling a cross-spectrum of insect pests including Lepidoptera, Dipteran leafminers, fruit flies, beetles, whiteflies, thrips, aphids, leafhoppers. The higher lettuce leaf counts observed in the Cyazypyr® enhanced treatments in the current study would imply that Exirel® and Verimark® were effective, thereby resulting in increased plant protection and overall leaf growth.
**Plant Weight**

For Trial 1 (2014) plant weights were recorded at 22, 36, and 50 DAP (Figure 7). At 22 DAP, there was a significant difference between treatments for plant weight in ounces ($F (4,12) = 19.40$, $p = <0.0001$). At 22 DAP, the Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly heavier heads than the grower’s standard (Admire Pro alone) and the Untreated treatments. At 36 DAP, there was a significant difference between treatments for plant weight in ounces ($F (4,12) = 37.74$, $p = <0.0001$). At 36 DAP, the Verimark® alone treatment had significantly heavier heads than the grower’s standard (Admire Pro alone) and the Untreated treatments. At 50 DAP, there was a significant difference between treatments for plant weight in ounces ($F (4,12) = 12.62$, $p = 0.0003$). At 50 DAP, the Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had approximately 28% heavier heads than the grower’s standard (Admire Pro alone) and the Untreated treatments.

![TRIAL 1 (2014) - PLANT WEIGHT OZ/PLANT](image)

*Figure 7. Trial 1 (2014) plant weight in ounces per plant*
For Trial 2 (2015) plant weights were recorded 22, 36, and 50 DAP (Figure 8). At 21 and 35 DAP, there was no significant difference among treatments for plant weight in ounces according to Tukey’s HSD at α=0.05. At 55 DAP, there was a significant difference between treatments for plant weight in ounces (F (4,12) =19.97, p = <0.0001). The Verimark® alone and Verimark® + Admire Pro treatments had significantly heavier heads than the grower’s standard (Admire Pro alone) and the Untreated treatments.

![Trial 2 (2015) plant weight in ounces per plant](image)

*Different letters are significantly different according to Tukey's test at α = 0.05.*

Figure 8. Trial 2 (2015) plant weight in ounces per plant

For both trials on the evaluation dates when there were significant differences between treatments in plant weight in ounces, the treatments that included Cyazypyr® had higher plant weights compared to the grower’s standard treatment (Admire Pro alone). This finding further supports DuPont’s early season pest control statement, in that Cyazypyr protects plants early on and lets them
grow more vigorously in conditions where there are pests present (Portillo et al., 2014).

Stand Counts

Stand counts (count of plants per 20 foot of row per plot) were conducted to ensure that none of the at plant treatments had a negative effect on crop emergence. For Trial 1 (2014), stand counts were taken 11, 15 and 22 DAP (Figure 9). At all three evaluation dates, the stand counts did not show any differences among treatments according to Tukey’s HSD at $\alpha=0.05$.

![Trials 1 and 2 Stand Counts](chart)

**Figure 9.** Trial 1 (2014) stand count per 20 feet of row

For Trial 2 (2015), stand counts were taken 11, 14 and 21 DAP (Figure 10). At all three evaluation dates, the stand counts did not show any differences among treatments according to Tukey’s HSD at $\alpha=0.05$. 
The data from these trials show that none of the at plant treatments in either year had a negative effect on crop emergence (stand count). These findings further endorse the mode of action of Cyazypyr® in that it impacts insect behavior by impairing muscle function, and is not expected to have any negative direct impact on seed emergence and the early establishment of lettuce in the Salinas Valley.

**Crop Response**

Visual phytotoxicity evaluations for crop response caused by the treatments were taken weekly for both trials, starting at 7 DAP until harvest. As was previously documented by Natwick (2012), no negative crop response was observed with any of the treatments in both trials at any of the evaluation dates (data not shown). This was as expected, since all of the products tested are registered for use in California and should not have caused any negative crop response.
Crop Vigor/NDVI

Visual Vigor (0-200) and NDVI

In Trial 1 (2014), visual vigor (0-200) and NDVI evaluations were conducted 15, 22, 29, 43, 50, and 57 DAP. At 15 DAP, there was a significant difference among the treatments for both the visual vigor (F (4,12) =9.70, p = 0.001) and NDVI evaluations (F (4,12) =41.38, p = <0.0001) (Figure 11).

![TRIAL 1 (2014) 15 DAP - VISUAL VIGOR & NDVI](image)

*Different letters are significantly different according to Tukeys test at α = 0.05.*

Figure 11. Trial 1 (2014) 15 DAP visual vigor and NDVI

The Verimark® + Admire Pro treatment had the highest visual vigor and NDVI ratings 15 DAP. At 22 DAP, there was a significant difference between treatments for both the visual vigor (F (4,12) =81.0, p =< 0.0001) and NDVI evaluations (F (4,12) =36.51, p = <0.0001) (Figure 12). The Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly
higher ratings for visual vigor and NDVI than the grower’s standard (Admire Pro alone) and Untreated treatments at 22 DAP.

Figure 12. Trial 1 (2014) 22 DAP visual vigor and NDVI

At 29 DAP, there was a significant difference between treatments for both the visual vigor (F (4,12) =3.58, p = 0.0383) and NDVI evaluations (F (4,12) =15.03, p = 0.0001) (Figure 13). The Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly higher NDVI ratings than the grower’s standard (Admire Pro alone) and Untreated treatments at 29 DAP. The Verimark® alone treatment had significantly higher ratings than the grower’s standard (Admire Pro alone) for the visual vigor evaluation at 29 DAP.
At 43 DAP, there was a significant difference between treatments for both the visual vigor (F (4,12) =19.28, p =< 0.0001) and NDVI evaluations (F (4,12) =19.13, p = <0.0001) (Figure 14). The Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly higher ratings for visual vigor and NDVI than the grower’s standard (Admire Pro alone) treatment at 43 DAP.

At 50 DAP, there was a significant difference between treatments for both the visual vigor (F (4,12) =10.35, p = 0.0007) and NDVI evaluations (F (4,12) =14.46, p = 0.0002) (Figure 15). The Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly higher ratings for visual vigor and NDVI than the grower’s standard (Admire Pro alone) treatment at 50 DAP.
Figure 14. Trial 1 (2014) 43 DAP visual vigor and NDVI

Figure 15. Trial 1 (2014) 50 DAP visual vigor and NDVI
At 57 DAP, there was a significant difference between treatments for the visual vigor evaluation \( (F(4,12) = 21.44, p < 0.0001) \) (Figure 16). The Verimark\(^\text{®}\) + Admire Pro, Verimark\(^\text{®}\) alone, and Admire Pro fb Exirel\(^\text{®}\) treatments had significantly higher ratings for visual vigor than the grower’s standard (Admire Pro alone) treatment at 57 DAP. At 57 DAP, there was no significant difference among treatments for the NDVI evaluation according to Tukey’s HSD at \( \alpha = 0.05 \).

**Figure 16. Trial 1 (2014) 57 DAP visual vigor and NDVI**

In Trial 2 (2015), visual vigor (0-200) and NDVI evaluations were conducted 21, 29, 35, 41, 48, and 55 DAP. At 21 DAP, there was a significant difference between treatments for the visual vigor evaluation \( (F(4,12) = 3.69, p = 0.0348) \) (Figure 17). The Verimark\(^\text{®}\) + Admire Pro treatment had a significantly higher visual vigor rating than the grower’s standard (Admire Pro alone)
treatment. At 21 DAP, there was no significant difference among treatments for the NDVI evaluation according to Tukey’s HSD at \( \alpha=0.05 \).

![Trial 2 (2015) 21 DAP - Visual Vigor & NDVI](image)

**Figure 17.** Trial 2 (2015) 21 DAP visual vigor and NDVI

At 29 DAP, there was a significant difference between treatments for both the visual vigor (\( F (4,12) =15.94, p = <0.0001 \)) and NDVI evaluations (\( F (4,12) =5.34, p = 0.0105 \)) (Figure 18). The Verimark\textsuperscript{®} alone treatment had a significantly higher visual vigor rating than the grower’s standard (Admire Pro alone) treatment at 29 DAP. For the 29 DAP NDVI evaluation, the Verimark\textsuperscript{®} + Admire Pro treatment had a significantly higher rating than the grower’s standard treatment (Admire Pro alone).
At 35 DAP, there was a significant difference between treatments for both the visual vigor (F (4,12) =4.46, p = 0.0193) and NDVI evaluations (F (4,12) =11.31, p = 0.0005) (Figure 19). The Verimark® alone treatment had significantly higher ratings for visual vigor and NDVI than the grower’s standard (Admire Pro alone) treatment at 35 DAP.

At 41 DAP, there was no significant difference among treatments for the visual vigor or NDVI evaluations according to Tukey’s HSD at α=0.05 (Figure 20).

At 48 DAP, there was no significant difference among treatments for the visual vigor evaluation according to Tukey’s HSD at α=0.05. However, at 48 DAP, there was a significant difference between treatments for the NDVI evaluation (F (4,12) =25.79, p =<0.0001) (Figure 21). The Verimark® + Admire Pro, Verimark® alone and Admire Pro fb Exirel® treatments had significantly higher NDVI ratings than the grower’s standard (Admire Pro alone) and Untreated treatments.
Figure 19. Trial 2 (2015) 35 DAP visual vigor and NDVI

Figure 20. Trial 2 (2015) 41 DAP visual vigor and NDVI
At 55 DAP, there was no significant difference among treatments for the visual vigor or NDVI evaluations according to Tukey’s HSD at $\alpha=0.05$ (Figure 22).

For both trials on the majority of evaluation dates when there were significant differences between treatments for the visual vigor and NDVI evaluations, the treatments that included Cyazypyr® had higher ratings when compared to the grower’s standard treatment (Admire Pro alone). The increase in NDVI values throughout the season for both trials was indicative of plant growth and accumulation of above ground biomass. This is based on the principle that vigorous vegetation absorbs a majority of the visible light that hits it while reflecting a large portion of the near-infrared light that it comes into contact with, whereas thinner or less vigorous vegetation reflects more of the visible light that hits it and less of the near-infrared light (Weier and Herring, 2000).
In addition to evaluating the effect of the various treatments on growth vigor, linear regression analyses were run in Excel v.2016 to determine the relationship between visual vigor (y) and NDVI (x) in 2014 (Table 2) and 2015 (Table 3), and to see if NDVI can be used to predict visual vigor. Based on the coefficient of determination ($r^2$) values and their overall closeness to 1 for both years, there is evidence that NDVI ratings obtained in the latter half of the lettuce crop growth can be used a reliable predictor of visual vigor.

Generally, as the plants developed the NDVI ratings appeared to be a better indicator of visual vigor. For example, in Trial 1 (2014), at 57 DAP the $r^2$ value was 0.897 compared to at 15 DAP when the $r^2$ value was 0.576. Based on the results from the current study, there is a positive linear relationship between 0-200 visual vigor evaluations and NDVI ratings. Similar positive relationships have been found between NDVI values and grain yield in rice, wheat, maize, and soybean (Ma et al., 2001; Boken and Shaykewich, 2002; Basnyat et al., 2004; Gutierrez-Rodriguez et al., 2004; Teal et al., 2006; and Holzapfel et al., 2009).
Table 2. Trial 1 (2014)- Relationship Between Visual Vigor (y) and NDVI (x) by Days After Planting (DAP)

<table>
<thead>
<tr>
<th>DAP</th>
<th>Linear Regression Equation</th>
<th>$r^2$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>$y = 102.51x + 88.68$</td>
<td>0.576</td>
<td>0.137</td>
</tr>
<tr>
<td>22</td>
<td>$y = 221.41x + 75.69$</td>
<td>0.751</td>
<td>0.057</td>
</tr>
<tr>
<td>29</td>
<td>$y = 50.26x + 85.10$</td>
<td>0.871</td>
<td>0.02</td>
</tr>
<tr>
<td>43</td>
<td>$y = 113.41x + 15.39$</td>
<td>0.784</td>
<td>0.046</td>
</tr>
<tr>
<td>50</td>
<td>$y = 219.72x - 66.51$</td>
<td>0.898</td>
<td>0.014</td>
</tr>
<tr>
<td>57</td>
<td>$y = 790.61x - 450.63$</td>
<td>0.897</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Table 3. Trial 2 (2015)-Relationship Between Visual Vigor (y) and NDVI (x) by Days After Planting (DAP)

<table>
<thead>
<tr>
<th>DAP</th>
<th>Linear Regression Equation</th>
<th>$r^2$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>$y = 446.17x + 18.15$</td>
<td>0.406</td>
<td>0.248</td>
</tr>
<tr>
<td>29</td>
<td>$y = 282.92x + 31.21$</td>
<td>0.466</td>
<td>0.204</td>
</tr>
<tr>
<td>35</td>
<td>$y = 127.84x + 51.86$</td>
<td>0.577</td>
<td>0.136</td>
</tr>
<tr>
<td>41</td>
<td>$y = 106.74x + 36.75$</td>
<td>0.938</td>
<td>0.007</td>
</tr>
<tr>
<td>48</td>
<td>$y = 108.45x + 15.08$</td>
<td>0.897</td>
<td>0.015</td>
</tr>
<tr>
<td>55</td>
<td>$y = 70.40x + 44.63$</td>
<td>0.915</td>
<td>0.011</td>
</tr>
</tbody>
</table>

In addition to examining the correlation between visual vigor and the NDVI values as a function of DAP, an attempt was also made to look at the relationship as function of the GreenSeeker® NDVI values (Table 4). Generally, the GreenSeeker® readings were less correlated with leaf N concentration at early stages of establishment due to plants small size and background noise. However, the goodness of fit ($r^2$) was at least 0.70 when the NDVI readings were at least
0.75. Furthermore, the \( r^2 \) values increased when the Visual Vigor vs NDVI data were fitted with a quadratic equation (Table 4), as have been conducted in related studies.

Table 4. Relationship Between Visual Vigor (y) and NDVI (x) Values Greater than 0.75

<table>
<thead>
<tr>
<th>Year</th>
<th>Model</th>
<th>Regression Equation</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Linear</td>
<td>( y = 256.6x - 95.33 )</td>
<td>0.70</td>
</tr>
<tr>
<td>2014</td>
<td>Quadratic</td>
<td>( y = -19257x^2 +30290x - 11803 )</td>
<td>0.96</td>
</tr>
<tr>
<td>2015</td>
<td>Linear</td>
<td>( y = 93.2x +26.91 )</td>
<td>0.84</td>
</tr>
<tr>
<td>2015</td>
<td>Quadratic</td>
<td>( y = 2184x^2 - 3337.6x +1373.9 )</td>
<td>0.88</td>
</tr>
</tbody>
</table>

In summary, while there have been studies on crops such as rice (Ali et al., 2014), wheat, oats (Erdle et al., 2011; Li et al., 2010; and Feng et al., 2008) and corn (Guo et al., 2008; and Freeman et al., 2007, among others) that examined the relationship between specific plant parameters and GreenSeeker® NDVI readings, the current study appears to be the first attempt in California to determine a relationship between lettuce crop growth visual vigor and the GreenSeeker® readings. Based on the \( r^2 \) values obtained, the recommendation would be that for the purpose of assessing the visual growth vigor of head lettuce with the use of GreenSeeker®, growers should rely on NDVI reading of 0.75 or greater. This information is also valuable for researchers looking for quantitative methods to replace the current qualitative visual vigor rating scales, and future work on head lettuce grown in the Salinas valley should also examine the relationship between NDVI values and parameters such Growing Degree Days (GDD), plant chlorophyll content, and Nitrogen (N) status of the crop.
Pea Leafminer

Pea Leafminer was present in both Trial 1 (2014) and Trial 2 (2015). For Trial 1 (2014) mine counts per plant caused by Pea Leafminer were counted 7, 22, 29, and 36 DAP (Figure 23). At 7 DAP, Pea Leafminer was not yet present in the trial (data not shown). At 22 DAP, there was a significant difference between treatments for the mine count (F (4,12) = 74.53, p =<0.0001) evaluation. The Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had approximately 97% less mines per plant than the grower’s standard (Admire Pro alone) treatment at 22 DAP.

Figure 23. Trial 1 (2014) Pea Leafminer mine count per plant

At 29 DAP, there was a significant difference between treatments for the mine count (F (4,12) = 28.81, p =<0.0001) evaluation. The Verimark® + Admire Pro and Verimark® alone, and Admire Pro fb Exirel® treatments had
approximately 95% less mines per plant than the grower’s standard (Admire Pro alone) treatment at 29 DAP. At 36 DAP, there was a significant difference between treatments for the mine count ($F(4,12) = 14.35$, $p = 0.0002$) evaluation. The Verimark® + Admire Pro and Verimark® alone, and Admire Pro fb Exirel® treatments had approximately 60% less mines per plant than the grower’s standard (Admire Pro alone) treatment.

For Trial 2 (2015) mine counts per plant caused by Pea Leafminer were counted 7, 14, 21, 29, and 35 DAP (Figure 24). At 7 and 14 DAP, Pea Leafminer was not yet present in the trial (data not shown). At 21 DAP, there was a significant difference between treatments for the mine count ($F(4,12) = 8.27$, $p = 0.0019$) evaluation. The Verimark® + Admire Pro and Verimark® alone, and Admire Pro fb Exirel® treatments had significantly less mines per plant than the Untreated Check.

![Figure 24. Trial 2 (2015) Pea Leafminer mine count per plant](image)
At 29 DAP, there was a significant difference between treatments for the mine count \( (F(4,12) = 26.54, p < 0.0001) \) evaluation. The Verimark\textsuperscript{®} + Admire Pro and Verimark\textsuperscript{®} alone treatments had approximately 97% less mines per plant than the grower’s standard (Admire Pro alone) treatment. At 35 DAP, there was a significant difference between treatments for the mine count \( (F(4,12) = 29.98, p < 0.0001) \) evaluation. The Verimark\textsuperscript{®} alone treatment had approximately 86% less mines per plant than the grower’s standard (Admire Pro alone) treatment.

For Trial 1 (2014) percent stippling damage per plot caused by Pea Leafminer was evaluated 7, 22, 29, and 36 DAP (Figure 25). At 7 DAP, there was a significant difference between treatments for the percent stippling per plot \( (F(4,12) = 34.10, p < 0.0001) \) evaluation. The Verimark\textsuperscript{®} + Admire Pro and Verimark\textsuperscript{®} alone treatments had significantly less stippling per plot than the grower’s standard (Admire Pro alone) treatment.

![Figure 25. Trial 1 (2014) Pea Leafminer percent stippling per plot](image-url)
At 22 DAP, there was a significant difference between treatments for the percent stippling per plot (F (4,12) = 28.81, p = 0.0001) evaluation. The Verimark® + Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly less stippling per plot than the grower’s standard (Admire Pro alone) treatment. At 29 DAP, there was a significant difference between treatments for the percent stippling per plot (F (4,12) = 40.76, p = <0.0001) evaluation. The Verimark® + Admire Pro and Verimark® alone treatments had significantly less stippling per plot than the grower’s standard (Admire Pro alone) treatment. At 36 DAP, there was a significant difference between treatments for the percent stippling per plot (F (4,12) = 6.51, p = 0.005) evaluation. The Verimark® + Admire Pro and Verimark® alone treatments had significantly less stippling per plot than the grower’s standard (Admire Pro alone) treatment.

For Trial 2 (2015) percent stippling damage per plot caused by Pea Leafminer was evaluated 21, 29, and 35 DAP (Figure 26). At 21 DAP, there was no significant difference among treatments for the percent Pea Leafminer stippling evaluation according to Tukey’s HSD at α = 0.05. At 29 DAP, there was a significant difference between treatments for the percent stippling per plot (F (4,12) = 19.82, p = <0.0001) evaluation. The Verimark® + Admire Pro treatment had significantly less stippling per plot than the grower’s standard (Admire Pro alone) treatment.

At 35 DAP, there was a significant difference between treatments for the percent stippling per plot (F (4,12) = 5.59, p = 0.0088) evaluation. The Verimark® + Admire Pro and Admire Pro fb Exirel® treatments had significantly less stippling per plot than the Untreated Check.
Pea Leafminer pressure was moderate in 2014 and low to moderate in 2015. The mine count and percent stippling damage data suggests that the addition of Admire Pro did not help the Verimark® treatment in controlling Pea Leafminer, as the Verimark® alone treatment provided better control than the Verimark® treatment with Admire Pro in both years. These results are similar to Dr. Palumbo’s findings in his previously referenced 2014 trial (Palumbo, 2014). Overall, the Verimark® alone soil surface band treatment provided the best control of Pea Leafminer in these trials.

Green Peach Aphid

Green Peach Aphids were present in Trial 1 (2014). For Trial 1 (2014) the number of Green Peach Aphid nymphs per plant were counted 7, 15, 22, 29, and 36 DAP (Figure 27). At 7 and 15 DAP, Green Peach Aphid nymphs were not yet present in the trial (data not shown). At 22 DAP, there was a significant difference between treatments for Green Peach Aphid nymph counts (F (4,12) =4.61, p
=0.0174). The Verimark® + Admire Pro and Admire Pro fb Exirel® treatments had significantly less Green Peach Aphid nymphs per plant than the Untreated Check.

![Graph showing average number of Green Peach Aphid nymphs per plant](image)

**Figure 27.** Trial 1 (2014) average number of Green Peach Aphid nymphs per plant

At 29 and 36 DAP, there was no significant difference among treatments for Green Peach Aphid nymph counts according to Tukey’s HSD at α=0.05.

For Trial 1 (2014) the number of Green Peach Aphid adults per plant were counted 7, 15, 22, 29, and 36 DAP (Figure 28). At 7 and 15 DAP, Green Peach Aphid adults were not yet present in the trial (data not shown). At 22 DAP, there was a significant difference between treatments for Green Peach Aphid adult counts (F (4,12) =7.46, p =0.0029). The Verimark® +Admire Pro, Verimark® alone, and Admire Pro fb Exirel® treatments had significantly less Green Peach Aphid adults per plant than the Untreated Check.
At 29 and 36 DAP, there was no significant difference among treatments for Green Peach Aphid adult counts according to Tukey’s HSD at $\alpha=0.05$.

Green Peach Aphid pressure was very low in 2014. On the dates where there were significant differences between treatments, the treatments with Cyazypyr® tended to have lower Green Peach Aphid counts than the Untreated Check, but similar results as the grower’s standard (Admire Pro alone) treatment. This is on trend with Dr. Palumbo’s 2013 trial where he found that two foliar applications of Exirel® reduced Green Peach Aphid numbers for 14 days. (Palumbo, 2013). If Green Peach Aphid population had appeared sooner and was heavier in the 2014 trial, and appeared in 2015, we might be able to make some definitive conclusions about which treatment had the best control of Green Peach Aphid, but based on the results of these studies no conclusions were made about Green Peach Aphid control.
Western Flower Thrips

Western Flower Thrips were present in Trial 2 (2015). For Trial 2 (2015) the number of Western Flower Thrips nymphs per plant were counted 7, 14, 21, and 35 DAP (Figure 29). At 7, 14 and 21 DAP, Western Flower Thrips nymphs were not yet present in the trial (data not shown). At 35 DAP, there was a significant difference between treatments for Western Flower Thrips nymph counts ($F (4,12) = 10.86$, $p = 0.0006$). The Verimark® + Admire Pro and Admire Pro fb Exirel® treatments had significantly less Western Flower Thrips nymphs per plant than Verimark alone and the Untreated Check.

![Figure 29. Trial 2 (2015) average number of Western Flower Thrips nymphs per plant](image)

For Trial 2 (2015) the number of Western Flower Thrips adults per plant were counted 7, 14, 21, and 35 DAP (Figure 30). At 7 and 14 DAP, Western Flower Thrips adults were not yet present in the trial (data not shown). At 21 DAP, there was no significant difference among treatments for Western Flower Thrips adult counts according to Tukey’s HSD at $\alpha=0.05$. 
At 35 DAP, there was a significant difference between treatments for Western Flower Thrips adult counts ($F (4,12) =11.30, p =0.0005$). The Verimark® alone treatment had significantly less Western Flower Thrips adults per plant than the grower’s standard (Admire Pro alone) and Untreated treatments.

Western Flower Thrips pressure was very low in 2015. Without Western Flower Thrips in the 2014 trial, and very low pressure in 2015, it is not feasible to determine which treatment provided the best control of Western Flower Thrips.

**Yield**

**Yield in Pounds Per Acre**

For both trials, the yield in pounds per plot was recorded so that per acre yield could be calculated (Table 5). For Trial 1 (2014), the plots were harvested 67 DAP. In Trial 1 (2014), there was no significant difference among treatments for yield in pounds per acre according to Tukey’s HSD at $\alpha=0.05$. For Trial 2 (2015),
the plots were harvested 66 DAP. In Trial 2 (2015), there was again no significant difference among treatments for yield in pounds per acre according to Tukey’s HSD at α=0.05.

Table 5. Trial 1 (2014) & Trial 2 (2015) Lettuce yield in pounds per acre

<table>
<thead>
<tr>
<th>Treatment Name</th>
<th>Year</th>
<th>2014 Yield Pounds / Acre</th>
<th>2015 Yield Pounds / Acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verimark 13.5 fl oz Band + Admire 10.5 oz Shank At Plant</td>
<td></td>
<td>53693.30</td>
<td>71193.40</td>
</tr>
<tr>
<td>Verimark 13.5 fl oz Band At Plant</td>
<td></td>
<td>54252.30</td>
<td>68187.10</td>
</tr>
<tr>
<td>Admire 10.5 oz Shank At Plant fb Exirel 20.5 fl oz 2 Foliar Apps</td>
<td></td>
<td>49605.20</td>
<td>70774.80</td>
</tr>
<tr>
<td>Admire Pro 10.5 oz Shank At Plant</td>
<td></td>
<td>46861.30</td>
<td>72856.10</td>
</tr>
<tr>
<td>Untreated Check</td>
<td></td>
<td>55609.70</td>
<td>70450.10</td>
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</table>

<table>
<thead>
<tr>
<th>CV</th>
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<tbody>
<tr>
<td>df</td>
<td>(Treatment, Error)</td>
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<tr>
<td>F value</td>
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</tr>
<tr>
<td>p-value</td>
<td>0.4212</td>
<td>0.3726</td>
</tr>
</tbody>
</table>

There were no significant differences between treatments for pounds per acre yield in 2014 or 2015, so all of the treatments produced similar yields. The lettuce yield in pounds per acre were much higher in 2015 than in 2014.

Yield in Boxes Per Acre

For both trials, the yield in number of boxes per plot was recorded so that per acre yield could be calculated (Table 6). In Trial 1 (2014) and Trial 2 (2015),
there was no significant difference among treatments for yield in pounds per acre according to Tukey’s HSD at α=0.05. All treatments in both years had similar yield in number of boxes per acre.

Table 6. Trial 1 (2014) & Trial 2 (2015) Lettuce yield in boxes per acre

<table>
<thead>
<tr>
<th>Treatment Name</th>
<th>Year</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Part Rated</td>
<td>Yield Number Boxes /Acre</td>
<td>Yield Number Boxes /Acre</td>
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<tr>
<td>Symptom</td>
<td>YIELD</td>
<td>Tukey</td>
<td>YIELD</td>
</tr>
<tr>
<td>Verimark 13.5 fl oz Band + Admire 10.5 oz Shank At Plant</td>
<td>1488.33</td>
<td>a</td>
<td>1516.91</td>
</tr>
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<td>Verimark 13.5 fl oz Band At Plant</td>
<td>1465.93</td>
<td>a</td>
<td>1474.44</td>
</tr>
<tr>
<td>Admire 10.5 oz Shank At Plant fb Exirel 20.5 fl oz 2 Foliar Apps</td>
<td>1395.21</td>
<td>a</td>
<td>1454.68</td>
</tr>
<tr>
<td>Admire Pro 10.5 oz Shank At Plant</td>
<td>1443.44</td>
<td>a</td>
<td>1545.47</td>
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<td>Untreated Check</td>
<td>1650.38</td>
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<td>1466.14</td>
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| CV 8.40731 | 4.43404 |
| df (Treatment, Error) | 4, 12 |
| F value | 2.26226 | 1.31957 |
| p-value | 0.1229 | 0.3179 |

Number of Culls (Unmarketable Heads) Per Plot

The number of unmarketable heads (culls) per plot at harvest was recorded by counting the number of heads that were left in the field after harvest (Figure 31). In Trial 1 (2014), there was a significant difference between treatments for number of culls per plot (F (4,12) =5.69, p =0.0083). The Admire Pro + Exirel® treatment had significantly less culls per plot than the grower’s standard (Admire
Pro alone) treatment. In Trial 2 (2015), there again was a significant difference between treatments for number of culls per plot ($F(4,12) = 4.61$, $p = 0.0173$). The Admire Pro + Exirel® treatment had significantly less culls per plot than the Untreated Check. The Admire Pro fb Exirel® treatment resulted in the least number of culls per plot in both years.

Figure 31. Trial 1 (2014) & Trial 2 (2015) number of culls per plot
CONCLUSIONS

A summary of the key findings and conclusions are listed below:

**Crop Health**

The treatments that included Cyazypyr® had higher leaf counts compared to the grower’s standard treatment (Admire Pro alone). The treatments that included Cyazypyr® had higher plant weights compared to the grower’s standard treatment (Admire Pro alone). None of the at plant treatments in either year had a negative effect on crop emergence (stand count). No negative crop responses or phytotoxicity issues were observed with any of the insecticide treatments.

**Crop Vigor/NDVI**

The treatments that included Cyazypyr® had higher visual vigor and NDVI ratings when compared to the grower’s standard treatment (Admire Pro alone). There was a positive correlation between the traditional 0-200 visual vigor evaluations currently used in head lettuce and GreenSeeker® NDVI ratings, with the NDVI readings of values of at least 0.75 providing a very strong ($r^2 > 0.8$) indicator of crop vigor at the post thinning and mature crop growth stages. More importantly, the current study appears to be the first attempt in California to determine a relationship between lettuce crop growth visual vigor and the GreenSeeker® readings. Based on the $r^2$ values obtained, the recommendation would be that for the purpose of assessing the visual growth vigor of head lettuce with the use of GreenSeeker®, growers should rely on NDVI readings of 0.75 or greater. This information is also valuable for researchers looking for quantitative methods to replace the current qualitative visual vigor rating scales, and future work on head lettuce grown in the Salinas valley should also examine the
relationship between NDVI values and parameters such as Growing Degree Days (GDD), plant chlorophyll content, and Nitrogen (N) status of the crop. Additional research should also focus on the assessment of any correlation between other visual vigor rating scales (e.g., 0-5, and 1-10 ratings) and the Greenseeker® NDVI values, for other crops of interest in the Salinas Valley.

Another significant accomplishment of this study was the establishment of a standardized procedure for the obtaining of NDVI readings with Trimble GreenSeeker® for a head lettuce crop (Objective 3) (see Appendix C). With the quantity and market value of this crop in the Salinas Valley, and in California in general, there is need to have a clearly defined protocol in anticipation that growers and researchers would adopt the use of this device as a monitoring tool for overall lettuce growth and plant stress detection. While there have been many studies in different countries that have used the Greenseeker® to measure NDVI for crops with specific growth characteristics, the “pull and walk” calibration and measurement technique defined in this study is unique for a lettuce crop.

**Insect Control**

The mine count and percent stippling damage data indicates that the addition of Admire Pro did not help the Verimark® treatment in controlling Pea Leafminer, as the Verimark® alone treatment provided better control than the Verimark® treatment with Admire Pro in both years. Overall, the Verimark® alone soil surface band treatment provided the best control of Pea Leafminer in these trials. There was no Green Peach Aphid pressure in 2015 and very low pressure in 2014, so no conclusions about what the best treatment for Green Peach Aphid control were made in this study. Without Western Flower Thrips pressure in the
2014 trial, and very low pressure in 2015, it is not feasible to conclude which
treatment provided the best control of Western Flower Thrips.

Yield

There were no significant differences among insecticide treatments for pounds per acre yield or number of boxes per acre in 2014 or 2015, so all the treatments produced similar marketable yields. However, the lettuce treated with two foliar applications of Exirel® had the lowest number of culls, and would therefore be the recommended application of Cyazypyr® for the head lettuce grown on the clay and clay loam soils in the Salinas region used in this study.
REFERENCES


Saltveit, M. E. 2014. Lettuce. USDA ARS.  


APPENDIX A: PROTOCOL FOLLOWED FOR TRIALS
**TITLE:**
Effects of Cyazypyr (cyantraniliprole), an anthranilic diamide insecticide, on early season pest control and impact on quality and yield of head lettuce in Salinas Valley

**VERSION:**

**OBJECTIVE:**

1. This study will examine insect control programs in lettuce using Cyazypyr or a typical grower’s standard program to determine their impact on insect control and identify any additional benefits on yield and quality.

2. To determine the fit of the Trimble GreenSeeker® Handheld device in the field to determine crop health and vigor by NDVI (Normalized Difference Vegetation Index). Also, to determine the benefit(s) of the Trimble GreenSeeker® Handheld device and define a standard procedure for using the device.

<table>
<thead>
<tr>
<th>TIMINGS</th>
<th>UNRCHK, UNTREATED</th>
<th>ATPLAN, SOIL BAND APPLICATION AT PLANT</th>
<th>FOLIAR, 14 DAYS AFTER PLANTING AND 14 DAYS LATER (broadcast)</th>
<th>ATPLAN, SHANK</th>
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**TRT** | **TREATMENT COMPONENT** | **FORMULATION** | **RATE** | **UNIT** | **TIMING** |
<table>
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<tr>
<td>1</td>
<td>Verimark Soil Band at plant 13.5 fl oz/A band+ Admire 10.5 oz/A shank</td>
<td>&gt;DPX-HGW86 20 SC (concentrated band)</td>
<td>SC 200.00 GL</td>
<td>13.50</td>
<td>FLOZ/A</td>
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<td></td>
<td></td>
<td>&gt;ADMIRE PRO (4.6SC) (shank)</td>
<td>SC 4.60 LG</td>
<td>10.50</td>
<td>FLOZ/A</td>
</tr>
</tbody>
</table>

| 2      | Verimark Soil Band at plant 13.5fl oz/A | >DPX-HGW86 20 SC (concentrated band) | SC 200.00 GL | 13.50 | FLOZ/A | 01 ATPLAN |

| 3      | Exirel foliar 14DAP fb Exirel foliar 14DA+Admire 10.5oz/A shank | >DPX-HGW86 10SE (foliar) | SE 100.00 GL | 20.50 | FLOZ/A | 02 FOLIAR |
|        |                        | >DPX-HGW86 10SE (foliar) | SE 100.00 GL | 20.50 | FLOZ/A | 02 FOLIAR |
|        |                        | >ADMIRE PRO (4.6SC) (shank) | SC 4.60 LG | 10.50 | FLOZ/A | 03 ATPLAN |

| 801    | Admire Pro SC 10.5 fl oz/A shank | ADMIRE PRO (4.6SC) (shank) | SC 4.60 LG | 10.50 | ZMA | 03 ATPLAN |

| 999    | Untreated check |

**AUTHOR:** Kylie Baker

**CREATED:** 10-30-2013

**REVISED:** 7-12-2014
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<tr>
<td>S</td>
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> = SUPPLEMENTAL CHEMICAL

1 trial location in 2014, 1 in 2015
Time planting when high leafminer pressure is anticipated

Crop Management: Provide nutrients and water to meet the crops needs per local practice. Use the Cyazypyr plots as the guide for defining the need for nutrients and water. Make nutrient and water applications to all plots at the same time.

Additional chemical applications may be needed to get trial to harvest. Use of standard maintenance chemicals is allowed. Record any chemicals and the rates used in the test records. Preferred grower’s standard fungicide program includes Fontelis banded post thinning +14DA for Sclerotinia and Revus or Previcur banded post thinning +14DA for Downy Mildew.

RATE UNITS

ZMA = OZ (FLUID) MATERIAL / ACRE

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<tr>
<th>DESIGN:</th>
<th>RANDOMIZED COMPLETE BLOCK DESIGN</th>
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<tr>
<td>NO. REPS:</td>
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</tr>
<tr>
<td>PLOT SIZE:</td>
<td>13.33 x 30 (80 inch) beds per plot</td>
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<tr>
<td>PLOT AREA:</td>
<td>SFT</td>
</tr>
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</table>

OBSERVATIONS/RATING

Make insect evaluations at 7, 14, 21,28 and 40 days after application. Begin application of a standard grower program across all test plots 30 days after trial initiation to maintain the crop until harvest.

If multiple rows are being used for each treatment, make evaluations from the middle row(s), at least 3-5ft from the end of the plots.

Evaluation needs:

OBTAINING PLANT HEALTH, VIGOR AND HARVEST DATA IS AS IMPORTANT AS PEST DATA.

1. Pest Evaluations - Count the number of insects alive at each early time point. Also make evaluations of % plant damage by leafminers (mines and stippling). Count all pests present: leafminers, aphids, leps, thrips

2. Plant Vigor - For leafy vegetables, plant vigor should be measured the following ways on the following days after planting: 14, 21, 28 DAP.
   - standard plant vigor rating as defined in this protocol. (UTC is defined as 100%, Trt is rated versus UTC on the basis of general plant vigor) every two weeks (0-200%)
   - plant stand count - after seedling emergence, count the number of seedlings that emerged per 20 ft per replicate plot and report as # plants per 20 ft at 10 and 20 days after planting and 7-10 days after thinning
   - NDVI READINGS - Use the “pull and walk” method for the Trimble GreenSeeker Handheld to produce a NDVI reading for each row/plot to use as subsamples. Report as NDVI/plot

3. Plant Evaluations
   - Take leaf counts after first true leaf emerges- weekly to harvest, 10 plants selected at random per plot
   - Take plant weights with root after first true leaf emerges- every other week to harvest, 10 plants selected at random per plot

4. Marketable Yield and Time to Harvest - Harvest a minimum of 25 ft. of row
   - Harvests should be made using an experienced/commercial harvest crew to determine acceptable harvest qualities.
   - Record harvest readiness or earliness (% plants with formed heads) at 40, 50 and 60 DAP. Use typical grower criteria for determining harvest timing.
   - Record yield per plot expressed as yield per acre.
   - Record yield pack out (boxes) per acre.
DESIGN: RANDOMIZED COMPLETE BLOCK DESIGN

- Record the number of unmarketable heads per plot at harvest. Count the number of heads that were left in the field at harvest by the harvest crew for various reasons (disease, pest damage, size, etc.) Report as number unmarketable heads per acre.
- Count total heads harvested per plot and weight so weight per head can be determined.

5. Rating on Phytotoxicity - Record data on Phyto CAUSED by the chemical at 5-7 days after each application. Note and document with photos any phytotoxic effects observed. Include a detailed description in comments of symptom development. If any crop response is seen, please notify Hector E Portillo (302-650-2294) immediately.

6. Record the soil pH and soil type in the study records and in the AgTrax report.

7. Photo Documentation - Please document by photographs any significant insect control, vigor, plant health or yield effects seen in the trial. Photos should be professional quality and print media ready.

MATERIALS & METHODS

1 trial in 2014, 1 in 2015
Time planting when high leafminer pressure is anticipated

* Block all treatments so pest pressure gradient is same across all treatments.

Try to limit border effects by placing trial away from field edges. Allow several buffer rows from field edge.

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# UNMARKETABLE HEADS PER ACRE

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CROP YIELD IN POUNDS PER ACRE

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CROP VIGOR VS UTC

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**BASIS DESCRIPTION**

- A = ACRE
- IND = INDIVIDUALS (PLANTS/INSECTS/ANIMALS ETC.)
- LF = LEAF
- PL = PLOT

**CALCULATION METHOD**

- ABM = ABBOTT / CHECK MEAN
- NO = EFFICACY (NO CALCULATION)

**CONCENTRATION UNIT**

- GL = GRAMS PER LITER
- LG = POUNDS PER GALLON
- NA = NOT APPLICABLE

**CONFIDENCE**

- --- = TO BE SELECTED
- H = HIGH CONFIDENCE IN TRIAL DATA

**DATA SOURCE**

- FLD = FIELD TRIAL
- INH = IN-HOUSE
- NA = NOT AVAILABLE / BLANK

**DMRT**

- 5% = 5% LEVEL OF SIGNIFICANCE
- NO = NO DMRT ANALYSIS

**EVALUATION METHOD**

- % = PERCENT (0-100% INDEX / SCALE)
- %U = PERCENT OF UNTREATED CHECK
- DAP = DAYS AFTER PLANTING
- IN = INCH
- LB = POUND (WEIGHT)
- NUM = NUMBER

**EVALUATION PART**

- ADU = ADULT
- ALL = WHOLE PLANT / INSECT / ANIMAL
- EGG = EGG
### BASIS DESCRIPTION

<table>
<thead>
<tr>
<th>Abbreviation</th>
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</tr>
</thead>
<tbody>
<tr>
<td>FRM</td>
<td>FRUIT (MATURE/RIPE)</td>
</tr>
<tr>
<td>HD</td>
<td>HEAD / EAR / PANICLE</td>
</tr>
<tr>
<td>IAV</td>
<td>INSECT - LIVING</td>
</tr>
<tr>
<td>LTO</td>
<td>LARVA - TOTAL</td>
</tr>
<tr>
<td>MIT</td>
<td>MINES - TOTAL</td>
</tr>
<tr>
<td>NYM</td>
<td>NYMPH</td>
</tr>
<tr>
<td>SD</td>
<td>SEED / GRAIN</td>
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### EVALUATION SYMPTOM

<table>
<thead>
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<th>Description</th>
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<tr>
<td>CNT</td>
<td>COUNT</td>
</tr>
<tr>
<td>CON</td>
<td>CONTROL/KILL</td>
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<tr>
<td>DMP</td>
<td>DAMAGE - PEST</td>
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<tr>
<td>GNS</td>
<td>GRADE/QUALITY - NUMERIC SCALE</td>
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<tr>
<td>PHY</td>
<td>PHYTO - GENERAL</td>
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<tr>
<td>SEV</td>
<td>SEVERITY (DEGREE OF INFECTION OR INFESTATION)</td>
</tr>
<tr>
<td>VIG</td>
<td>VIGOR</td>
</tr>
<tr>
<td>YLD</td>
<td>YIELD</td>
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### FORMULATION TYPE

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<tr>
<td>SC</td>
<td>SUSPENSION CONCENTRATE/FLOWABLE</td>
</tr>
<tr>
<td>SE</td>
<td>SUSPO EMULSION</td>
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</table>
APPENDIX B: APPLICATION EQUIPMENT
Figure 32. Tractor-pulled single bed planter that has been modified for small plot trials
Figure 33. At plant soil surface band nozzles (green) and 2 inch bands behind planter
Figure 34. Shanks on planter used for at plant shank application
Figure 35. CO₂ powered backpack sprayer used for foliar Exirel® applications
APPENDIX C: TRIMBLE GREENSEEKER® STANDARD PROCEDURE
Standard Procedure-Trimble GreenSeeker® Handheld to Determine NDVI and Crop Vigor

**Purpose:** To learn how to effectively use the Trimble GreenSeeker® Handheld device in the field to determine crop health and vigor by NDVI (Normalized Difference Vegetation Index)

**Materials:**
1- Trimble GreenSeeker® Handheld Crop Sensor
1-replicated randomized complete block field trial (4 replications preferred for statistics)
1-Notebook for recording data

**Procedure:**
1. Charge GreenSeeker® for 4 hours before use in the field.
2. Manually remove all weeds (or other crops that are not to be evaluated) from the trial area. Spraying herbicides and leaving dead weeds in the plot will hinder the NDVI reading for the crop so all weeds must be manually removed.
3. To ensure the GreenSeeker® will pick up the differences in NDVI for the crop you are evaluating, first take a measurement for a visibly less vigorous area of the field, and then take a reading for a visibly more vigorous area in the field. The measurement for the visibly more vigorous area should be greater than the less vigorous area.
4. Starting with the untreated check, use the “pull and walk” method and take a reading for the entire plot (in cases where there is more than one row for a single plot take a reading for each row)
5. Record the NDVI reading shown on the GreenSeeker® display screen for the correct plot/row that is being evaluated
6. Take photo documentation of vigor differences between treatments as well as the untreated check

**Safety Information:**
- Do not enter the field trial before the proper re-entry interval after the crop has been treated with any pesticides
- Wear appropriate field work clothes (boots, long pants, hat, etc.)

**Interferences:**
- Weeds must be removed in the entire trial. The GreenSeeker® cannot differentiate between weeds and crops so in order to get a correct reading of crop NDVI, weeds cannot be present
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**Kylie Baker**

Type full name as it appears on submission

**October 28, 2016**

Date