2023

CALIFORNIA LEAFY GREENS
RESEARCH PROGRAM

RESEARCH REPORT SUMMARIES

Executive Director's Comments

Reflecting on the past year, we observed a silver lining amid the challenges of Impatiens Necrotic Spot Virus (INSV). Most notably, we were fortunate to avoid the significant losses experienced in previous years. The lower INSV incident rate raises several important questions: Why? How? And what can we learn from this? Fortunately, we have an incredible team of researchers diligently collaborating together to answer these complex questions.

In March, the Board voted to increase assessment rates to ensure ongoing support for vital research projects that benefit the Leafy Greens Industry. The increased assessments demonstrate the industry's commitment to advancing California Leafy Greens. This financial support enables the funding of innovative research projects aimed at driving progress, mitigating risks, and ensuring high-quality standards for Leafy Greens.

Looking ahead, we are excited to share the research results from this year's funding. The Annual Research Conference is scheduled for March 19, 2024 at The Cliffs Hotel and Resort in Pismo Beach, where we will have the opportunity to hear directly from researchers working on Thrips, INSV, disease and pest management, soil health, and lettuce breeding. We anticipate that the insights and outcomes shared by these researchers will underscore the importance of industry-funded research.

Our Request for Proposals for the 2024-2025 funding cycle has been sent out, and we eagerly await submissions.

On November 3, 2023, the USDA-ARS in Salinas held a Ribbon-Cutting Ceremony for the long-awaited Sam Farr United States Crop Improvement and Protection Research Center. We are grateful to former Congressman Farr and Congressman Panetta for their dedication to this project and continued support. This state-of-the-art facility will allow USDA-ARS to house more research scientists, fostering continued collaboration. We look forward to promising future collaborations with USDA-ARS.

The full research reports are available on our website:

www.calgreens.org

Richard W. Michelmore, María José Truco
Breeding crisphead and leafy lettuce

The research program focuses on improving disease resistance in crisphead and four leaf lettuce varieties for California. New sources of resistance to downy mildew, Verticillium and Fusarium wilts have been identified and are more effective than known resistance genes. The program also aims to minimize susceptibility by deploying multiple new sources of resistance. Additionally, advanced breeding lines with resistance to Fusarium wilt have been released, along with the genomic positions of the resistance genes. Efforts have been initiated to develop resistance to INSV and Globisporangium (Pythium) uncinulatum.

Richard W. Michelmore
Genetic variation in lettuce

This project focuses on enhancing lettuce through genomics and biotechnology. Our research has identified the high-expression promoter of the lettuce ubiquitin gene and is confirming its stability across generations using the RUBY transgene. We extensively utilize high-throughput sequencing, marker technologies, and genome editing for gene identification. We've uploaded an improved reference genome assembly (Version 11) to GenBank, and a gapless v14 assembly is nearly complete. Currently, we're generating T2T genome assemblies for approximately 150 additional lines to build a lettuce pangenome and catalog resistance gene candidates. Genome sequences are explored for genes related to disease resistance and development. Ongoing programs address corky root resistance, Verticillium wilt resistance, and salinity tolerance in lettuce. We continue to curate databases containing genetic, molecular marker, phenotypic, and sequence data for lettuce, with key sequences accessible on GenBank.

Ivan Simko
Lettuce breeding, genetics, and physiology

The Lettuce Breeding, Genetics, and Physiology Project is dedicated to enhancing the quality of crisphead, mixed lettuce, and spring mix varieties suitable for coastal California and low desert conditions. Simultaneously, we're advancing knowledge and tools to maximize the potential of our germplasm. This year's report (2022-2023) focuses on postharvest quality, genetics, and physiology of yellow spot malady, resistance to bacterial leaf spot, and the impact of silicon on lettuce performance. Field tests persist in evaluating resistance and developing improved breeding lines for lettuce drop, lettuce dieback, downy mildew, bacterial leaf spot, impatiens necrotic spot virus, and extending the shelf-life of fresh-cut salads. Throughout all our programs, we prioritize horticultural traits and adaptation.

Renée L. Eriksen
Understanding the molecular mechanisms of resistance to insv+pythium wilt and tipburn in support of the usda lettuce breeding program

Our goal is to support the USDA lettuce breeding program by understanding the physiological and molecular mechanisms of tipburn and diseases, such as INSV and other soil-borne pathogens, and developing molecular markers for marker-assisted selection of stacked resistance to tipburn and disease. We made substantial progress in understanding the growing needs of lettuce and are optimistic that we will be able to mimic field conditions for inducing tipburn in future experiments to investigate the molecular mechanisms of tipburn. We are also confident that the inoculation methods we learned will continue to be optimized and yield an improved understanding of the molecular mechanisms of disease in lettuce.

Gail Taylor
Improving the post-harvest quality and shelf-life of lettuce: qtl to candidate genes and markers and testing proof of concept

Our primary research goal is to enhance the quality and extend the shelf life of both whole head and spring mix lettuce, ultimately reducing post-harvest losses and improving nutritional value. We've successfully identified the genetic factors governing lettuce leaf shelf life and have developed molecular markers for further investigations. A secondary objective involves using extreme commercial and GWAS lines to evaluate the impact of contrasting leaf surface traits and microbiomes on the presence of human pathogenic bacteria in field conditions. We recognize that post-harvest leaf quality and shelf life are likely influenced by multiple genes, rather than a single one. Combining data from various field trials in Davis, Salinas, greenhouse studies, and prior European research, we've pinpointed a set of QTLs and associations that are helping us uncover the molecular underpinnings of shelf life and nutritional quality.

David W. Still
Selecting for improved water and nitrogen uptake by focusing on root characteristics

Our hypothesis centers on the potential for improving

Water Use Efficiency (WUE) and Nitrogen Use Efficiency (NUE) through increased root biomass. In our recent trials, we utilized a newly developed mapping population comprising 279 F7 families from the D221 x W28 recombinant inbred line. These families were subjected to two different nitrogen treatments: limited (N50) and non-limited (N100). We assessed root and leaf biomass, root length, and leaf nitrogen and carbon concentrations at market maturity. On average, we observed a decrease in leaf biomass and an increase in root biomass in response to limited nitrogen. Notably, leaf nitrogen concentration only decreased by 12% despite a 50% reduction in nitrogen supply. We identified seven F7 families that significantly boosted their root biomass under limited nitrogen conditions, and these families will undergo further field evaluations with the aim of releasing them as genetic lines for enhancing root biomass under limited nitrogen conditions.

E. Charles Brummer, Allen Van Deynze, Allison Krill-Brown, Daniel Pap and Steve Klosterman
Breeding baby leaf spinach for California growers

Our spinach breeding project aimed at creating commercial cultivars with broad-spectrum resistance to downy mildew continued. Over the past year, we conducted trials in Salinas and El Centro, CA, involving the creation and evaluation of 79 breeding populations. Some of these experimental populations exhibited very low downy mildew incidence and severity, making them strong candidates for release. Additionally, our breeding program is focused on identifying the genetic basis of low cadmium uptake. To facilitate this, we've developed a hydroponic system for more efficient plant evaluation and selection based on this trait, pending field comparison results. We've also devised a method for growing plants under a long photoperiod using LED lights in a growth chamber, reducing seed production time by a month. In terms of genetic analysis, we're utilizing genetic markers for major downy mildew genes and employing genome-wide markers to assess genetic diversity, identify genetic relationships, and enhance our selection process.

Kelley L. Richardson
Usda lettuce breeding for improved resistance to INSV, Pythium, Verticillium, and Fusarium

We conducted greenhouse experiments to validate INSV results and examined resistance mechanisms in our most resistant germplasm through thrips and virus-only experiments. Sixteen promising USDA breeding lines were selected for advancement in our breeding program. We successfully validated field Pythium results through an in-vitro assay and made progress in developing a greenhouse Pythium evaluation protocol. Field and greenhouse evaluations were carried out on USDA breeding lines combining Verticillium Race 1 and Race 2 resistance in preparation for germplasm release. USDA breeding lines with combined Fusarium and Verticillium wilt resistances underwent field and greenhouse trials, with selections made for germplasm release. All these research efforts contribute to our overarching goal of releasing lettuce varieties with enhanced resistance to diseases that affect production.

Maeli Melotto
Editing the lettuce genome to reduce human pathogen load on leaves

The ability of E. coli O157:H7 to grow on/in edible leaves varies according to different lettuce types. This discovery presents an opportunity to pinpoint lettuce genes that create a favorable environment for this pathogen's survival. Leveraging CRISPR/Cas9 gene editing precision, we can potentially disrupt these genes from the lettuce genome, leading to the development of lettuce varieties less susceptible to contamination by human pathogens. We've identified a promising gene family, known as JAZ, as strong candidates for gene editing. In the Salinas cultivar, we've characterized the six members of this family, examined their expression in various plant tissues in response to E. coli O157:H7 and Salmonella enterica, and devised a process for individually knocking out each JAZ gene. This process is intricate, involving multiple controls for robust genetic analysis and the necessity to advance plant lines through at least three generations. Nevertheless, we've already completed this work for one JAZ gene, and the progress is underway for the others in the pipeline.

Ali Montazar
The impact of irrigation and fertilizer management practices on enhancing resource-use efficiency in the low desert lettuce production systems

This study's goal is to adapt the CropManage (CM) web-based tool for lettuce in the desert region. We conducted four trials in drip-irrigated lettuce fields in the Imperial and Coachella Valleys from October 2022 to February 2023. These trials revealed variable rates of water and nitrogen (N) application. In general, iceberg lettuce fields, especially those with sandy soil textures, received higher water and N application rates compared to romaine lettuce fields. Notably, there was a significant difference between the N and water appliction rates recommended by CM and the actual practices observed in the field trials with sand-dominated soil textures.

This two-year study suggests optimal lettuce growth may be achieved with seasonal N fertilization and irrigation water application rates lower than current typical practices, even in drip-irrigated fields. However, further data from ongoing replicated trials at the Desert Research and Extension Center is needed to confirm this preliminary conclusion. As a freely accessible decision-making tool, CM holds the potential to assist local growers in maximizing lettuce production and improving the efficiency of N and water utilization.

Ian Grettenberger Daniel K. Hasegawa
Novel technologies for effective and sustainable management of thrips and aphids in lettuce: precision insecticide applications

Emerging technologies offer promising opportunities to enhance aphid and thrips control. Specifically, the increasing adoption of automated thinners in lettuce cultivation presents a novel avenue for more precise insecticide applications. Our research focused on testing the efficacy of this innovative technology for managing aphids and thrips in lettuce. We assessed the advantages of precision sprays delivered by an automated thinner in organic lettuce, using various active ingredients for aphid and thrips management. We compared these precision applications to traditional broadcast methods. We also investigated the benefits of precision spraying for thrips management in conventional lettuce, exploring multiple application rates of a key active ingredient (Radiant/spinetoram) and contrasting them with standard broadcast application techniques. In combination, these objectives aimed to explore a technology that has the potential to advance integrated pest management strategies for aphids and thrips in lettuce cultivation.

Alex Putman
Monitoring the population of the lettuce Fusarium wilt pathogen in California and occurrence of the disease with Pythium wilt

Fusarium wilt of lettuce, caused by the soil inhabiting fungus Fusarium oxysporum f. sp. lactucae (FOL), has historically posed a pest challenge in California's fall-to-spring production regions. However, its incidence and severity have recently increased in coastal production areas. Due to the limited recent data on the FOL population in these regions, we are conducting an updated assessment. Additionally, it's unclear whether FOL is impacting the INSV-Pythium wilt epidemic. To date, we have collected 278 F. oxysporum isolates from 47 locations. A novel race variant was discovered, characterized by its resistance to Banchu Red Fire and susceptibility to the commercial cultivar San Miguel. In our survey of 23 location-cultivars, we observed visible symptoms of either internal vascular discoloration (indicative of Fusarium or Verticillium wilts) or external root rot (indicative of Pythium wilt) at all locations. In many cases, lab analyses couldn't confirm the suspected disease based on visual symptoms. Ongoing work aims to further confirm the novel race variant and evaluate isolates from more locations. Our analysis of Fusarium-Pythium coinfections is ongoing, with initial results indicating the need for a new study design in 2023.

Steven Koike
Race characterization of Verticillium isolates recovered from lettuce growing regions

The lettuce Verticillium wilt pathogen, Verticillium dahliae, is known to comprise various races. Among these, Race 1 is the most prevalent, but the occurrence of non-Race 1 isolates remains uncertain. In our project, we collected V. dahliae isolates from lettuce and other coastal crops, characterized them by molecular methods to determine their race, and mapped the distribution of these races. We analyzed 131 plant samples from 85 field locations, resulting in the identification of 646 Verticillium isolates. Notably, Verticillium dahliae Race 1 was the most frequently encountered. Race 2 was recovered from 7% of the samples, primarily in lettuce, but also in artichoke and tomato. Race 3 was found in 18% of the samples, mainly in strawberry, with occurrences in pepper and cabbage. Additionally, we identified isolates that were confirmed as V. dahliae but did not belong to any of the established races. These isolates may potentially represent a new, novel race. Of particular significance is the confirmation of Verticillium wilt of lettuce in Santa Maria by the TriCal Diagnostics lab, an area that previously did not have this disease.

Frank Martin
Development of a molecular assay for Fusarium oxysporum f. sp. lactucae

We've developed genomic resources for Fusarium oxysporum f. sp. lactucae, Race 1, the causal agent of fusarium wilt in North American lettuce. These resources have enabled the creation of molecular diagnostic assays for pathogen detection. Two distinct assays have been developed and shared with the research community. The first assay, based on real-time PCR, is highly sensitive and regarded as the

"gold standard" for diagnostic testing. The second assay utilizes recombinant polymerase amplification (RPA) technology, enabling field-based testing without DNA extraction. This approach can deliver results in as little as 20 minutes. Additionally, the dataset has proven valuable for genome assembly and developing resources for genotyping isolates. This advancement enhances our capacity to assess pathogen variation and monitor the movement of specific strains.

Frank Martin
Development of a molecular diagnostic assay for Pythium uncinulatum

While numerous Pythium species are commonly found in soil, most of them do not pose a threat to lettuce plants once they've surpassed the seedling stage. However, Pythium uncinulatum stands as an exception, increasingly impacting lettuce production. The rapid identification of this pathogen, without the need for complex culturing or taxonomic expertise, is crucial for effective management strategies. A specific molecular diagnostic tool has been developed, demonstrating sensitivity even in low pathogen quantities. This technique employs real-time PCR with DNA extracted from infected plant tissue. Multiple labs, including those in Monterey County, are currently testing the tool to verify its specificity and accuracy in pathogen detection. Additionally, there's a move to transfer the assay to a different detection chemistry, enabling results in as little as 20 minutes without the requirement for DNA extraction.

Jim Correll
Race diversity and the biology of the spinach downy mildew pathogen

During the 2022-2023 period, spinach downy mildew disease remained low, similar to the past three years. Nineteen distinct races of the disease were identified and evaluated against a standard set of spinach differentials (Table 1 in the report). Due to the limited disease pressure, only previously known races were examined, primarily from conventional production fields. Currently, several new isolates are being discussed by the IWGP for potential classification as new races. Nonetheless, as of October 2023, no new race designation has been officially established. Additionally, findings regarding downy mildew disease progression in the San Juan Bautista Spinach Field Trial for September-October 2022 and the Yuma, AZ Spinach Field Trial for January-March 2023 are presented in the final report.

Steven J. Klosterman
Downy mildew detection, epidemiology, and biopesticide evaluation

Downy mildew diseases, affecting lettuce (caused by Bremia lactucae) and spinach (caused by Peronospora effusa), pose significant challenges to crop production. Plant isolator experiments confirmed that spinach downy mildew disease was transmitted from seeds infested with the oospores. We also found that spinach downy mildew can be transmitted from oospore-infested leaf debris incorporated into the soil of the isolators. Ongoing research involves quantifying the airborne spores of both P. effusa and B. lactucae in California's Imperial, Coachella, and Salinas Valleys. These studies have revealed a correlation between spore quantities and crop acreage planted. However, there was a noteworthy period in October lasting for several weeks weeks in the Imperial Valley where spores were not detected. This suggests that seed or soilborne oospores may serve as sources of primary inoculum in this region. These findings underscore the importance of developing practical solutions to limit the introduction of downy mildew via spinach seeds Furthermore, they provide valuable information for downy mildew forecasting in lettuce and spinach crops.

Nicholas LeBlanc
Application of organic soil amendments for improved soil health and management of Fusarium wilt of lettuce

The application of organic soil amendments can alleviate pressure from soilborne diseases and impact various soil health factors. In this project, we investigated the effects of plant-based amendments, including green manures and compost, on Fusarium wilt of lettuce and examined the chemical and microbial aspects of the soil. Additionally, we explored whether the pathogen responsible for Fusarium wilt of lettuce could infect non-lettuce crops. This project highlights the potential use of compost for managing Fusarium wilt of lettuce, emphasizes the role of green manures and compost in promoting soil health, and underscores the importance of considering non-lettuce crops as potential hosts for the fusarium wilt pathogen.

William Horwath
Continued monitoring of nitrogen management strategies for leafy greens using soil health metrics

Our project is dedicated to enhancing knowledge about sustainable nitrogen (N) management and advocating

for tools to improve soil health without compromising agronomic efficiency, particularly in the challenging lettuce production systems. The benefits of improving soil health in lettuce production include increased levels of organic N in the soil, reduced dependence on N fertilizers, enhanced crop nitrogen use efficiency (NUE), and heightened resilience of crops to extreme weather events. Our project builds on our ongoing field trials and outreach efforts focused on sustainable N management. Our primary objectives revolve around adopting tools and techniques to increase sustainability and enhance soil health in lettuce production systems. To attain these goals, we continue to gather additional data on crop performance and N loss from our multi-year field site to monitor and model N cycling.

Arun D. Jani

Carbon nanoparticle addition to fertilizers as a strategy to improve lettuce yields and nitrogen recovery while reducing nitrate leaching

Our project explores the potential of using low-cost, plant-based carbon nanoparticles (CNPs) as a liquid fertilizer additive to enhance romaine lettuce yields, nitrogen recovery, and mitigate nitrate leaching in Salinas Valley and Imperial Valley soils. Plant-based carbon nanoparticles possess high porosity and can temporarily sequester nitrate-N (NO3-N) within their pore structure. Once absorbed by plant roots, CNPs break down, releasing NO3-N for plant utilization. Additionally, research indicates that CNPs may stimulate root growth, thereby increasing the plant's capacity to absorb NO3-N. Our investigations involve greenhouse pot experiments on various soil types, serving as a foundation for future field experiments. The preliminary findings from sandy loam and clay loam soils suggest that CNPs can enhance yields and crop N recovery in soils with low NO3-N concentrations.

Michael Cahn

Water treatment technology using polyacrylamide (PAM) to mitigate sediment and pesticides in agricultural run-off and improve chlorine treatment of tailwater

Leafy green growers face growing challenges due to stricter water quality regulations, notebly from fields with irrigation run-off carrying sediment and pesticides, and compliance with the Leafy Green Marketing Agreement (LGMA) that mandates farming operations storing water in open reservoirs or reusing tailwater for irrigation to maintain generic E. coli levels below 10 MPN/100 ml. Our project has focused on developing and testing cost-effective methods for reducing sediment losses from sprinkler-irrigated leafy greens fields. This involves treating irrigation water with polyacrylamide (PAM) using an applicator designed for pressurized irrigation systems. Additionally, we have developed and tested equipment designed to apply PAM to irrigation run-off that drains into tailwater ponds. This process aims to lower the concentration of suspended sediments, potentially enhancing the effectiveness of chlorine treatment for reducing E. coli and coliform bacteria levels.

Michael Cahn

Evaluation of equipment to improve water and nitrogen management in lettuce

Prior research has indicated that optimizing drip irrigation systems in leafy greens requires improved pressure regulation and meticulous monitoring of water application through flowmeters. Additionally, precise measurement of liquid fertilizer injection into the drip systems can enhance crop management. This project evaluated the accuracy, ease-of-use, and suitability of commercially available flowmeters, pressure reducing valves, and pressure sensors for commercial vegetable growing operations. The primary goal was to identify equipment that can aid growers in achieving improved water and nutrient management for lettuce cultivation.

Steve Fennimore

Soil disinfestation with steam for leafy greens

Enhanced weed control reduces the need for manual weeding and contributes to optimizing labor efficiency, a critical factor for the long-term economic sustainability of vegetable production. Injecting steam into the soil proves effective in killing weed seeds within the seedbank, ensuring efficient weed control within the steam-treated area. In the 2022-2023 period, we assessed the performance of a steam applicator designed to apply steam in bands prior to lettuce seeding. This device effectively managed weeds in the treated area, reducing both manual weeding efforts and the incidence of Pythium in the soil.

Funding received through SB170

Richard Smith and Jose Pablo Dundore-Arias
Screening of romaine and head lettuce varieties for tolerance to Pythium Wilt

In the summer of 2022, a lettuce variety trial was conducted at a site historically afflicted with Pythium wilt (Pythium uncinulatum) in lettuce. Visual assessments of wilting or deceased lettuce plants revealed several head lettuce varieties exhibiting significant tolerance to Pythium wilt, notably 22PT/07, Paraiso, Telluride, Molera, and 22PT/08. While romaine varieties generally displayed greater sensitivity to Pythium wilt, certain varieties that resisted the disease included Copious, Patton, 1024 Momentus, and SR2-21-33B. Additionally, two green leaf varieties, Clerac and Loubressac, exhibited resilience to Pythium wilt. It was observed that the incidence of Impatiens necrotic spot virus (INSV) was highly correlated (89%) with Pythium incidence. The most Pythium-resistant varieties also displayed the highest resistance to INSV. Crisphead lettuce varieties, on average, demonstrated significantly lower INSV severity and incidence compared to romaine varieties. However, laboratory testing revealed that a larger proportion of crisphead samples tested positive for the virus compared to romaine samples. Laboratory assessments confirmed that the dominant soilborne disease at the site was Pythium wilt, even in plants with no visible above-ground symptoms. In separate evaluations on commercial farms, Pythium wilt levels surged to extremely high levels by the end of the production season in the Salinas Valley in 2022. Even varieties that exhibited high tolerance in the variety trial displayed significant susceptibility under severe late-season conditions.

Daniel K. Hasegawa, Jose Pablo Dundore-Arias
Enhanced surveillance strategies of thrips vectors and understanding the interplay between INSV and Pythium Wilt of lettuce

In this report, we present the development of a novel diagnostic tool designed to detect and quantify INSV and Pythium uncinulatum in co-infected lettuce roots. This advancement significantly improves our capability to assess the timing of co-infections by INSV and P. uncinulatum, both in field and controlled greenhouse settings. Furthermore, we introduce a second innovative tool that enables the identification of western flower thrips and their role as vectors for INSV. Both tools have undergone field testing and provide valuable support to various projects funded by the California Leafy Greens Research Program.

Jose Pablo Dundore-Arias
Assessing the frequency of Pythium wilt and INSV co-occurrence, and the effect of temperature on Pythium wilt incidence in Monterey County's lettuce production

We conducted on-farm field trials to investigate the occurrence of co-infections of Pythium wilt and INSV in commercial lettuce fields. Visual assessments of aboveground plant symptoms revealed that INSV infections were frequently observed before any signs of Pythium wilt became apparent. In clinical evaluations, we detected INSV infections in both leaf and root tissues. In contrast, Pythium wilt infections were detected in root tissues several weeks before aboveground wilting symptoms appeared. We also explored the influence of temperature on Pythium wilt incidence and pathogen development. Our experiments demonstrated that all Pythium uncinulatum isolates consistently grew at temperatures of 9°C and 21°C but not at 25°C or 28°C. In contrast, P. aphanidermatum and oligandrum isolates exhibited broader temperature adaptability, growing well across the temperature range.

Daniel K. Hasegawa
Developing RNA interference (RNAi) technology to manage thrips and viruses in lettuce

In this study, we explored the potential of RNA interference (RNAi) technology to combat thrips and viruses in lettuce. Our findings reveal the successful uptake of double-stranded RNAs (dsRNAs) by lettuce plant roots in field conditions. These dsRNAs moved systematically throughout the plant and remained detectable in leaf tissue for at least seven days post-application. Because the natural incidence of INSV infections in the field was low during the trial, we were unable to establish a conclusive link between dsRNA treatments and INSV infection levels. Notably, our investigation demonstrated that repeated, high-concentration dsRNA applications had no adverse effects on lettuce plants' overall growth and biomass. Future research is essential to uncover the potential suppressive effects of dsRNAs on INSV infection, and these investigations will be a priority in the upcoming year.

CALIFORNIA LEAFY GREENS
RESEARCH PROGRAM

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