

# **International Spinach Conference**

**Murcia, Spain  
February 14-15, 2018**







# 2013 International Spinach Conference Guangzhou, China



# 2015 International Spinach Conference Yuma, Arizona



Welcome to the 2018

**International Spinach Conference  
Murcia, Spain**

**February 14-15, 2018**

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February 14, 2018

On behalf of the organizing committee, we would like to welcome everyone to Murcia, Spain for the 2018 International Spinach Conference dedicated to the international exchange of information for the benefit of the spinach community. We have over 125 participants and have an exciting program and field day planned. This should be a valuable venue for anyone interested in spinach.

Alec Roberts is this year's program chairperson and local organizer. We would like to thank Alec for all of his efforts associated with the meeting.

We also would like to thank the many people for their time and effort in convening the meeting and the dedicated sponsors for their financial support. In particular, we would like to thank Shelby Hanson, Kim Keeney, and Chunda Feng for all of their exceptionally hard work in helping organize the conference.

We would also like to thank Sakata Seed Co. and David Samuels for their willingness to host the spinach field trial for the conference.

Welcome to the meeting and welcome to Murcia!

We hope everyone enjoys the meeting and finds it productive.

Sincerely,

Jim Correll, Alec Roberts, Shelby Hanson, Kim Keeney, and Chunda Feng  
Department of Plant Pathology  
University of Arkansas  
Fayetteville, AR 72701

2018 International Spinach Conference Program Sponsors

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**Murcia, Spain**

**PROGRAM**

**Tuesday, February 13, 2018**

- 3:00 – 5:00 pm** Early Registration (Hotel Lobby)
- 7:00 - 9:00 pm** Welcome reception at the hotel Occidental Murcia Siete Coronas  
**Wednesday February 14, 2018**  
**Occidental Murcia Siete Coronas**
- 7:00 – 8:00 am** Pick-up registration materials
- 8:00 - 8:15 am** Welcome to the 2018 International Spinach Conference  
Jim Correll, University of Arkansas and Alec Roberts, Tozer Seed Co.
- 8:15 – 10:15 am** **I. Challenges of Spinach Seed / Product Production**  
**Jim Correll, presiding**
- 8:15 - 8:30 am** Spinach seed production in the Pacific Northwest USA.  
Lindsey du Toit, Washington State University
- 8:30 – 8:45 am** Can French Coastal areas be a new solution for spinach seed  
production? Anthony Gorin, Haden Seeds
- 8:45 - 9:00 am** Spinach seed production in New Zealand.  
Jay Schafer, Schafer Ag Services
- 9:00 - 9:15 am** Spinach production in the European Union.  
Alec Roberts, Tozer Seed Co.
- 9:15 – 9:30 am** Conventional and organic spinach production in the US; Perspective  
from an organic grower / shipper.  
Ramy Colfer, Earthbound Farm DanoneWave
- 9:30 – 9:45 am** California Seed Association’s Organic Spinach Committee’s Support  
of the Organic Spinach Community.  
Dale Krolikowski and Donna Boggs, Germain’s Seed Technology and  
California Seed Association
- 9:45 – 10:00 am** Developing a new spinach breeding program for California.  
E. Charles Brummer, Allen Van Deynze, Juliana Osorio-Marin, Rachel  
Greenhut, Steve Klosterman, Steve Koike, Richard Smith. University  
of California, Davis.
- 10:00– 10:15 am** Water challenges in vegetable production in California.  
Amy L. White, MPP, Two Girls Ranch, LLC
- 10:15 – 10:30 am** **Break**

- 10:30 – 1:00 pm**      **II. Spinach Molecular Approaches and Downy Mildew**  
**Lindsey du Toit, presiding**
- 10:30 – 10:45 am**      Genetic diversity, genome-wide association study and genomic selection in spinach.  
Ainong Shi, Jun Qin, Yuejin Weng, Jim Correll, Chunda Feng, Gehendra Bhattarai, Waltram Ravelombola, Bazgha Zia, Wei Zhou, and Beiquan Mou. University of Arkansas, Fayetteville, AR USDA-ARS, Salinas, CA
- 10:45 – 11:00 am**      Spinach genome and its transcriptome variation provide insights into evolution, domestication and important nutrient traits.  
Zhangjun Fei, Cornell University
- 11:00 – 11:15 am**      Combining high-throughput genotyping and phenotyping to improve spinach breeding efficiency.  
Carlos A. Avila, Juan Enciso, Jinha Jung, Thiago Marconi, and Henry Awika
- 11:15 -11:30 am**      Molecular markers for spinach sex determination gene.  
Chunda Feng, Bo Liu, Braham Dhillon, Maria Isabel Villarroel-Zeballos, Burt Bluhm, Ainong Shi, James Correll. University of Arkansas, Fayetteville, AR
- 11:30 – 11:45 am**      A reference genome sequence for *Peronospora effusa*: towards the molecular dissection of race specificity.  
Burt Bluhm, Chunda Feng, Jim Correll. University of Arkansas, Fayetteville, AR
- 11:45 -12:00 pm**      Overview of downy mildew disease resistance and race diversity.  
Jim Correll and Chunda Feng, University of Arkansas, Fayetteville, AR
- 12:00 – 12:15 pm**      Whole genome variation in 2016 field populations of spinach downy mildew.  
Kurt Lamour, Sandesh Shrestha, Bo Liu, Chunda Feng, and Jim Correll  
University of Tennessee and the University of Arkansas
- 12:15 – 12:30 pm**      Oospore production, viability, and incidence on spinach seed.  
Shyam Kandel, Beiquan Mou, Sridhara G. Kunjeti, Krishna V. Subbarao, Steven J. Klosterman, USDA/ARS, Salinas, CA.
- 12:30 – 12:45 pm**      Seed health protocol development for spinach downy mildew.  
Sierra L. Hartney and Philip Brown, Sakata Seed, Mt. Vernon, WA
- 12:45 – 1:00 pm**      Evaluation of oospores on spinach seed.

Bo Liu, Chunda Feng, Jim Correll, University of Arkansas,  
Fayetteville, AR

- 1:00 – 2:00 pm**      **Lunch**
- 2:00 – 3:45 pm**      **III. Diseases and Management**  
**Larry Stein, presiding**
- 2:00 – 2:15 pm**      Characterization of spinach leaf spot pathogens and fungicide efficacy.  
Liu, B., Feng, C., Cochran, K., Stein, L., du Toit, L. J., and Correll, J.  
C., University of Arkansas, Fayetteville, AR, and Texas A&M  
University
- 2:15 - 2:30 pm**      Determining the genetic basis of pathogenicity of *Fusarium oxysporum*  
f. sp. *spinaciae* on spinach.  
Alex Batson, Tobin Peever, and Lindsey du Toit, Washington State  
University
- 2:30 -2:45 pm**      Soilborne disease management in Australia.  
Len Tesoriero
- 2:45 - 3:00 pm**      *Rhizoctonia*: seedling disease or web blight in Texas spinach?  
Cochran, K.A., Spurlock, T.N., Stein, L., and Drury, D., Texas A&M
- 3:00 – 3:15 pm**      A multidisciplinary approach to improve damping-off tolerance in  
spinach.  
K.J.H. (Kim) Magnée, S.P.C. (Steven) Groot, J. (Joeke) Postma, E.T.  
(Edith) Lammerts van Bueren, O.E. (Olga) Scholten, Wageningen  
University & Research, Netherlands
- 3:15 – 3:30 pm**      Development of a spinach white rust management strategy in Texas  
Larry A. Stein, Texas A&M
- 3:30 – 3:45 pm**      White rust: beyond borders.  
Jim Correll, Chunda Feng, Ainong Shi, University of Arkansas,  
Fayetteville, AR
- 3:45 - 4:15 pm**      **Break**
- 4:15 – 5:45 pm**      **IV. Spinach Research (potpourri)**  
**Alec Roberts, presiding**
- 4:15 – 4:30 pm**      Tissue culture approaches for spinach research on disease resistance.  
Maria Isabel Villarroel-Zeballos, Braham Deep Singh Dhillon, Chunda  
Feng, Jim Correll, University of Arkansas, Fayetteville, AR
- 4:30 – 4:45 pm**      Genome sequencing of spinach Near Isogenic Line 1 and candidate  
resistance genes.

Braham Deep Singh Dhillon, Chunda Feng, Ainong Shi, Quighua Pan,  
and Jim Correll, University of Arkansas, Fayetteville, AR

- 4:45 – 5:00 pm** Evaluation of natamycin seed treatments for *Stemphylium botryosum* and other necrotrophic fungi on spinach seed.  
Lindsey du Toit and Michael Derie, Washington State University
- 5:00 – 5:15 pm** PurGrow: a novel methodology for cleaning seed and managing diseases.  
Chunda Feng and Jim Correll, University of Arkansas, Fayetteville, AR
- 5:15 – 5:30 pm** Subterranean collembola, a challenging pest of spinach seed production.  
Beverly Gerdeman, G. Hollis Spitler, and Lynell Tanigoshi, Washington State University
- 5:30 – 5:45 pm** Food safety strategies for preventing the contamination of spinach with foodborne pathogens-A Texas and global approach.  
Marcel Valdez, Texas A&M University
- 5:45 – 6:00 pm** Insights into nitrogen assimilation and oxalic acid metabolism in spinach.  
Vijay Joshi, Madhumita Joshi, Daniel Leskovar, Diwas Silwal  
Texas A&M AgriLife Research and Extension Center, Uvalde, Texas 78801. USA.
- 7:00 pm** Group Dinner  
Occidental Murcia Siete Coronas  
Short Program (Sponsorships, Industry Service Award, Field Day)

**Thursday, February 15, 2018**  
**Sakata Hosted Spinach field Trial**

<b>8:30 am</b>	Bus departs Occidental Murcia Siete Coronas for field trial
<b>10:00 am</b>	Arrival at Sakata Field Trial
<b>Noon</b>	Box lunch at Sakata Field Trial
<b>1:00 pm</b>	Bus departs to Occidental Murcia Siete Coronas
<b>2:00 pm</b>	Arrive at hotel
<b>Dinner</b>	On your own

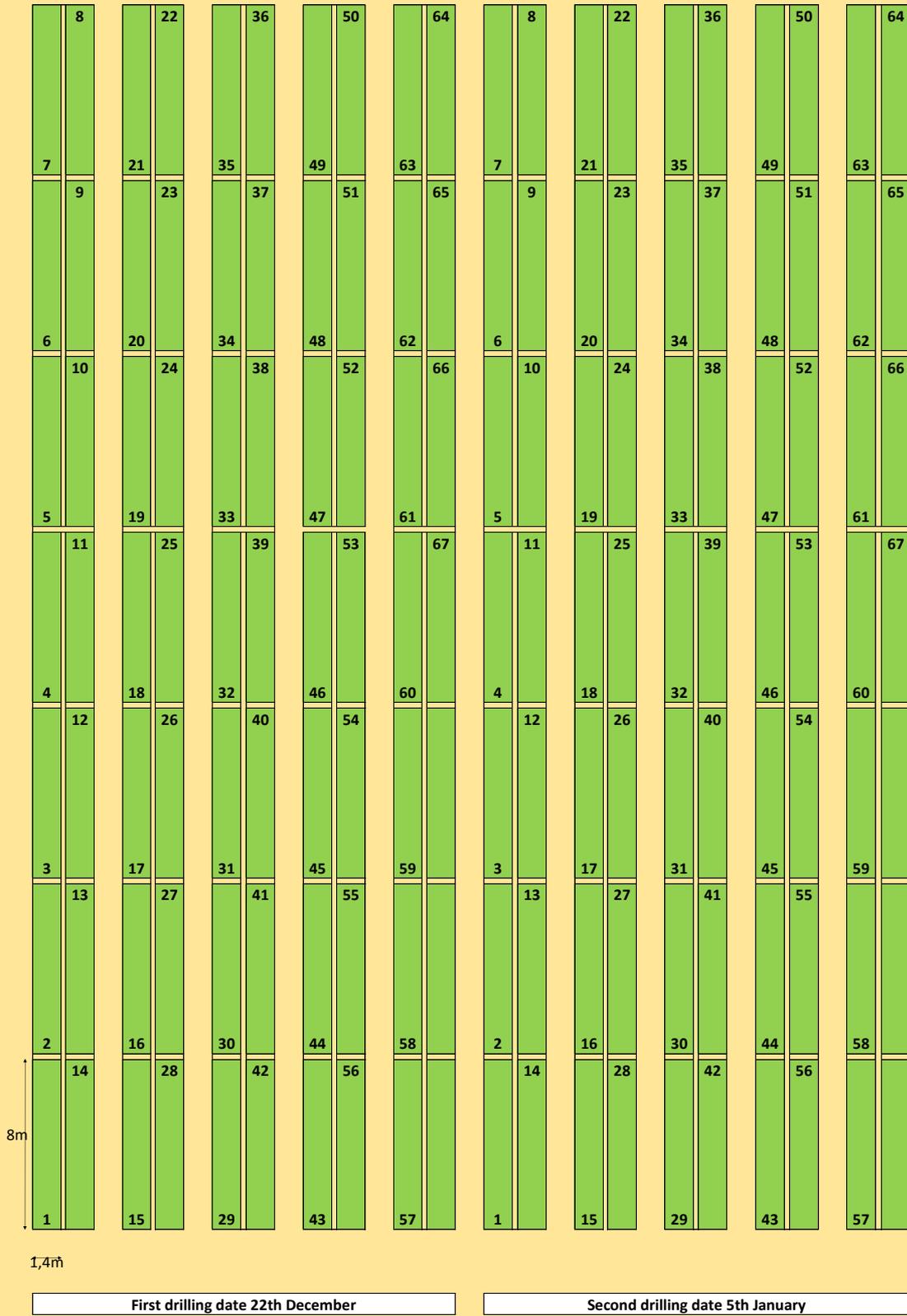
**Sakata Field Trial  
Murcia, Spain  
February 15, 2018**

**List of varieties**

Code	Variety	Company	Code	Variety	Company
1	Woodpecker RZ (51-335)	Rijk Zwaan	35	Rebound	Bejo
2	Canary RZ (51-343)	Rijk Zwaan	36	Reflect	Bejo
3	Cassowary RZ(51-352)	Rijk Zwaan	37	Region	Bejo
4	Parakeet RZ (51-715)	Rijk Zwaan	38	Renegade	Bejo
5	Platypus RZ (51-707)	Rijk Zwaan	39	Responder	Bejo
6	Mandril RZ (51-332)	Rijk Zwaan	40	Space	Bejo
7	Merkaat RZ (51-333)	Rijk Zwaan	41	Spiros	Bejo
8	Coati RZ (51-331)	Rijk Zwaan	42	Pungi	Syngenta
9	Finwhale RZ (51-348)	Rijk Zwaan	43	El Prado	Syngenta
10	Kolibri RZ (51-710)	Rijk Zwaan	44	El Caballo	Syngenta
11	Shelby F1	Enza Zaden	45	Volans	Nunhems
12	Yukon (E03D.0593) F1	Enza Zaden	46	Serpens	Nunhems
13	Sioux F1	Pop Vriend	47	Hydrus	Nunhems
14	Monterey F1	Pop Vriend	48	Cepheus	Nunhems
15	Aztec F1	Pop Vriend	49	Antalia	Nunhems
16	Napa F1	Pop Vriend	50	Alcor	Nunhems
17	Inyo F1	Pop Vriend	51	Canopus	Nunhems
18	PV-1295 F1	Pop Vriend	52	Virgo	Nunhems
19	PV-1245/Yuma F1	Pop Vriend	53	Cursa	Nunhems
20	Viroflay	Pop Vriend	54	Andromeda	Nunhems
21	Amador F1	Pop Vriend	55	Apollo	Sakata
22	Alameda F1	Pop Vriend	56	Helios	Sakata
23	PV-1194 F1	Pop Vriend	57	Soyuz	Sakata
24	Sioux F1	Pop Vriend	58	Sputnik	Sakata
25	Monterey F1	Pop Vriend	59	SPI02089 (Lakeside)	Sakata
26	Aztec F1	Pop Vriend	60	SVVC5810	Seminis
27	Napa F1	Pop Vriend	61	SVVC5044	Seminis
28	Inyo F1	Pop Vriend	62	Magnetic	Seminis
29	PV-1295 F1	Pop Vriend	63	SV2146VB	Seminis
30	PV-1245/Yuma F1	Pop Vriend	64	Rapanui	Seminis
31	Viroflay	Pop Vriend	65	SVVC5663	Seminis
32	Amador F1	Pop Vriend	66	Java	Seminis
33	Alameda F1	Pop Vriend	67	SVVC8155	Seminis
34	PV-1194 F1	Pop Vriend			

13-23 Treated seed

# Plot design



# **PROGRAM ABSTRACTS**

## **Spinach seed production in the Pacific Northwest USA**

Lindsey J. du Toit

Washington State University, 16650 State Route 536, Mount Vernon, WA 98273-4768, USA

Few areas of the world have the climatic conditions required to produce high quality spinach seed, i.e., long summer day length for uniform bolting and flowering, mild summer temperatures for this heat-sensitive species, and dry conditions during the summer to minimize the risk of fungal and bacterial pathogens infecting the developing seed. As a result, the few countries in which spinach seed is produced commercially include Denmark (>5,000 ha annually), the USA (~1,500 ha annually in the maritime region of western Washington and western Oregon), France (~220 ha), Italy (~250 ha), Holland (~500 ha), and New Zealand (~200 ha). In addition, seed crops of 'Asian' spinach types (*Tetragonia* spp.) are produced on ~8,000 ha annually in China for eastern markets. An overview of spinach seed production in the Pacific Northwest USA will be presented to illustrate the complexity of producing high quality spinach seed of cultivars that meet the requirements of growers and various markets or consumers in the USA and other countries. Aspects of spinach seed production presented include some of the primary restrictions on spinach seed production, crop rotation, cross-pollination isolation standards, field preparation and fertility, insect and disease management, weed control, and seed harvest, cleaning, health and quality testing, conditioning, and treatments.

## **Can French Coastal Areas be a new solution for spinach seed production?**

Anthony Gorin

Deleplanque, 35bis rue des Canus, 78603 Maisons-Laffitte, France

anthony.gorin@hadenseeds.com

France is a major actor in seed production worldwide with a total acreage of 379 000 ha out of which 11 000 ha are dedicated to vegetable seed multiplication. Variability of suitable climates, professional seed production companies and experienced seed growers as well as official regulatory systems to avoid cross pollinations are well in place to ensure production of high quality seeds on this vast acreage. Spinach seed production is however very limited with 248 ha in 2016 compared to the leading production areas such as Denmark (from 5 to 10 000 ha) or USA (1 to 2000 ha). The spinach seed production area in France is mainly located close to Paris in the Beauce, a traditional area with large farms and experienced growers but with possible heat waves during summer time not suitable to this heat sensitive crop. Only very few areas in the world are suitable to produce high quality spinach seeds. It requires a mild climate as found in Denmark or the maritime regions of Washington allowing a long flowering period. North West French Coasts offer similar mild climate conditions as Denmark or Skagit Valley with long days and sufficient rains. Relying on its experience and its seed grower network, Deleplanque has conducted some experiments of spinach seed production in this area in 2017 showing very good results in terms of seed yield, seed quality and seed health at a competitive production price. Further seed production contracts will allow to confirm the opportunities of this new potential area as well as its challenges.

## **Spinach seed production in New Zealand**

Jay Schafer  
Schafer Ag Services, LLC.

New Zealand has favorable climatic conditions and light requirements for spinach production in the Southern Hemisphere. Although limited in scope, spinach seed production in New Zealand opens up a production window during the winter for the Northern Hemisphere. Approximately 200 ha of spinach seed production occurs annually in New Zealand. Similar to other seed production areas of the world, constraints include insects, diseases, and environmental conditions. An overview of spinach seed production in New Zealand will be presented.

## **Spinach Production in Europe: Changes in the production over the last 30 years from a grower's point of view**

Alec Roberts, Tozer Seeds Ltd.

From blocks of frozen spinach, bunched big leafed spinach, and occasionally loose leafed spinach found on the supermarket shelf, we have seen, over the last 30 years, a massive move to the production of intensively produced baby leaf spinach for the bagged fresh salad market. What instigated this move, and what have and what could be the consequences of this change in growing practices. How sustainable is this form of production is a question as we have seen bean seed-fly, leaf minor, and downy mildew as major constraints making growing spinach difficult over the last few years. In addition, we now have several types of leaf spotting pathogens making production increasingly problematic. What approaches can be followed to safeguard spinach production into the future?

## **Organic Spinach Production in the US, Perspective from an Organic Grower / Shipper, Earthbound Farm**

Ramy Colfer, Ph.D.  
Director Agricultural Research and Pest Management  
Agricultural Operations, Earthbound Farm  
DanoneWave  
1721 San Juan Highway, San Juan Bautista, CA 95045  
[Ramy.Colfer@whitewave.com](mailto:Ramy.Colfer@whitewave.com)

Organic spinach production in the United States continues to grow. However, organic spinach production has key problems that sometimes limit organic spinach volumes. Most notably, downy mildew disease in spinach remains the greatest challenge to organic spinach production. I discuss how this disease negatively affects organic growers and shippers. I explain strategies used to help manage downy mildew disease in organic spinach production. I also discuss some potential directions in organic spinach production and spinach breeding that could lead to more stable organic spinach volumes in the future. Finally, I present some industry trends information from the California Leafy Greens Research Board. I discuss how the downy mildew disease in spinach is viewed generally by the leafy greens industry based in the United States.

## **California Seed Association's Organic Spinach Committee's Support of the Organic Spinach Community**

Dale Krolikowski<sup>1</sup> and Donna Boggs<sup>2</sup>  
Germaines Seed Technology, Inc., Gilroy, California<sup>1</sup>  
California Seed Association, Sacramento, CA<sup>2</sup>

The California Seed Association members formed a special committee to assist the organic spinach growers in finding solutions that impact their ability to supply the U.S. market with organic baby leaf spinach for the fresh produce market. An overview of the formation of the committee and the projects that are currently underway will be presented.

## Breeding Baby Leaf Spinach for California Growers

Juliana Osorio-Marin<sup>1</sup>, Rachel F. Greenhut<sup>1</sup>, Steve Klosterman<sup>2</sup>, and Richard Smith<sup>3</sup>, Allen Van Deynze<sup>1</sup>, E. Charles Brummer<sup>1</sup>

<sup>1</sup>University of California, Davis, CA, <sup>2</sup>USDA-ARS, Salinas, CA, <sup>3</sup>UC Cooperative Extension, Salinas, CA

Downy mildew (*Peronospora effusa* (Grev.) Rabenh.) is endemic to all areas of spinach (*Spinacea oleracea* L.) production in California with new virulent races appearing every 18 months on average. In addition to the aggressive nature of the pathogen, current horticultural practices for spinach provide optimum conditions for downy mildew pathogenicity and pathogen diversification such that race-specific genetic resistance, although effective until now, is becoming a losing battle. Our breeding program at the University of California, Davis is focused on providing broad spectrum downy mildew resistance in market quality spinach cultivars. Additionally, we are also selecting for low cadmium (Cd) uptake because spinach grown on soils high in cadmium can accumulate the metals to levels higher than those deemed acceptable by regulatory agencies. High Cd soils exist in parts of the Salinas Valley, which is otherwise an excellent environment for growing baby leaf spinach. Thus, spinach cultivars that have lower levels of Cd in their leaves would be desirable. While we are initially focused on developing open pollinated cultivars, we do not preclude the possibility of developing hybrids as the project progresses. We are developing our program so that we can develop and potentially commercialize OP cultivars, population hybrid cultivars, or, potentially, true F1 hybrids from inbred lines. We are assessing genetic divergence among populations, trait complementarity in different populations, and seed production potential of different crosses. We are developing segregating populations to genetically map major downy mildew resistance genes and other traits of horticultural importance. We expect to provide new solutions to conventional as well as to organic growers, releasing cultivars with broad genetic resistance to downy mildew, lower Cd uptake, and other desirable traits like leaf shape and color, shelf life and yield.

## **Water challenges in vegetable production in California.**

**Amy L. White, MPP**

**Two Girls Ranch, LLC**

Vegetable production in California faces many challenges. A major concern to production is the volatility of water availability. Although water availability changes from year-to-year, and represents a major variable to producers, there are a number of factors producers confront on an annual basis. These variables include an increasing demand on water supplies for environmental uses, water quality concerns, the 2014 Sustainable Groundwater Management Act and its unknown implications, flood risk, unreliable State funding for infrastructure projects, and reduced snowpack in the Sierra Nevada.

California will remain one of the major producers of vegetables, including leafy greens, for the foreseeable future. However, the sustainability of production of leafy greens, including spinach, will depend on a number of regulatory issues, water being one of the most important variables. Short-term water issues focus on funding for new infrastructure projects, water quality, and annual flood risks. Long-term water issues focus on the implementation of the 2014 Sustainable Groundwater Management Act and catastrophic water supply interruptions due to earthquakes, levee failures, and flooding. If California is to continue to be the largest producer of leafy greens in the U.S., the State must provide reliable and adequate funding to new infrastructure projects, fairly balance environmental and economic water allocations, and reduce vulnerability to water supply interruptions. Vegetable producers must focus efforts on providing input into the newly developing groundwater management plans under the 2014 Sustainable Groundwater Management Act.

## Genetic Diversity, Genome-wide Association Study, and Genomic Selection in Spinach

Ainong Shi(1), Jun Qin(1), Yuejin Weng(1), Jim Correll(2), Chunda Feng(2), Gehendra Bhattarai(1), Waltram Ravelombola(1), Bazgha Zia(1), Wei Zhou(1), and Beiquan Mou(3). (1)Department of Horticulture, University of Arkansas, Fayetteville, AR 72701, USA; (2) Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA; and (3) Crop Improvement and Protection Research Unit, US Department of Agriculture, Agricultural Research Service (USDA-ARS), 1636 E. Alisal Street, Salinas, CA 93905, USA.

Spinach (*Spinacia oleracea* L.,  $2n=2x=12$ ) is an economically important vegetable crop worldwide. The objective of this research was to assess genetic diversity, conduct genome-wide association study, and estimate breeding value for genomic selection in spinach. Genetic diversity and population structure have been assessed in three sets of spinach genotypes: 1) 268 USDA GRIN spinach germplasm accessions originally collected from 30 countries; 2) 45 commercial spinach F1 hybrids from three countries; and 3) 30 US Arkansas spinach breeding lines. Genome-wide association study has been conducted for disease resistance to white rust (*Albugo occidentalis*) and Stemphylium leaf spot (*Stemphylium botryosum* f. sp. *spinacia*); for pest tolerance to leafminer (*Liriomyza* spp.); for morphological traits: leaf texture, leaf base color, bolting, and height; for oxalate; and for mineral elements: boron (B), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), sulfur (S), and zinc (Zn). The general linear mode (GLM) and mixed linear mode (MLM) from TEASSEL, GAPIT, and FarmCPU were used for association analysis. The genetic diversity and population structures were related to the spinach origin (geography) and genetic backgrounds. The improved commercial F1 hybrids and Arkansas cultivars/lines had the different population structures from the USDA germplasm, and the US Arkansas breeding program had a unique genetic background. So far, SNP markers were identified to be associated with resistance to white rust, Stemphylium leaf spot, leafmine, leaf oxalate concentration, leaf traits (surface texture, petiole color, and edge shape, bolting, tallness, erectness, and mineral elements). The best linear unbiased estimator (BLUE) plus best linear unbiased prediction (BLUP) was used as prediction of breeding value using both genome-wide SNP set and the only associated SNP markers for each trait. The SNP markers identified from this study will provide breeders with a tool to select associated traits in spinach breeding programs through marker-assisted selection.

## **Spinach genome and its transcriptome variation provide insights into evolution, domestication and important nutrient traits**

Zhangjun Fei

Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, USA

Spinach, *Spinacia oleracea*, is an important green leafy vegetable and a rich source of carotenoids, folate, vitamin C, calcium and iron. The annual worldwide production of spinach was approximately 26.7 million tonnes in 2016, of which around 91.8% was produced in China. As a minor crop, currently genomic and genetic resources, which are critical for crop improvement, are very limited for spinach. We have *de novo* assembled a draft genome of spinach using high throughput Illumina sequences, combined with high-resolution genome maps constructed using the BioNano Irys system. The final assembly has a total length of ~996 Mb, with an N50 scaffold length of 919,290 bp and the longest of 9.3 Mb, and contains 25,495 protein-coding genes. The genome is highly repetitive with 74.4% of its content being transposable elements. Genome synteny analysis between spinach and sugar beet suggests substantial inter- and intra-chromosome rearrangements during the Caryophyllales genome evolution. Furthermore, we performed transcriptome sequencing of 120 cultivated and wild spinach accessions of three different groups: *S. oleracea*, *S. tetrandra*, and *S. turkestanica*. By aligning the RNA-Seq reads to the assembled spinach genome, we identified more than 420K variants. Population genomics analyses suggest that *S. turkestanica* is likely the direct progenitor of cultivated spinach, and spinach domestication has a weak bottleneck. We identify 93 domestication sweeps in the spinach genome, some of which are associated with important agronomic traits including bolting, flowering and leaf numbers. Further improvement of the reference spinach genome using emerging novel technologies will be discussed.

## Combining high-throughput genotyping and phenotyping to improve spinach breeding efficiency

Carlos A. Avila<sup>1,2\*</sup>, Juan Enciso<sup>1,3</sup>, Jinha Jung<sup>4</sup>, Thiago Marconi<sup>1</sup>, and Henry Awika<sup>1</sup>

<sup>1</sup>Texas A&M AgriLife Research, Weslaco, TX 78596

<sup>2</sup>Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843

<sup>3</sup>Biological and Agricultural Engineering Dept. Texas A&M Univ., College Station, TX 77843

<sup>4</sup>School of Engineering and Computing Sciences, Texas A&M-Corpus Christi, Corpus Christi, TX 78412

\*Email: [carlos.avila@ag.tamu.edu](mailto:carlos.avila@ag.tamu.edu)

Spinach (*Spinacia oleracea* L.) is an economically important leafy green crop grown worldwide. Spinach production must thrive in a dynamic environment constantly challenged by abiotic and biotic stresses. Such stresses have a profound effect on plant growth and development, ultimately reducing yield and quality. Therefore, the current challenge in Spinach production is to increase crop productivity by improving crop resistance and tolerance to diseases and environmental stresses, respectively. With the advancements in next-generation sequencing tools and the completion of the reference spinach genome, as well as the identification of a large panel of SNPs by high-throughput genotyping, it is now possible to identify markers associated to traits of interest to improve breeding efficiency. However, to precisely link these markers, it is required to phenotype large and diverse populations. Traditionally, phenotyping has been performed by manual measurements at single time points, but the task is very time consuming and results in high variability due to human error. Therefore, it is necessary to develop high throughput phenotyping methods to combine with current genotyping capabilities to improve breeding efficiency. An unmanned aerial vehicle was used to measure plant growth and development weekly on a spinach population with diverse leaf shapes, colors, and bolting time. Canopy cover (CC) and canopy volume (CV) was measured over the growing season and data was fitted in a sigmoid function to generate CC and CV growth curves. From growing curves, several growth parameters were calculated to precisely characterize spinach lines. Principal component and regression analysis allowed us to estimate spinach biomass, color, and bolt resistance. Genome-wide association analysis will be performed to develop molecular markers associated with plant growth variables correlated to yield and quality. It is expected developed high-throughput genotyping-phenotyping methodology will have significant applications in breeding as well as in crop management.

## **Development of molecular markers for spinach sex determination genes**

Chunda Feng, Bo Liu, Qianghua Pan, Maria Isabel Villarroel-Zeballos, Braham Dhillon ,  
Burt Bluhm, Ainong Shi, James Correll  
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Hybrid spinach cultivars are used for virtually 100% of all commercial spinach production in the U.S. and most areas of the E. U. The advantages of using hybrid spinach cultivars seed are hybrid vigor and the ability to combine the desired traits from two parents, particularly resistance to the down mildew pathogen. As spinach is dioecious, containing either predominantly male or female plants, producing hybrid cultivars is relatively straightforward. Female plants produce small amounts of pollen and can self-pollinate if no pollen from other plants is available. This phenomenon makes it possible for spinach breeders to develop pure female lines by self-pollination of female plants. Molecular markers associated with spinach sex determination genes will help facilitate the breeding and selection process. For example, breeders could select female plants before flowering, thus reducing the population size. In this research, single nucleotide polymorphisms (SNPs) have been identified from four female and four male plants of the spinach cultivar Viroflay by using genotyping-by-sequencing approach. Primers were developed based on the SNPs between the male and female plants. Six pairs of primers were found to each amplify one fragment specific to the male plants. And one pair of primers can amplify one monomorphic band from all plants and an additional fragment specific to the male plants. These dominant and co-dominant markers were tightly linked to or co-segregated with the spinach sex determination genes. These markers can be used to expedite the development of pure female lines as well as access the uniformity of femaleness.

## **A reference genome sequence for *Peronospora effusa*: towards the molecular dissection of race specificity**

Burt Bluhm, Chunda Feng, Jim Correll. University of Arkansas, Fayetteville, AR

Downy mildew, caused by *Peronospora effusa* (= *P. farinosa* f. sp. *spinaciae*), is the most economically important disease of spinach. To date, 16 races of *P. effusa* have been identified, 13 of which were described in the past 25 years. The rapid emergence of new races may be due to several factors, including the dramatic increase in spinach planting densities, selection pressure imposed by modern resistant hybrids, novel mutations in pathogen populations affecting virulence, sexual reproduction resulting in new virulence combinations, or loss of heterozygosity occurring during asexual reproduction. The genomes of three strains of *P. effusa*, representing races 12, 13, and 14, were sequenced with the Illumina platform. The sequence depths were 34x to 153x as estimated with the filtered reads. A draft genome of 25 Mb was assembled, while the total genome size was predicted to be approximately 48 Mb, of which approximately 8% was repetitive elements. About 8,000 protein-coding genes have been predicted, including numerous pathogenicity-related genes and more than 200 putative secreted effectors. The presence of approximately 15,000 single nucleotide variant (SNV) sites among the three strains was indicative of sexual recombination, and asexual loss of heterozygosity (LOH) may have generated novel genotypes with unique virulence phenotypes. These findings have implications for monitoring *P. effusa* and developing improved disease management strategies.

## Overview of Downy Mildew Disease Resistance and Race Diversity.

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Downy mildew disease, caused by the obligate pathogen *Peronospora farinosa* f. sp. *spinaciae* (Pfs) (= *P. effusa*), is the most economically important disease of spinach. A total of 16 races have been identified based on disease reactions on a standard set of spinach differentials including Near Isogenic Lines (NILs) which have been developed in cooperation with the International Working Group on Peronospora (IWGP). In addition to the 16 described races, a number of deviating strains have been identified and new deviating strains continue to appear in spinach production areas. The emergence of new races in recent years may be the result of a more favorable environment for the pathogen in the current spinach production system, the selection pressure of modern resistant hybrids, and the point mutation or sexual reproduction of the pathogen. We continue to evaluate the virulence variation of the pathogen based on disease reactions on differential sets of spinach germplasm with known resistance genes, as well as novel germplasm with uncharacterized resistance genes. Fortunately, many of the known sources of resistance are effective against a subset of the races of Pfs including the most recently characterized races. Novel strains which aggressively attack all known sources of resistance have not been identified. Examination of isolates in recent years has indicated that quite a few “novel” or “deviating” races were the result of a mixture two or more known races. However, deviating isolates of Pfs continue to be identified. One isolate in particular, UA1014APLP, was able to infect cotyledons and true leaves of all of the standard differentials, the cotyledons of most lines tested, but only infect the true leaves of certain spinach lines. In addition, observations indicate the disease reactions are somewhat temperature sensitive and the qualitative disease reaction can vary depending on temperature. Cooperation with the IWGP continues to nominate races of concern to the spinach industry.

## Whole genome variation in 2016 field populations of spinach downy mildew

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Extensive sampling of field populations of the downy mildew disease caused by *Peronospora farinosa* f. sp. *spinaciae* (Pfs) (= *P. effusa*) was conducted in 2016 in Arizona and California, USA. Initially, infected plant tissue was analyzed using targeted genetic sequencing to determine multi-locus genotypes. Following genetic analysis, a subset of 20 samples were further analyzed using whole genome sequencing. The focus was on genotypes new as of 2016 and included those thought to be the products of sexual recombination and those which may contain variation introduced during asexual epidemic development. The findings are still being processed. An initial assessment suggests ploidy varies between field isolates with evidence that the pathogen genome may experience expansions to higher levels of ploidy followed by contractions where individual chromosomes do not maintain the original allele dosages across the genome (e.g. loss of heterozygosity). This same phenomenon is also being documented in the similar, but more tractable oomycete vegetable pathogen, *Phytophthora capsici*, and the implications for field studies and the breeding of durable resistance will be discussed.

## **Downy mildew of Spinach: Oospore production, viability, and incidence on seed**

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Downy mildew caused by *Peronospora effusa*, is the major disease threat for spinach production in the US and worldwide. The formation of oospores, sexual spores of *P. effusa* on spinach seed was described in the early 1980's. Studies about viability of oospores and their dispersal through spinach seed are important to manage seed-borne inoculum and potentially reduce the increasing number of virulent races that are appearing in US spinach production areas. One hundred sixty-eight spinach seed lots (1,000 seeds per lot) were assessed to estimate the levels of seed-associated oospores, and determine the germination potential of newly formed oospores in infected spinach leaves. Oospores were counted using a compound microscope. Nineteen percent (32/168) of seed lots were infested with oospores. Viabilities of oospores from five seed lots were determined by plasmolysis in hypersaline condition, and staining with trypan blue. About 59% of the oospores were viable by the plasmolysis test, and 45% by trypan blue staining. The oospores were plasmolysed in saline solution (4 M NaCl) and deplasmolysed in water, displaying an active and viable cell membrane. Oospores from infected leaves incubated at 4°C for about a month germinated and produced coenocytic mycelium. These germinating oospores indicate that oospores likely serve as primary inoculum to initiate the disease in field conditions.

## **Seed as a pathway: Spinach seed health assay protocol development for *Peronospora effusa***

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Seed health testing protocol development and/or validation is primarily the aim of organizations such International Seed Testing Association (ISTA), National Seed Health System (NSHS), and the International Seed Health Initiative – Vegetables (ISHI-Veg). ISHI-Veg brings together seed companies, public sector institutions and private laboratories that collaborate in developing detection methods for economically important seed-borne pathogens of vegetable crops. ISHI-Veg works closely with ISTA, NSHS and others to ensure that the detection methods are reliable and robust. The priority for initiating the development of detection methods for seed-borne pathogens within ISHI-Veg is contingent in part on the International Seed Federation (ISF) regulated pest list database (<https://pestlist.worldseed.org/public/pestlist.jsp>).

The publication of the regulated pest list database on the ISF website recognized spinach seed as a pathway for *Peronospora effusa* (*P. farinosa* f. sp. *spinaciae*). Combined with the ISF pest list that is based on scientific peer reviewed publications, there has been increased pressure within the industry to have a standardized testing protocol for the presence of *P. effusa* on spinach seed. A draft test plan (protocol) was presented at ISHI-Veg meeting in November of 2017. The current test plan includes a seed wash assay followed by an oospore viability test, and if viable oospores are detected the final confirmation step would most likely be grow out based. Once the test plan is finalized and there is sufficient positive seed available, a rigorous comparative test will be organized and performed. The goal is to have a robust protocol that can be publish on the ISF website ([www.worldseed.org](http://www.worldseed.org)) and ultimately be accepted as an ISTA and NSHS accredited protocol.

## **Evaluation of oospores on spinach seed**

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Downy mildew of spinach, caused by *Peronospora farinosa* f. sp. *spinaciae* (= *P. effusa*), is the most economically important disease of spinach in most spinach production areas of the world, including the U.S. Downy mildew was mainly transmitted by asexual spore sporangia through water splashing and wind. However, sexual stage oospore was detected on spinach seed surface, which suggest seed transmission might also be a transmission approach for spreading downy mildew to other areas. The objective of this research was to develop protocols to quantify the oospores on spinach seed surface, and examine the existence of oospores in commercial seed lots. A total of 16 targeted commercial seed lots were examined with the seed wash assay and six seed lots were detected with oospores on the seed surface. The oospore numbers in the contaminated seed lots ranged from 3 – 231 oospores / gram of seed. Based on commercial planting densities, this would translate into the introduction of approximately 300,000 to 25 million oospores / ha<sup>1</sup>. Estimates of oospore viability based on a trypan blue stain assay and a NaCl plasmolysis assay ranged from 22.2 to 46.3% and 38.5 to 55.9%, respectively for the contaminated seed lots. Germination tests for the isolated oospores from commercial seed lots are in progress, as are seed treatments to try and reduce oospore viability.

## Characterization of spinach leaf spot pathogens and fungicide efficacy

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Leaf spot diseases of spinach, which reduce yield and quality, have become a major concern in spinach production in Texas. Determining the causal agents of the various leaf spot diseases on spinach, their relative frequency, pathogenicity level and the fungicide efficacy to these pathogens is vital for effective disease management. Spinach leaves with leaf spot symptoms were collected in the Winter garden area of Texas from 2016 to 2017. Leaf samples were incubated in a moist chamber and isolations were performed on PDA. Fifty lesions were evaluated for each of the 47 samples. Fungal and bacterial colonies were recovered to pure cultures for pathogenicity test. Fungal isolates were identified to species based on morphology and molecular analysis, including DNA sequencing of the ITS region, GS and GPDH intron and 16S rRNA and PCR with pathogen-specific PCR primers. The survey demonstrated that anthracnose (*Colletotrichum dematium* f. sp. *spinaciae*) and Stemphylium leaf spot (*Stemphylium botryosum*) were the predominant diseases. Several additional fungi were recovered from leaf spot lesions at limited frequency, and were demonstrated to be pathogenic to spinach, including a *Colletotrichum coccodes*, *C. truncatum*, *Cercospora* sp. and *Myrothecium verrucaria*. None of the bacterial isolates recovered was pathogenic on spinach. Pathogenicity test showed that *C. dematium*, *C. coccodes*, *C. truncatum* and *S. botryosum* can caused significant leaf damage on spinach leaves. Moreover, fungicides Bravo WeatherStik, Dithane F-45, Cabrio, and Merivon were completely effective in preventing leaf spot symptoms from developing when inoculated with *C. dematium*, *S. botryosum*, or the combination of both pathogens.

## Determining the genetic basis of pathogenicity of *Fusarium oxysporum* f. sp. *spinaciae* on spinach

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The maritime Pacific Northwest (PNW) is the only region of the United States suitable for production of spinach seed, which requires a long day length and cool, dry summers to yield high quality seed. The most significant disease affecting spinach seed crops in this region is Fusarium wilt, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *spinaciae* (*Fos*). Resistance to spinach Fusarium wilt is quantitative, with no known races of *Fos*. Furthermore, the genetic basis of pathogenicity of *Fos* is unknown. Fourteen effector genes called *Secreted in Xylem* (*SIX*) genes, originally discovered in *F. oxysporum* f. sp. *lycopersici*, the causal agent of tomato Fusarium wilt, have been demonstrated to be major determinants of pathogenicity to tomato. *SIX* genes have been identified in other *F. oxysporum* formae speciales (ff. spp.), and each f. sp. examined to date appears to have a unique *SIX* gene profile. To determine if *Fos* carries *SIX* genes and has a different *SIX* gene profile compared to the profiles of other ff. spp., PCR assays for the 14 *SIX* genes were used to test 38 isolates of *F. oxysporum* representing ff. spp. *cepae*, *ciceris*, *lycopersici*, *pisi*, and *spinaciae*. Of the 19 *Fos* isolates tested, 2 carried only *SIX8*, 11 carried only *SIX14*, and 6 carried both *SIX8* and *SIX14*. The isolates of ff. spp. *cepae*, *ciceris*, *lycopersici*, and *pisi* tested all had the same *SIX* gene profiles as published previously. Phylogenetic analyses of the *SIX8* and *SIX14* DNA sequences indicated that the *Fos* isolate sequences are divergent from those of other ff. spp. tested. Furthermore, the predicted *SIX14* amino acid sequence was unique for *Fos* compared to other ff. spp. carrying this gene. To our knowledge, this is the first report of *SIX* genes in *Fos*. Characterization of the *SIX* effector profile of *Fos* and identification of other potential effector genes unique to this pathogen will assist with developing molecular diagnostic tools for the pathogen and breeding spinach cultivars with greater levels of resistance to Fusarium wilt.

## Managing damping off in baby-leaf spinach in Australia

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Field production of baby-leaf spinach surged in Australia over the past decade. It is currently valued at AU\$55M annually. Year-round supply requires a network of growers spanning diverse regions and production environments. Damping off has become a significant constraint particularly where multiple crops are grown without rotation. Diseased plants have yellow or wilted leaves which contaminate harvested product. Furthermore when plants are damped-off the remaining healthy plants have a flatter habit making machine harvesting more difficult and resulting in broad cuts across leaf blades rather than at the petiole. This reduces shelf life and failure to meet processor specifications. Our current study has identified and confirmed the key pathogens causing damping off in different production regions. They are: various species of *Pythium*, namely *P. aphanidermatum*, *P. ultimum* var *ultimum* and *P. irregulare*; *Rhizoctonia solani*; and *Fusarium oxysporum* f.sp. *spinaciae*. They occur either as single pathogens or more commonly as disease complexes. Greenhouse and field trials are evaluating chemical, cultural and biological control options. Chemical soil drenches at sowing or dressed seed with certain chemicals has successfully controlled *Pythium* but not always *Rhizoctonia* rots. To date microbial biological control products tested have yielded similar results highlighting our challenge to find robust management options for *Rhizoctonia* rots.

## **Rhizoctonia: seedling disease or web blight in Texas spinach?**

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*Rhizoctonia solani* (Kuhn) is a common soilborne fungal pathogen of many economically important crops, including spinach. In the Texas Winter Garden spinach production area, *R. solani* has historically been considered a seedling disease that was not a significant challenge to yield and profitability. However, in the 2017-2018 winter production season, three field locations were noted to have not only seedling disease symptoms, but also mature plants displaying symptoms similar to those seen in web blight. Symptoms on mature plants observed and collected in the field at all three locations included stunting of the plant, deep lesions on the stems that were brownish-red in color near the crown tissue extending upward, and water soaked leaf lesions from the outer edges inward. Leaf lesions enlarged rapidly and coalesced, resulting in the collapse of large areas of leaf tissue with whitish-tan mycelium evident when humidity was especially high for long periods of time. Leaves on mature plants had extensive lesions on the leaf blade area. In screenhouse grown spinach plants that were inoculated with isolates of *R. solani* from field grown spinach plants, similar symptoms were observed. While applications of the fungicide azoxystrobin have been reported to provide satisfactory control in the past, there is an increasing interest in organic production where most fungicides cannot be applied, including azoxystrobin. Additionally, later season symptoms in mature plants are likely under reported in the field, especially in cases with stunting and not complete plant death. The field observations and results from this work indicate that *R. solani* can have a greater impact in spinach production than previously thought, particularly in organic systems.

## **A multidisciplinary approach to improve damping-off tolerance in spinach**

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Damping off is a common disease of spinach in agricultural fields worldwide. Damping off can be classified into pre-emergence damping off, including seed rot and root rot before seedling emergence, and post-emergence damping off, including seedling rot and wilting after emergence. These symptoms can be caused by a complex of soil-borne pathogens that belong to different genera: *Aphanomyces*, *Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia*. Several studies have confirmed the importance of *Pythium ultimum* as it is the most abundant damping off causing pathogen in tested spinach fields. In conventional spinach production, fungicides can be used to reduce damping-off symptoms. However, in organic production fungicides are not allowed. In recent years, food safety awareness of consumers has increased, restricting the use of fungicides in conventional production as well. As a result, the problems of damping off becomes more apparent. Therefore, we need spinach that is better able to resist or tolerate damping off causing pathogens. Spinach breeders have observed variation in damping-off tolerance between spinach cultivars as well as between seed lots of same cultivars. In addition, high soil moisture level and high temperatures can increase the severity of damping off. We hypothesize that the variation in damping-off tolerance level is caused by a combination of factors, including the genotype, environmental conditions and seed vigour. In order to phenotype damping-off tolerance level in a reliable and reproducible way, we need to take these factors into account. At this moment, we are developing a phenotyping method for pre-emergence damping off. In the mean time we are studying seed vigour in relation to moisture, temperature and oxygen level. Our hypothesis is that a higher seed vigour, in terms of fast germination and/or emergence from the soil, contributes to a better tolerance to damping off. We aim to find genetic variation for seed vigor components that increase damping-off tolerance in spinach. Eventually, our research will contribute to the development of a more tolerant or even resistant spinach cultivar.

## Development of a Spinach White Rust Management Strategy in Texas

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After trial plantings of spinach in 1917 and 1918, spinach became a major crop in Texas in the early 1930s with upwards of 33,000 acres being planted. In the late 1930s white rust (*Albugo occidentalis*) became a major production problem. At one time white rust was considered the most damaging Texas spinach disease. Losses are due primarily to reduction in quality and increased costs resulting from increased sorting time, and rejection of loads when the level of white rust is too high. Initial outbreaks often follow hard rains. Plants infected with the white rust fungus are weak and collapse quickly under warm, humid, or wet conditions. Free moisture on a leaf surface is the key to rust spore germination and development. The disease appears as a small yellowish spot on the upper surface of the leaves. As these lesions develop, glassy white pustules form which eventually release spores. Tissue next to the pustules may turn brown and an entire leaf may become infected and die. Growers struggled with this issue and gradually there was a decline of the industry. In 1972 there were cooperative breeding efforts between Texas A&M and Arkansas to overcome these challenges and in 1976 Dainello established a white rust screening nursery at Uvalde to screen varieties for tolerance of this problem. In 1984 University of Arkansas spinach breeder, Teddy Morelock, joint ventured with Dainello to screen new lines for resistance to white rust. Resistant plants were dug and used for breeding and great strides were made in developing white rust tolerant varieties. In addition, fungicide trials were being conducted. Researchers learned that an in-seed furrow application of metalaxyl at planting would provide several weeks of control. In addition, screening of new generation fungicides has resulted in the identification of promising white rust control compounds and subsequent spinach labels like Quadris and Cabrio. Furthermore, various foliar materials continue to be evaluated for control of this problem. Currently, economic control of this disease is being achieved with the implementation of a systems approach including the use of resistant varieties, in-seed furrow application of metalaxyl at planting and the use of foliar fungicides.

## White rust: beyond borders

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White rust, caused by the obligate oomycete pathogen *Albugo occidentalis*, is an important spinach pathogen in some production areas around the world. Although white rust was identified in Texas as early as 1930, and has been endemic to Texas, Arkansas, and east coast production areas for some time, the pathogen has more recently been found in other production areas of the world. For example, white rust was reported in Greece in 2013, in Mexico in 2017, and in Turkey 2018. In addition, there is a poorly documented reported of white rust on spinach in Iran in 2000. The white rust pathogen has a somewhat similar lifestyle to the related downy mildew oomycete pathogen *Peronospora farinosa* f. sp. *spinaciae* (= *P. effusa*) producing asexual sporangia. However, unlike Pfs where sporangia germinate to produce a germ tube, sporangia of *A. occidentalis* release swimming zoospores. Also, *A. occidentalis* produces copious amounts of oospores in older lesions and senescing spinach leaves. The mechanism by which *A. occidentalis* has been introduced into these newer geographical areas is unknown as no spinach seed production occurs in areas where white rust is present. Thus, the potential for the white rust pathogen to be introduced into major production areas where it is not present exists. Although there is no known qualitative resistance known in spinach, there is quantitative resistance (governed by QTL, or quantitative trait loci) in some material. Del Monte Seed Co. and the University of Arkansas breeding programs from many years ago to first identify and characterized this type of QTL resistance. Current efforts are underway to genetically characterize QTL resistance in spinach.

## **Tissue culture approaches for disease resistance research in Spinach**

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Identification and cloning of disease resistance genes is critical to develop a better understanding of resistance-gene function. Molecular characterization and functional validation of a resistance gene function can be achieved by transforming a susceptible plant line with the candidate R-gene and subsequently screening for the resistance phenotype. The objective of this research was to develop tissue culture system for spinach to examine resistance gene function. Three spinach cultivars, Viroflay, Lion, Fall Green, and three Near Isogenic Lines, NIL1, NIL2 and NIL3, were used in the tissue culture system. Seeds were surface sterilized and allowed to germinate on water agar for 10 days. Explants derived from three different tissues, hypocotyl, cotyledonary, and true leaves, were subcultured to initiate callus formation. For proof of concept, a vector (pCAMBIA1300) containing the screenable reporter gene green florescent protein (GFP) will be introduced into the callus using biolistic and agrobacterium transformation approaches. Once the protocol is established in the lab, candidate resistance genes will then be introduced into the callus obtained from different susceptible spinach cultivars. Successful establishment of callus from explants, and regeneration of transformed adult spinach plants will aid in the functional validation of several candidate resistance genes.

## Genome sequencing of spinach Near Isogenic Line 1 and candidate resistance genes

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The *Peronospora*-spinach pathosystem has been characterized by the occurrence of cultivars that carry distinct resistance genes capable of recognizing specific races of the downy mildew pathogen. In order to understand the genetic basis of downy mildew resistance, research has focused on the identification and characterization of Resistance Gene Analogs (RGAs) in spinach. For this purpose, several genetic and molecular resources were generated. Six Near Isogenic Lines (NILs) containing distinct resistance loci named, Resistance to *Peronospora farinosa* (RPF) were developed by utilizing different resistance sources and recurrently backcrossing the susceptible line to these cultivars and selecting for resistance. In-house draft genome sequence for the NIL1 line was instrumental in the development of molecular markers that co-segregate with the RPF1 resistance locus. Subsequently, the RPF1 locus was narrowed down to a region containing a cluster of multiple RGAs in the NIL1 draft genome sequence. Currently, multiple approaches are being followed for the functional validation of the candidate RGAs at the RPF1 locus in spinach. In one approach, the RGAs are being used to transform a susceptible spinach genotype to determine if any of the RGAs impart resistance in an otherwise susceptible line. Another approach to assess functionality is to knock-down RPF1 function in the resistant genotype using an RNAi approach. A third approach focuses on the CRISPR/Cas9 system to disrupt the RPF1 locus and subsequently assess for disease susceptibility. The ultimate goal is to have a robust system to validate RPF gene function in spinach to improve the durability of resistance to the downy mildew pathogen.

## Evaluation of natamycin seed treatments for *Stemphylium botryosum* and other necrotrophic fungi on spinach seed

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Natamycin is an antifungal compound used widely in the food industry. A spinach seed lot naturally infected with *Stemphylium botryosum* and other necrotrophic fungi was used to evaluate natamycin for control of these fungi on spinach seed. Natamycin treatments (0.5, 1.5, and 4.5 g/kg seed) were evaluated in combination with three durations (short, medium, and long) of a proprietary seed priming treatment. Non-primed seed served as a control treatment, and metalaxyl + thiram applied in combination with each priming duration were compared with the natamycin treatments. The freeze-blotter seed health assay revealed 68.8% of the seed infested with *S. botryosum*, 74.3% with *Verticillium* spp., 81.0% with *Alternaria* spp., and a diversity of other necrotrophic fungi at incidences ranging from <1 to 30%. All of the priming + natamycin treatments were highly effective at preventing development of these fungi on seed. The treatments completely prevented *S. botryosum* from developing on seed except for 0.3% of the seed subjected to the short priming duration + 0.5 g natamycin/kg seed, and 1.0% of seed with the long priming duration + metalaxyl + thiram. Likewise, the treatment combinations prevented development of *Verticillium* spp. and *Gibbelulopsis nigrescens* (formerly *V. nigrescens*), prevented development of *Fusarium* spp., and prevented development of *Alternaria* spp. (except 0.3% of the seed with the medium priming duration + metalaxyl + thiram). Actinomycetes, which can be beneficial on seed, developed on 16.3% of the control seed. The incidence of seed with actinomycetes was not reduced by the short priming duration + natamycin (any rate of application) or by the medium priming duration + natamycin at 4.5 g/kg seed. All other treatments reduced the incidence of seed with actinomycetes. Seed germination measured after 7, 14, and 21 d was affected adversely by the medium and long durations of priming combined with the higher rate of natamycin, and the incidence of seed with abnormal germination was increased by the long priming duration with natamycin at 1.5 or 4.5 g/kg seed. The results indicate spinach can be subjected to short or medium priming durations with natamycin applied at 0.5 or 1.5 g/kg seed for control of seedborne necrotrophic fungi without reducing seed quality. Various durations of priming and rates of natamycin application are being evaluated with additional seed lots for potential use in conventional and certified organic spinach production.

## Use of PurGrow for spinach disease management

Chunda Feng, and James Correll

Current high-density spinach production provides a conducive environment for disease development. Even though spinach is a short season crop, often being harvested in 4-5 weeks, many diseases, such as downy mildew, white rust, and several leaf spots can cause severe reductions in yield and quality. Although some fungicides can be used to manage diseases in conventional production, few options are available for organic production. As a result, some novel disease management materials are being evaluated. In this research, PurGrow was evaluated for managing spinach diseases. Although a single application of PurGrow one day prior to inoculation with *Peronospora effusa* (*Pe*) had no effect on downy mildew disease development, it was effective as a curative in killing active lesions. After spraying the solution once a day for five consecutive days, downy mildew or white rust diseases were inactivated and plants recover from the infections. PurGrow could suppress the germination of *Pe* spores, and instantly kill the zoospores of *Albugo occidentalis*. In seed wash experiments, the results indicated that PurGrow is highly effective as a surface disinfectant and can kill the microbes, including pathogens, on the seed surface. There was no negative effect of the treatment on seed germination rate. These results were compatible with that of seed washed with 40% household bleach. Thus, PurGrow can be used as an effective seed disinfectant. Evaluation of PurGrow for spinach leaf spot diseases and efficacy on *Pe* oospores is on-going. In the future, PurGrow will be tested for soil sanitation and managing spinach diseases by addition to spinach irrigation.

## **Subterranean collembola, a challenging pest of spinach seed production**

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Subterranean springtails (Onychiurini), members of the Subclass Collembola, can be a serious pest of spinach and spinach seed production. In 2013 a spinach seed field on Fir Island in Skagit County, Washington State, suffered severe stand loss as a result of damage from subterranean collembola. A field trial was conducted in the same ground the following year to investigate effective insecticides. Laboratory experiments were also performed to determine effects of subterranean collembola on germinating spinach seed. Laboratory experiments revealed the collembola would enter the pericarp, feed on the germinating spinach seed and lay eggs in this protected location. Collembola were also highly attracted to the spinach root hairs which they fed upon and cotyledons exhibited characteristic holes as a result of collembola nibbling.

The field trial occupied approximately 0.2 hectare and consisted of 12 different insecticides plus the untreated in a randomized split plot design (treated side-by-side with untreated). Soil samples were processed using a modified Berlese -Tullgren funnel to extract the collembola. Collembola counts were made for each of the 72 treated and corresponding 72 untreated replicates for a total of 144 samples. The limited number of funnels (24) and 3-day extraction process, stretched sampling across 2 weeks. Additional analyses included stand count/plot, plants/acre, dried biomass/10 plants, dried biomass lbs/acre and final plant height. The number of collembola extracted/sample verified their presence but failed to clearly identify efficacious products resulting from either toxicity or repellency. Due to the complexity of variables including soil moisture, spotty infestation and mobility of the collembola among others, results of the field trial were not conclusive.

## **Food Safety for the Spinach Industry-A Global Agenda**

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A Multistate outbreak of Shiga Toxin-Producing *Escherichia coli* 0157:H7 linked to Organic Spinach and Spring Mix Blends in 2012 in the United States, and in 2011 The European Commission dealt with the E-coli outbreak(shigatoxin-producing *Escherichia coli* O8:H19 stx2+) in organic cucumbers are just 2 examples of the potential risk of food borne illnesses caused by several food borne pathogens which must be addressed. In the United States Good Agricultural Practices (GAPs), and now the 2011 Food Safety Modernization Act(FSMA) will have a major impact on how food safety education and compliance procedures and strategies are managed by growers, processors, shippers, packers and harvesters of spinach and other leafy greens. The European Food Safety Authority's new strategy for 2020 and beyond will guide the EFSA in carrying out its mission of protecting European consumers from health risks in the European food chain. Knowing that these protocols are in place or soon will be, the spinach industry needs to take a proactive global approach to insure a safe product at all levels of the spinach industry. This proactive global approach will insure that everyone in the food chain from growers, harvest crews, packing shed workers, shippers retailers and consumers must be trained on food safety if we are going to have a safe food source in America, Europe and the World.

## **Insights into nitrogen assimilation and oxalic acid metabolism in spinach**

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Spinach needs significant amounts of nitrogen to sustain a rapid vegetative growth during its short growing cycle. However, spinach has poor nitrogen use efficiency and is inefficient in nitrate reduction resulting in accumulation of high amounts of free nitrates. Besides many health-promoting compounds, spinach accumulates high amounts of anti-nutritional oxalates that could inhibit mineral (Ca and Mg) bioavailability. Studies have confirmed increased accumulation of oxalates and lower ascorbates in spinach leaves with increasing amounts of available nitrates. Organic spinach production is also profoundly reliant on the ability of plants to uptake the limited nitrogen, due to the inconsistent and unpredictable mineralization of nitrogen. We screened 355 spinach accessions for nitrogen uptake (free nitrates and ammonia) and assimilation (free amino acids) in the open field conditions under organic and conventional farming. Preliminary analysis confirmed that the conventionally produced spinach had higher nitrate levels than organic in almost all the accessions tested. There was significant variation in the amount of the nitrates and the proportion of  $\text{NO}_3/\text{NH}_4$  in both the systems. These results suggested the possibility of identifying accessions that are efficient in harnessing higher total nitrogen in the form of proteins and amino acids rather than free nitrates for each farming system. A set of accessions with high and low leaf oxalates was used for transcriptomic and metabolomics studies to understand the correlations between oxalic acid, free nitrates, amino acids and associated gene network. Expression analysis and metabolic profiling of the selected accessions validated the impact of nitrates on oxalate content; implying their utility as bio-markers for trait improvement and introgression breeding.

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