Final Report

International Onion Researcher Delegation

Project leader:

Lechelle Earl

Delivery partner:

Onions Australia Pty Ltd

Project code:

VN17001

Project:

International Onion Researcher Delegation – VN17001

Disclaimer:

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Summary

Australia's onion growers need to be kept up to date with international R&D findings and production practices in order to manage inevitable challenges such as those posed by pests and diseases, and to further boost productivity. This project, carried out in 2018, sought to develop Australia's onion industry by engaging international visitors to come to Australia to share their knowledge during a series of events.

The project meets the recommendations of the Onions Strategic Investment Plan 2017-2021, that the industry needs to boost productivity through better engagement with industry R&D investment and outcomes as well as greater access to international expertise. Outcome 3 of the Onions Strategic Investment Plan states that:

- The best way to reduce costs is to improve yield to have more marketable product for the same input cost
- Pest and disease management is the biggest factor in improving yield. There needs to be ongoing work to provide cost-effective solutions to the key pest and disease challenges.

To meet this Outcome, two overseas experts were brought to Australia to meet with producers and others along the supply chain.

Louis de Kock from South Africa, one of the world's largest growers of onions, is renowned internationally for consistently producing superior quality produce in a sustainable and environmentally friendly way.

Louis was invited to present at the Hort Connections event, held in Brisbane 18-20 June 2018. Hort Connections attracts around 2000 delegates with 50 onion levy payers (90 per cent of the industry production) expected to attend the 2018 event.

Onions Australia also arranged for Louis to attend a farm tour of Queensland's Lockyer Valley, which allowed levy payers who were not able to attend the Brisbane-based convention to benefit from his expertise.

The second expert was plant pathologist **Dr Fred Crowe** from the US, who was invited to travel to Tasmania to impart his knowledge of best practice for control and prevention of onion white rot, a serious soil borne disease limiting production in parts of Australia, including Tasmania and South Australia.

Fred was asked to speak at a conference in Tasmania in October 2018, attend the onion industry information day where 75 growers and stakeholders were expected, and appear at the Forthside Vegetable Research Facility open day, scheduled to attract over 200 people.

The project enabled a mix of one on one meetings, group discussions, question and answer sessions, farm visits, technical presentations, international perspectives and tips for best practice on farm.

The project was completed both on budget and within specified timeframes.

Evaluation revealed that the international experts were well received and it was clear that audience members made the most of the opportunity to further their knowledge of both national and international world best research and development practices. Past delegations of this kind have demonstrated practice change as a result of the new information imparted.

The visits successfully provided access for Australian onion growers to world best practice, including white rot management, and provided insights into boosting productivity and targeting international markets.

Keywords

Onions, extension, tour, communication, delegation, onion white rot, biosecurity, industry development

Introduction

Australia's onion growers need to be kept up to date with international R&D findings and production practices in order to manage inevitable challenges, such as those posed by pests and diseases, and further boost production to supply overseas markets. This project, carried out in 2018, sought to develop Australia's onion industry by engaging international visitors to come to Australia.

The onion industry at a glance

The Australian onion industry is a mature industry with stable production. It is the fourth largest vegetable crop produced in Australia and the second largest vegetable category exported. Onion production occurs almost year round with the peak harvest season for all varieties occurring between summer and autumn.

Production of onions has grown steadily over the past two decades, with the main type of onion grown in Australia being the traditional brown onion, which accounts for 79 per cent of fresh production.

Onions are grown in most states of Australia; however, South Australia and Tasmania together produce 71 per cent of the Australian crop. Key onion production locations are the Lockyer Valley in Queensland; north-eastern regions of South Australia and the Adelaide plains; and the Devonport/Launceston region of Tasmania.

Pest and disease management is an important issue for onion growers, not just because of the impact on yield and quality, but also because chemicals used to control them add significantly to production costs.

In 2015-16, Australia exported 45,000 tonnes of onions with a value of $29 million, with half sent to Europe, particularly Germany. Markets in the Middle East and Asia are expected to take more Australian onions in the near future, due to lower freight costs. Exported produce commands higher prices that the domestic market, offering considerable opportunity to onion growers.

Looking to the future

According to the Onions Strategic Investment Plan 2017-2021, the industry needs to boost productivity through better engagement with industry R&D investment and outcomes as well as greater access to international expertise.

This project sought to address this recommendation by supporting two aspects of Outcome 3 of the Onions Strategic Investment Plan:

- The best way to reduce costs is to improve yield to have more marketable product for the same input cost
- Pest and disease management is the biggest factor in improving yield. There needs to be ongoing work to provide cost-effective solutions to the key pest and disease challenges.

Making use of overseas expertise to boost profitability for Australian growers

Two international experts were invited to visit Australia at different times during 2018 to impart their knowledge to growers. Both visits were arranged to coincide with large events attended by the majority of onion growers, to ensure maximum knowledge transfer to Australian growers. In addition, one on one meetings, group discussions, question and answer sessions, and farm visits were scheduled to provide a variety of opportunities for growers to learn from the delegation.

The first visitor was one of the world's largest growers of onions, Louis de Kock from South Africa. Louis is renowned internationally for consistently producing superior quality produce in a sustainable and environmentally friendly way.

Louis was invited to present at the Hort Connections event, held in Brisbane 18-20 June 2018. Hort Connections attracts around 2000 delegates with 50 onion levy payers (90 per cent of the industry production) expected to attend the 2018 event.

Onions Australia also arranged for Louis to attend a farm tour of Queensland's Lockyer Valley, which allowed levy payers who were not able to attend the Brisbane-based convention to benefit from his expertise.

The challenges posed by onion white rot

Growers in parts of northern Tasmania face additional production challenges due to the presence of onion white rot, a fungal disease that reduces the yield of commercial onion crops. This soil borne disease is caused by *Sclerotium cepivorum* and affects Allium species including garlic, leek, chives and spring onion.

Once a bulb is infected, it rots and the fungus forms small black reproductive structures called sclerotia. These can remain viable in soil for up to twenty years and are not controlled by fungicides.

Production for growers affected by white rot is commonly based on integrated pest management involving fungicides, which help to control the disease in developing crops.

Since onion white rot poses a considerable management challenge for these growers, Onions Australia sought to bring an white rot expert to Australia to speak to growers in Tasmania, ensuing that South Australia growers were notified of the visit.

Plant pathologist Fred Crowe, from the US, was invited to travel to Tasmania to impart his knowledge of best practice for control and prevention of onion white rot. An itinerary was devised to ensure that growers had the best chance to interact and learn from him.

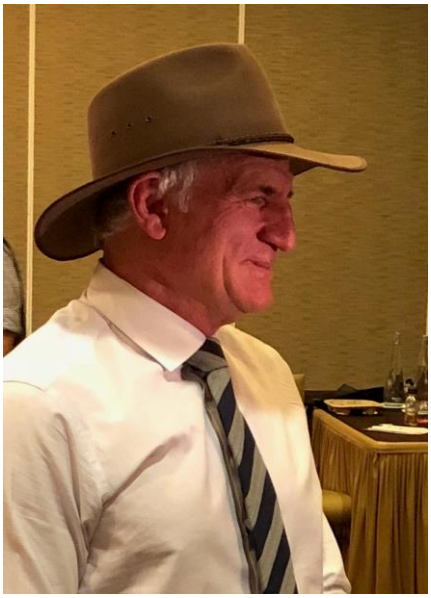
Fred was asked to speak at the Onions Australia Levy Payer Information Day in Tasmania in October 2018, attend the onion industry information day where 75 stakeholders were expected to attend, and appear at the Forthside Vegetable Research Facility open day, scheduled to attract over 200 people.

The visits by these international experts will give Australian onion growers access to world best practice and provide an insight into targeting international markets, as well as diversifying their product. The project followed the format of a highly successful international delegation in 2017 (see VN16001)

Methodology

The international onion delegation consisted of two visits by overseas experts.

Louis de Kock 's visit to Queensland in June 2018



Thirty years ago Louis de Kock purchased rundown farming operations on the banks of the Vaal River near Barkly West in the Northern Cape Province of South Africa and transformed it into a highly successful enterprise that sets international benchmarks for onion production.

Key to the success of the farm is the use of more than 70 centre pivot irrigators that are used to grow onions as well as beetroot, carrots, potatoes, maize and wheat.

Production land of 3750 hectares per year is under irrigation, with 1810ha under vegetables. It produces 75 - 100t per hectare of onions.

Louis spent ten days in Australia from 14 to 23 June 2018. He featured as keynote speaker at the Onions Australia Onion Levy Payer meeting in Brisbane on 18 June 2018 which was well attended by growers. He spoke for more than an hour, addressing 45 industry representatives.

Louis' expertise in shaping the land at optimal gradients was a key to his success and inspirational for Australian growers. This shaping ensured maximum water infiltration with ideal run-off conditions, and relied on laser levelling.

In addition to the centre-pivot systems, he added pumps, new pipe networks and pump-stations. Each possible area of the farm that has the potential to be productive underwent careful planning and sustainable soil preparation for future crops. Growers can learn more about Louis' Wildeklawer enterprise on his website

As part of the visit, Louis spoke at the Hort Connections event in Brisbane on 19 June, about his successful operations and accessing international markets.

He also travelled to regional Queensland to meet with groups of onion levy payers, accompanied by Onions Australia CEO Lechelle Earl and other members of the Onions Australia Executive Committee.

Louise made three farm visits:

- At Kalbar in southeast Queensland, he met with ten key growers representing half of the state's production, and toured crops and on-farm packing facilities. He also met with seed breeders.
- In the Lockyer Valley he met with local growers on two onion farms and production lines.
- He visited vegetable growers Kalfresh to discuss diversification of products.

Louis de Kock inspects produce in Queensland

Dr Fred Crowe's visit to Tasmania in October 2018

Dr Fred Crowe is an emeritus plant pathologist with Oregon State University, famous for his work on controlling onion white rot using garlic juice.

Fred is an international expert on the biology and control of soil and seed borne diseases of onion and other crops. His main clients are large Western US garlic and onion companies together with many small garlic producers both domestically and internationally.

Since 2007, Fred has been technical advisor to the California Onion and Garlic Research Advisory Board. He also served as superintendent of the Central Oregon Agricultural Research Center from 1984 to 1998.

Onions Australia was honoured to host Fred's visit to Tasmania to meet with growers and speak about his many years in the onion industry. During his 12 day stay, Fred had a packed agenda that allowed him to engage with many onion growers and other onion industry stakeholders.

Key among them were:

- Speaking at the Onions Australia Levy Payer Information Day held in Ulverstone on 19 October
- Featuring at the Tasmanian Institute of Agriculture's open day on 10 October
- Explaining the soil temperature model to around 40 industry personnel
- Giving an interview to the ABC at Forthside
- Presenting his findings on the longevity of white rot in soil to the Tasmanian onion agronomy group, including a delegation of NZ onion growers and agronomists
- Visiting many key growers on the North West Coast and North East Tasmania to discuss production practices including managing white rot
- Meeting with the Tasmanian Onion Agronomy Group Discussing collaborations with Tasmanian Institute of Agriculture scientists
- Presenting to 20 people on white rot biology and control in Forth
- Providing an interview to the University of Tasmania, University College.

Onion growers and agronomists learned the latest on managing white rot in Kindred, Tasmania.

Onions Australia ensured that growers from South Australia also affected by white rot would attend the Tasmanian sessions. Fred's presentation *Update on Allium White Rot Activities in the U.S.*, is at Appendix 1.

Outputs

Both visits were met with acclaim by onion growers who took part in the various events. Onions Australia executives, agronomists and growers gained a greater understanding of how the industry and individual farms can improve productivity including through better management of white rot.

Each event had good turnout, confirming the benefits of the visits for growers and other stakeholders. It is estimated that the Hort Connections presentation by Louis De Kock was attended by growers covering 80 per cent of production.

Fred Crowe's events in Tasmania were attended by 95 per cent of all onion industry production, representing exceptionally high audience coverage for an outreach program.

Each of the major outreach activities was evaluated by attendees, who confirmed a high level of satisfaction among levy payers. Comments received in evaluations included that Fred's presence provided "timely advice from a leading expert" and that Louis was "an engaging speaker with plenty of interesting information".

Growers and agronomists were particularly pleased that they had the chance to approach the delegates and have their particular questions answered.

Louis De Kock's visit and his successful venture in South Africa was covered in an article in Good Fruit & Vegetables magazine.

Fred Crowe's presentation, *Update on Allium White Rot Activities in the U.S.*, is at Appendix 1.

Outcomes

The international delegation from South Africa and the US successfully extended R&D and international production practices to the majority of onion growers in Australia.

The Australian industry was exposed to new information that may be considered for on-farm and supply chain practice change including changes in insect and disease control, fertiliser and irrigation practices, supply chain and consumer markets.

It is envisaged that onion growers will seek to maximise their access to international markets after learning best practice methods for international supply.

The knowledge gained will also inform the development of an Australian Onion Industry Strategic Export Plan.

Also, growers are expected to take advantage of new information on pest and disease management within a short timeframe with on-farm adoption.

Monitoring and evaluation

Evaluation surveys were conducted with attendees at the four major events held over the course of the international delegation: Hort Connections, regional Queensland on-farm tour, Onions Australia Levy Payer Day, Onion Industry Information Day, and the Tasmanian Onion Agronomy Group.

Results showed that, overwhelmingly, attendees were very happy with the delegation and the knowledge transfer they achieved. Growers valued the access that they were provided to the overseas experts, particularly the chance to ask questions about their own operations. Many reported heading back to their farms with renewed enthusiasm as well as knowledge.

Specifically, growers and other stakeholders indicated that:

- The information presented at the events, including by the two invited speakers was relevant and useful
- The Levy Payer Day guest speaker, Dr Fred Crowe, was engaging and his information on managing white rot was highly informative for those affected by the disease and others aiming to avoid infection
- They obtained an understanding of R&D priorities for the onion industry
- They had an opportunity to participate and contribute to conversations around the effectiveness of Onions Australia as the representative organisation for the industry
- They met and networked with like-minded industry representatives.

Recommendations

Given the high level of satisfaction among levy payers and the successful knowledge transfer, future international delegations should be arranged to ensure that the Australian onion industry is kept up to date with overseas expertise.

A similar format for future delegations is recommended, to again provide different events during which onion growers can engage with the delegation, including having the chance to ask questions.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

Appendices

Professor Fred Crowe's presentation Update on Allium White Rot Activities in the US – pdf.

Update on Allium White Rot Activities in the U.S.

Dr. Fred Crowe

Emeritus Professor, Dept. Botany & Plant Pathology
Oregon State University

Fred Crowe Consulting

Partner, Deerfield Farm Seed Garlic (recently retired)

The annual farm-gate value of onion and garlic in the US >$1.5 billion, with >$6 billion in added-value after processing.

>20% of the world's onion seed is produced in the US and is valued >$100 million annually.

Annual value of U.S. potato crop is $4.5 billion.
Annual value U.S. carrot crop $1 billion

Research support federal/state

National research funding:

- Potatoes (3 cents/100wt) $20 million federal
  - Idaho $15 million, Washington $3 million, many other states with significant $$
- Onions – nothing. A few, small regional grants

Within California:

- Calif strawberries, grapes, citrus… “many millions”/yr each
- Calif lettuce >$600k/yr
- Calif Tomatoes $500k
- Celery $350k
- Onions/garlic 100k (recent)
- Carrots $200k

> mid-1970’s, no USDA pathologists full time on onions.

States with dedicated onion pathologists:

New York, until retirement of Jim Lorbeer (leaf diseases)

Georgia, until retirement of Ron Gitaitis (bacteria)

Colorado, until retirement of Howard Schwartz (extension)

States with researchers with strong focus onion pathology (1/3?)

Washington: Lindsey Du Toit, seed crops pathologist

Allium production US (Acres/1000)

	ONION	Garlic
• CA	60	34
• WA/OR* (Columbia Basin)	30	1
• ID/OR (Treasure Valley)	23	
• GA	12	
• TX	10	
• NY	9	
• NM	6	
• NV	4	2
• CO	4	
• OR (WV, So. OR)	4	4
• Other	5	<<1
• [*OR total	20^{+}	6]

California Allium

- Fresh & fresh processed onion bulbs 25k acres
  - Low desert, San Joaquin Valley, Coastal Valleys, High desert

- Dehydration onions 35k acres
  - All regions including far No Cal/So OR (???k acres)

• Garlic fresh	6k acres	mid-southern regions
• Garlic dehydration	20k acres	all regions
• Garlic seed	4k acres	far north and high desert

California Allium

- Fresh (and fresh processed) onion bulbs 25k acres
  - Low desert, San Joaquin Valley, Coastal Valleys, High desert

- Dehydration onions 35k acres
  - All regions including far north

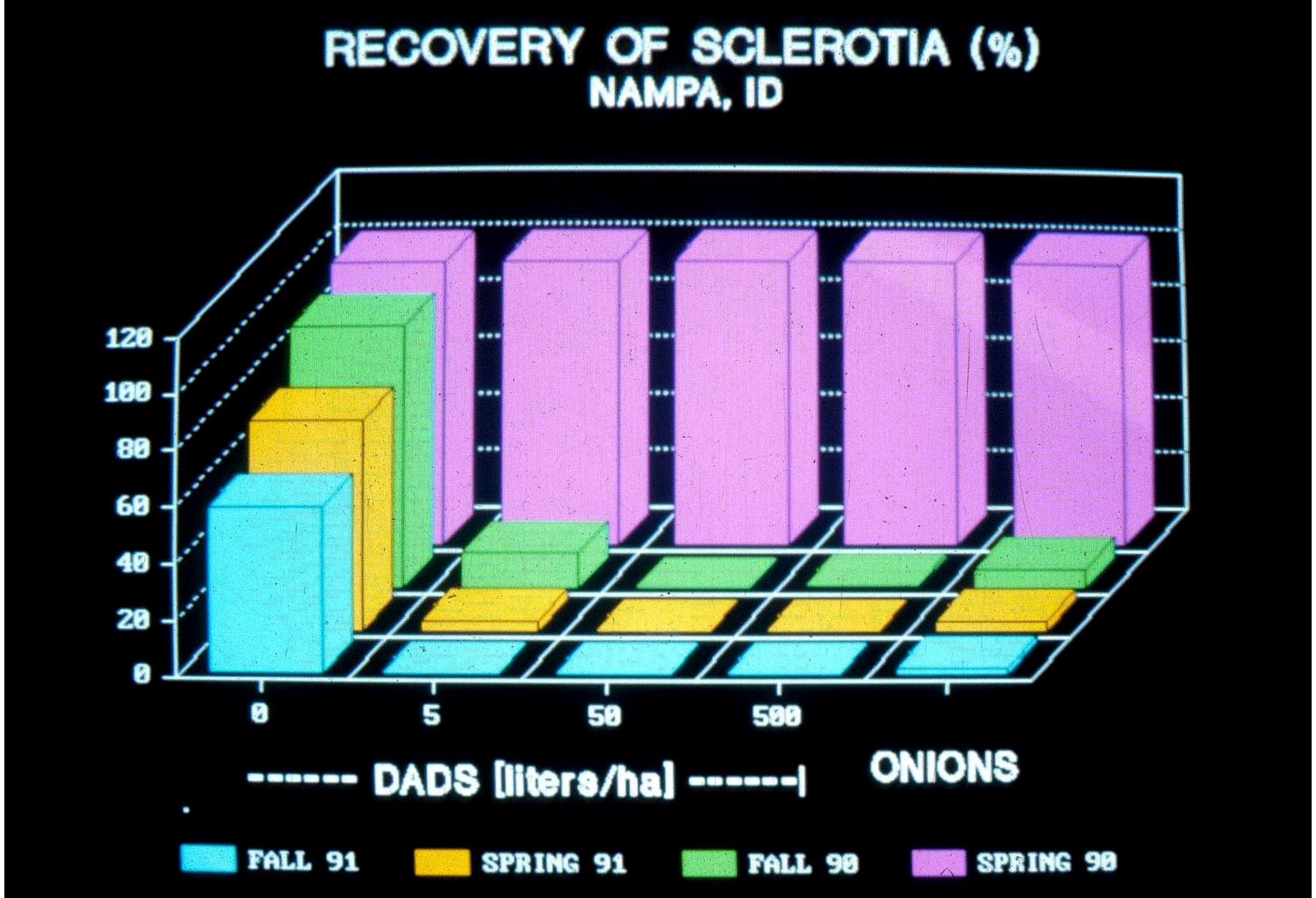
- Garlic fresh 6k acres
- Garlic dehydration 20k acres
- Garlic seed 4k acres

Research support for Allium in California <2005

- $25-40k/yr by onion and garlic processors (ADOGA), sometimes with input from a few growers.
    - My thesis 1974-78: Special funds arranged by contract from processors and farmers strictly for investigation into Allium White Rot.
- **Crowe, F.J**., D.H. Hall, A.S. Greathead, and K.G. Baghott. 1980. Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. Phytopathology 70:64-69.
- **Crowe, F.J**., and D.H. Hall. 1980. Vertical distribution of sclerotia of *Sclerotium cepivorum* and host root systems relative to white rot of onion and garlic. *Phytopathology* 70:70-73.
- **Crowe, F.J**. and D.H. Hall. 1980. Soil temperature and moisture effects on sclerotium germination and infection of onion seedlings by *Sclerotium cepivorum*. *Phytopathology* 70:74-78.

Research support for Allium in California <2005

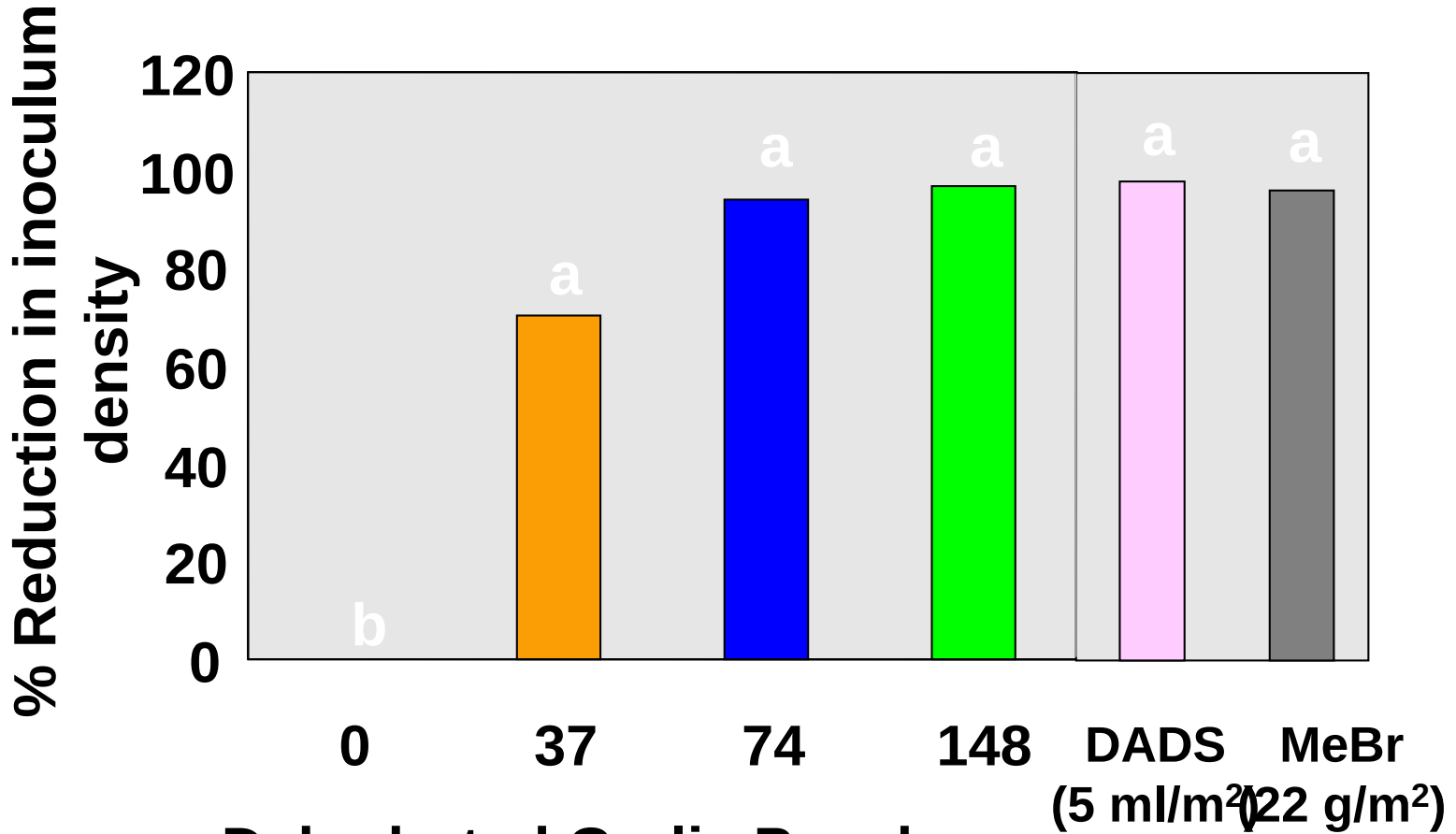
- 1985-2005: Stimulated germination (also included $$ from 3 other onion regions and Phillips Petroleum, later UAP)
  - Crowe, F.J., J. Debons, E. Redondo, T. Darnell, D. McGrath, J. Laborde, P. Koepsell, and M. Thornton. 1990. "Use of germination synthetic stimulants of sclerotial germination as a method of inoculum management of the Allium white rot fungus." 4th International Allium White Rot Workshop, Neustadt/Weinstrasse, Fed. Rep. Germany.
  - Crowe, F.J., J. Debons, T. Darnell, M. Thornton, D. McGrath, P. Koepsell, J. Laborde, and E. Redondo. 1994. "Control of Allium white rot with DADS and related products." 5th International Workshop on Allium White Rot, Cordoba, Spain.
  - Registration of DADS "AlliUp" by UAP late '90's-2003; "DADS^{tm}" Aceto 2007-16 but no product on market after 2010 or so…


RECOVERY OF SCLEROTIA (%)
NAMPA, ID
120
100
80
60
40
20
0
0
5
50
500
------ DADS [liters/ha] ------|
ONIONS
FALL 91
SPRING 91
FALL 90
SPRING 90

Research support for Allium in California <2005

- Mike davis publication on g.p.
    - Garlic powder work with Mike Davis, Jason Dennis
- Fungicide testing

Bakersfield



1998

Research support for Allium in California >2005

- Marketing order, part of the Allium crops assessed on a weight yield basis. Approx. $100,000^{+}$/yr

  California Garlic and Onion Research Advisory Board (CGORAB). Frequently, NV growers contribute to this fund. Recently, some Washington growers, also.

  Mission: Fund research projects of potential benefit to the California garlic and onion production. Weeds, insects, disease, quality issues…. $\geq$ Half of funds directed towards White Rot efforts.

Robert Ehn, Technical Manager CGORAB

- http://www.cagarlicandonion.com/
- e-mail: robertehn@sbcglobal.net

- **R3 Ag Consulting, LLC**
- 1823 Shaw Ave., Suite 103
  Clovis, CA 93611
- Phone: 559-297-9322
- Fax: 559-297-9341

Recent past CGORAB support WR

- 2003-2007: Crowe & Davis work on stimulated germination, fungicide trials, etc…
- 2009-11: My immediate successor at OSU, but he left OSU prematurely w/o accomplishments.
- 2003-2015: Mike Davis UCD. Fungicide trials and germination stimulants. One grad student PhD completed 2014 but no research articles published from this.
- 2003-present: Two county agents with fungicide and stimulant trials on garlic and onions. Latter included fungicide application methodology with onion seeding.

Recent past CGORAB support WR

- 2006-2012: Colin Eady, NZ Institute for Plant & Food Research, Lincoln NZ. [Allison Stewart, Lincoln Univ.] Genetically engineering onion and garlic to express oxalate oxidase and oxalate decarboxylase to degrade oxalic acid from *S. cepivorum.* Initial lab/greenhouse successes were reported until program was discontinued due regulatory problems, loss of corporate sponsors and Eady's shift to non-Allium project…..just before transformed plants were to be evaluated in the field in California.

- 2014-present: CGORAB approached regularly by an Israeli private/-govt consortium seeking partnerships (i.e. funding support) towards breeding improved garlic, led by Rina Kamenetsky, Agr. Res. Org., Inst. Plant Sciences, Volcani Center… To date, CGORAB sees no benefit in joining this effort.

- 2017-18: Lead in pushing for national grant funding. Recent successes after a decade of effort to convince other regions.

Immediate past, present & future activities

- The current Allium White Rot “team”
- Dr. Jerimiah Dung. Assistant Professor OSU, Dept Botany & Plant Pathology
- Dr. Michael Qian: Professor OSU, Department of Food Science & Technology.
- Tom Turini: Fresno County Farm Advisor, Univ. Calif. MS Pest Management.
- Rob Web: Intermountain Research & Extension, Univ. Calif, Tulelake. MS Pest Management/Weed Science.

Tom Turini
Farm Advisor, Cooperative Extension
Fresno County, University of California
taturini@ucanr.edu

Pest management of all commercial vegetable and melon production in the most major such county in the U.S.

Field trials on garlic, onions, etc… Currently, takes lead on testing of fungicides and germination stimulants against WR of garlic.

Seeding rate: 18-24 cloves/bed ft

2 Seed lines/bed:

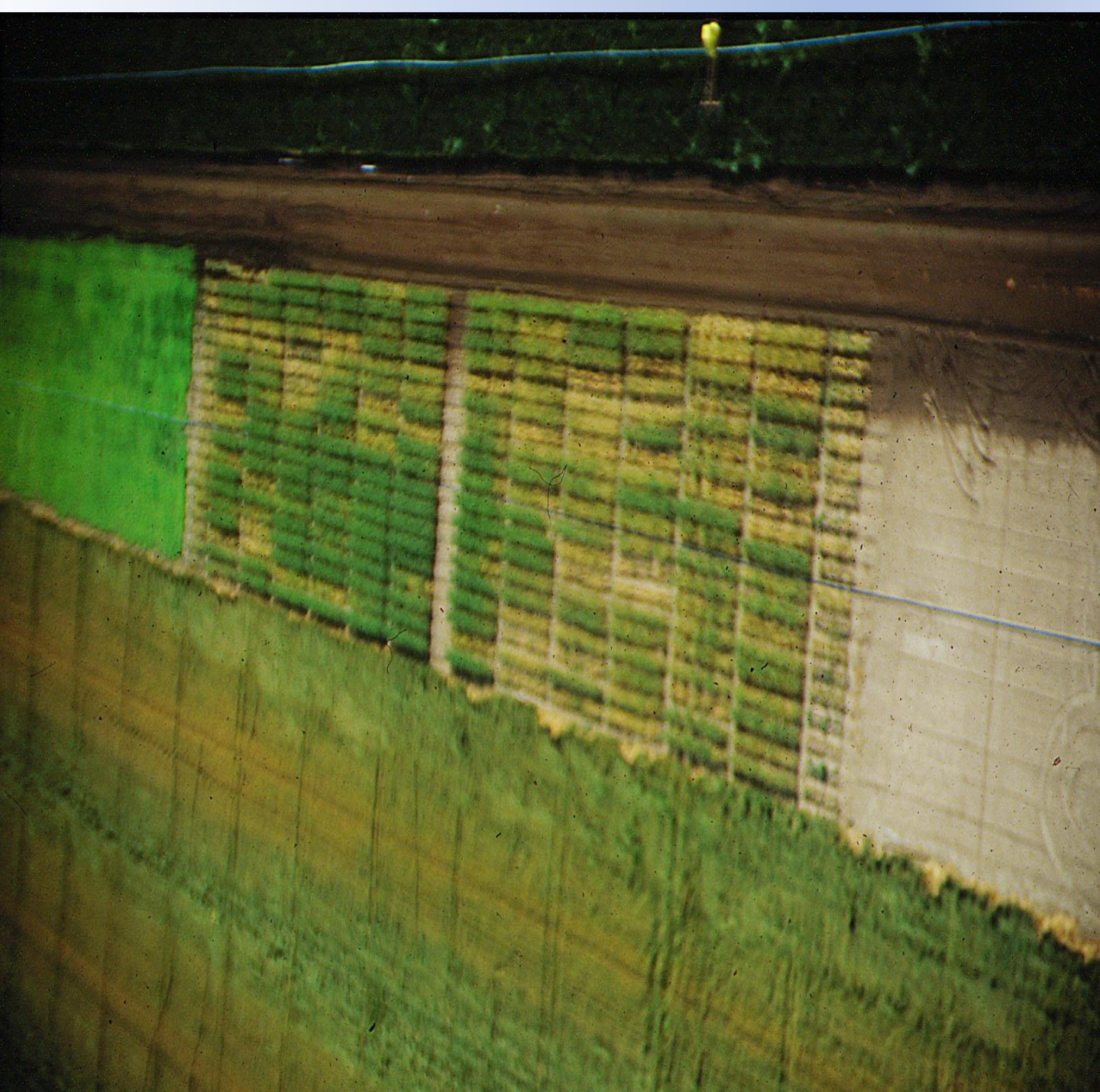
Beds: 36-40”

Plant population: 240k-350k/ac

Ave: 1 ton/ac
Range: 1,500-3,000 lb/ac

Garlic White Rot Control

- Folicur in furrow still the standard. Sprayed onto seed and across opened seed furrow as seed dropped behind opener and in front of closer. Labeled allowance (20.5 oz/ac = 1.5 L/H) a bit high; some yield depression. Most use a bit less (14 oz/ac = 1 L/H). Also controls Botrytis porri if on/with planted cloves.
- Registered but less used:
  - Penthiopyrad (Fontelis) 24 oz/ac
  - Boscalid (Endura) 6.8 oz/ac
  - Fludioxanil (Cannonball) 7 oz/ac



Rob Wilson
Farm Advisor, ANR Intermountain Research Center
Tulelake, CA 96134
rgwilson@ucanr.edu
http://irec.ucanr.edu/

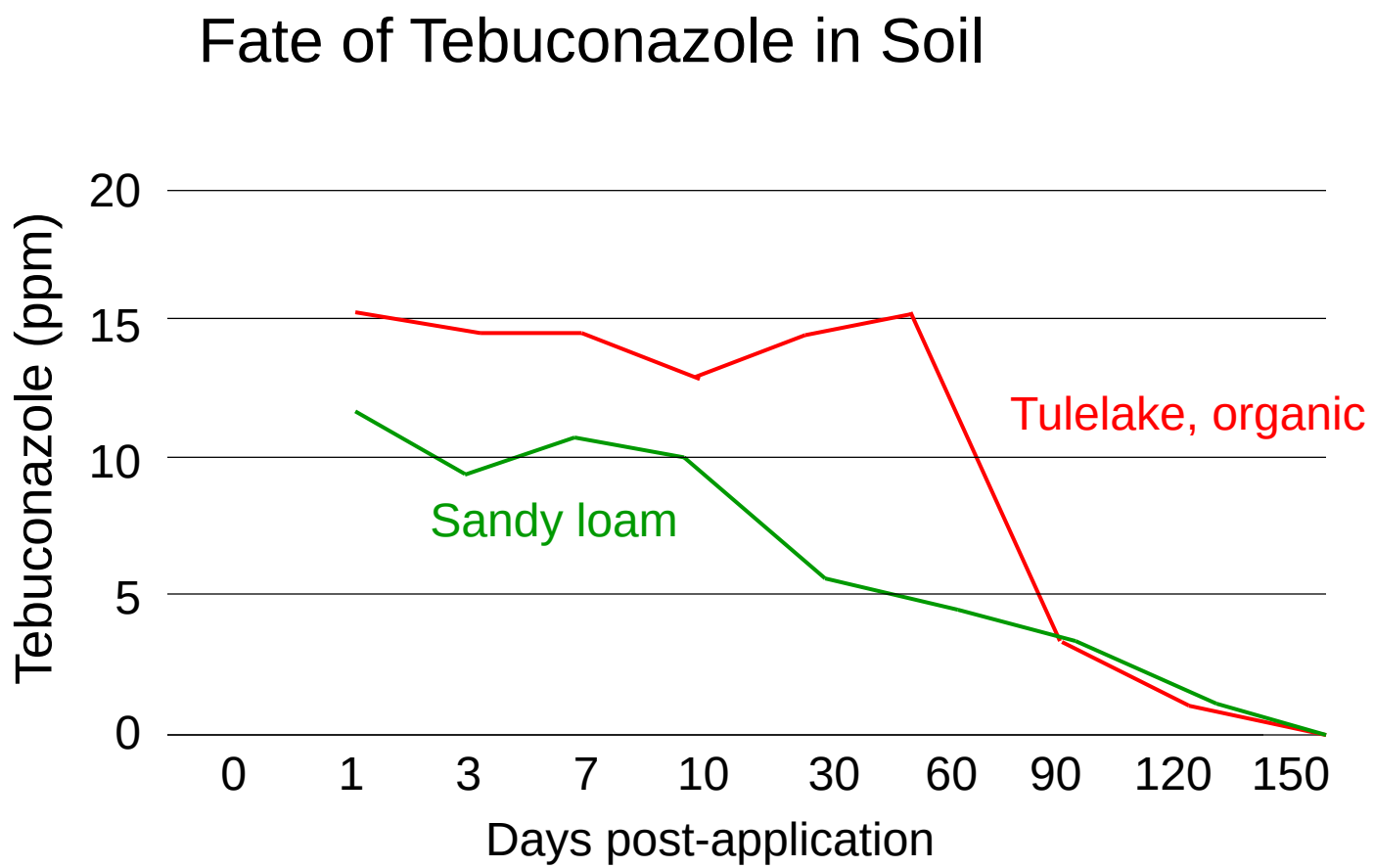
IPM potatoes, onions, grain,….. MS in weed science. Particularly good with chemical application and planting techniques. The WR team leader on testing of fungicides and germination stimulants on onion.

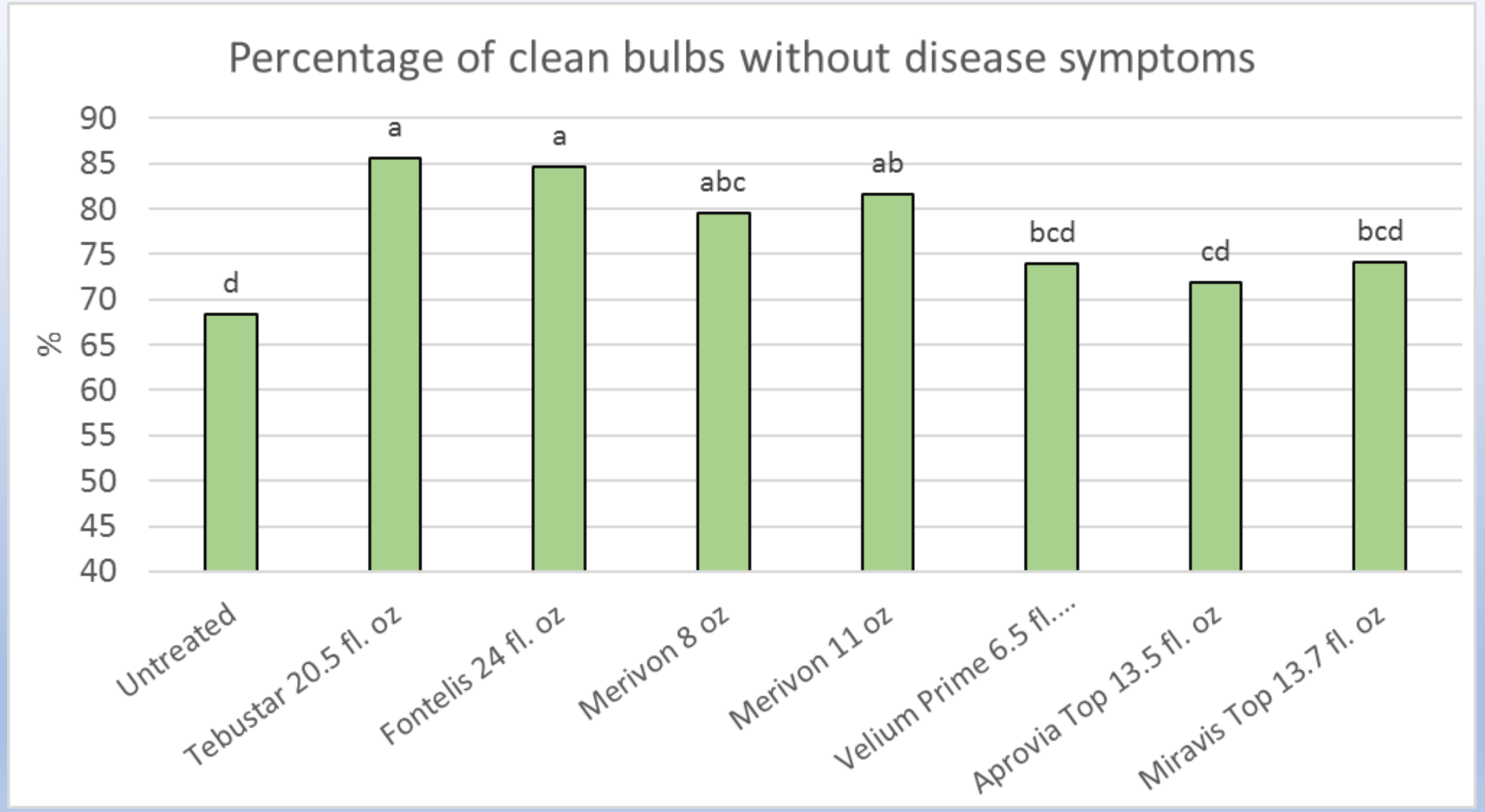
- Tebuconazole (Tebustar) and penthiopyrad (Fontelis) were most effective in CA testing
- Fungicides work best when sclerotia populations are low and conditions are not optimal for rapid disease development

- Folicur 1.5 L/H (same as for garlic). Per seed line this is less than on garlic.
- Tricky to avoid plugging seeding drop with 4 planted seed lines per 36” (91 cm) bed, 6” (15 cm) separation btw seedlines.
- Recent spread of onion smut in far No. Calif, could be as problematical as White Rot. Seed treatment.
  - Penflufen (another carboxamide) works but not labeled yet.
  - Carboxin doesn’t work very well but labeled
  - Difenconazole doesn’t work very well
  - Mancozeb fair but perhaps limited in future?

In-furrow

Banded on soil surface


Fate of Tebuconazole in Soil
Tebuconazole (ppm)
20
15
10
5
0
0
1
3
7
10
30
60
90
120
150
Days post-application
Tulelake, organic
Sandy loam


Percentage of clean bulbs without disease symptoms
%
90
85
80
75
70
65
60
55
50
45
40
d
a
a
abc
ab
bcd
cd
bcd
Untreated
Tebustar 20.5 fl. oz
Fontelis 24 fl. oz
Merivon 8 oz
Merivon 11 oz
Velium Prime 6.5 fl....
Aprovia Top 13.5 fl. oz
Miravis Top 13.7 fl. oz

Cassandra Swett ????
Ph D UC Davis 2013; Post-doc; hired 2017
clswett@ucdavis.edu
http://swettlab.faculty.ucdavis.edu/

Fungal pathogen ecology in vegetables and field crops; Soil borne diseases, effects of adaptive water use on plant-pathogen interactions.

Extension specialist all vegetable and field crops. Many to choose from.... Unsure yet if she'll address Allium crops.

Jerimiah Dung

Assistant Professor, OSU Dept. Botany & Plant Pathology
Central Oregon Agricultural Research Center
850 NW Dogwood Ln, Madras OR 97741
Jeremiah.Dung@oregonstate.edu

PhD Washington State Univ; post-doc OSU; Hired OSU-COARC 2014

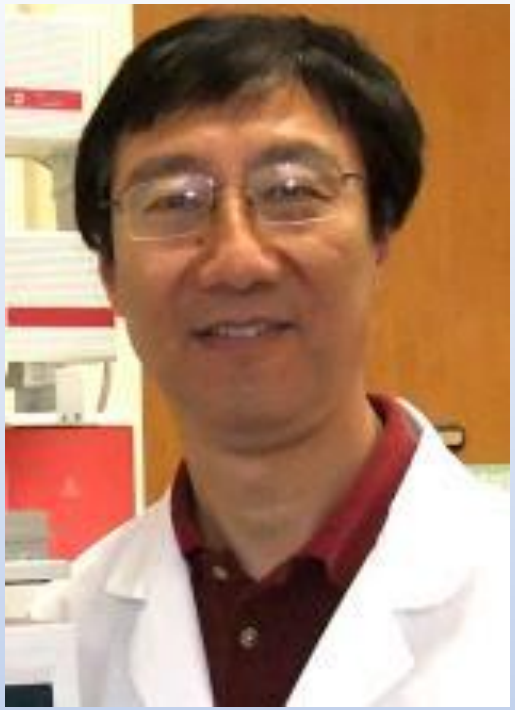
Pathology projects on peppermint (verticillium wilt), grass seed (ergot), and Allium (white rot). Has not accomplished much yet re AWR, but by backing up field efforts of AWR activities in Calif, he has developed a good, basic understanding WR biology and techniques.

APS Early Career Award 2016. On track for tenure next year.

The WR leader on $3.3 million USDA-IPM grant 2018-2022: ***DEVELOPMENT AND DELIVERY OF INTEGRATED MANAGEMENT PACKAGES FOR THE MOST SERIOUS PEST AND DISEASES THREATENING US ALLIUM INDUSTRIES***
US Allium industries and states agree in advance that all $$ will go to AWR and IYSV.
Jerimiah will have a post-doc and perhaps grad students working on AWR

Michael Qian

Professor, OSU Food Science and Technology



PhD Univ. Minnesota; hired OSU 2000

2018, Distinguished Service Award from the Agr. and Food Chemistry Division, of the American Chemical Society

Flavor chemistry & technology of grapes/wine, beer hops, dairy….
"Solventless sample preparation techniques such as solid phase micro-extraction, solid phase dynamic extraction, stir bar sorptive extraction and instrumental analysis with an emphasis on GC, fast GC, HPLC, GC-MS, GC-MS/olfactometry, and multi-dimensional GC/GC-MS analysis."

Sub-specialty: Detection and quantitative measurements of organic sulfur compounds. Now is an established member of the California-Oregon WR team, CGORAB and USDA-IPM grant funding.

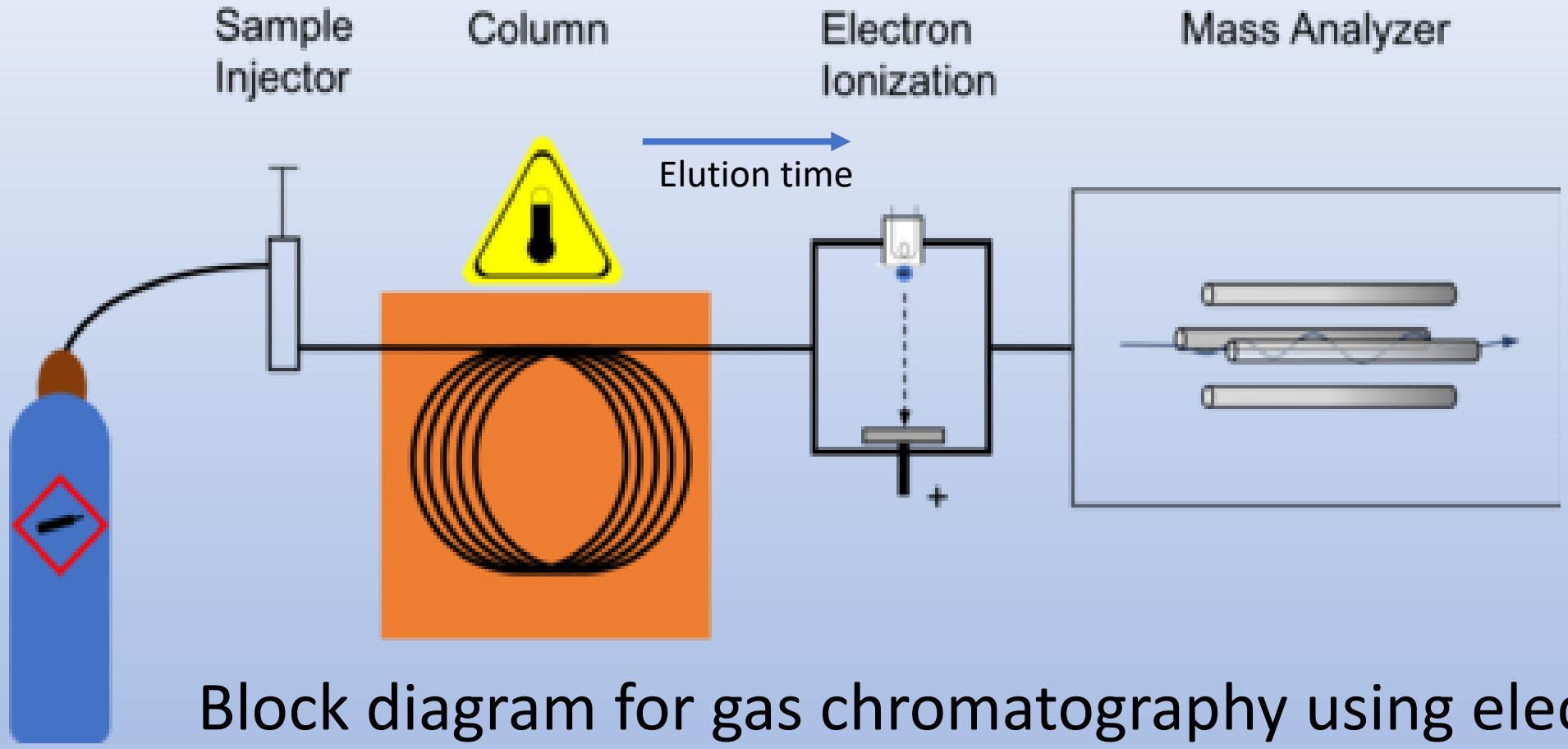
Objectives

- Identify natural sulfur-organic compounds in onion and garlic
- Develop analytical method(s) to quantitate the concentration of volatile sulfur compounds
- Search for potential sources of biostimulates
  - High concentration of volatile sulfur compounds
  - Economical
- develop a sustainable approach using garlic/onion oil or waste products as biostimulants to manage onion and garlic white rot (long term goal)

The GC-MS is composed of two major building blocks: gas chromatograph and the mass spectrometer.

The gas chromatograph utilizes a capillary column as well as the phase properties. The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

https://en.wikipedia.org/wiki/Gas_chromatography%E2%80%93mass_spectrometry



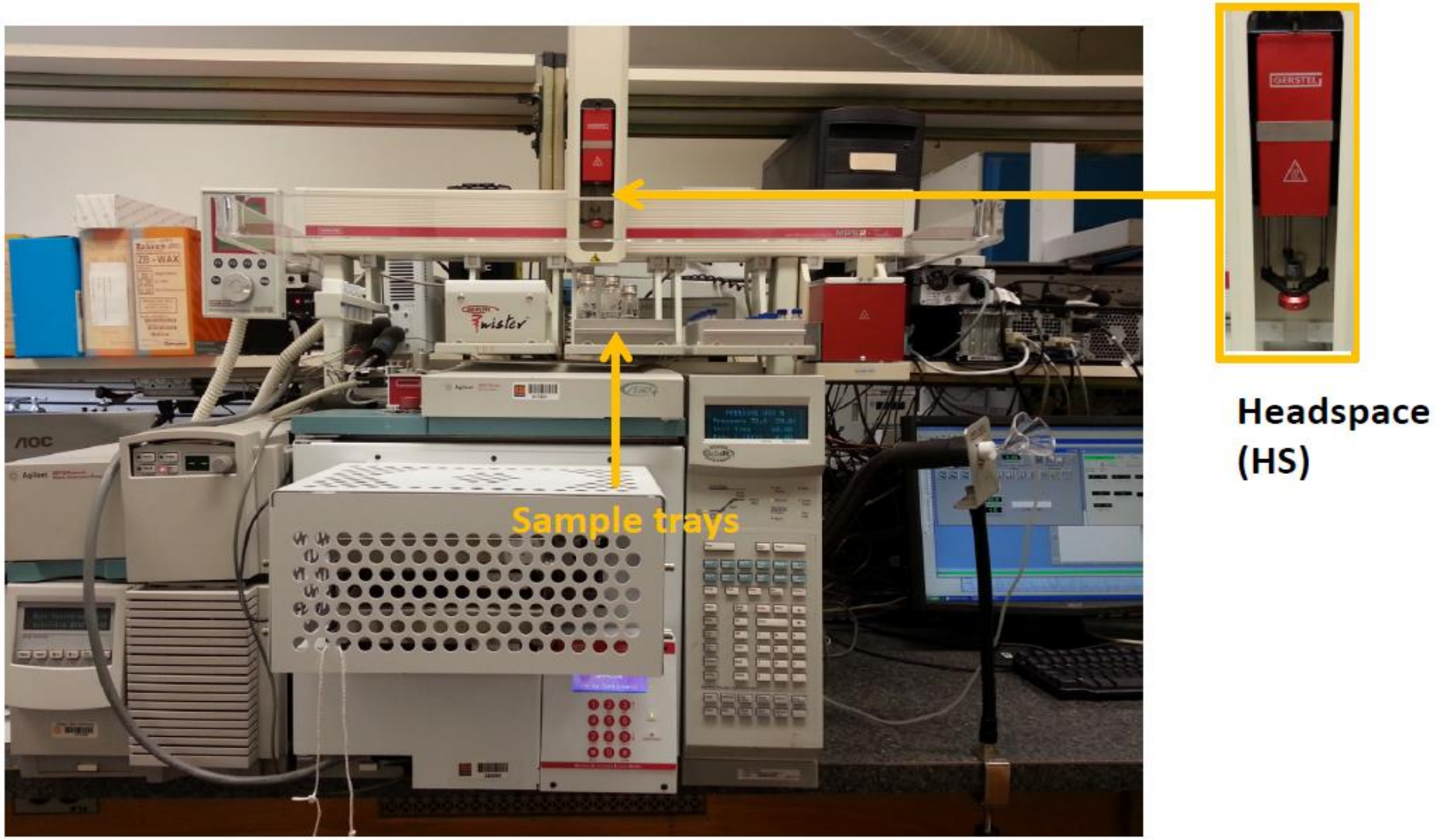
Block diagram for gas chromatography using electron ionization for collecting mass spectrum

https://www.restek.com/pdfs/59895B.pdf

Since 1959, regular evolution of miniaturization, lower cost, reduced time, confidence in results….and the number of chemists trained.

“Affordable”, perhaps $150k both 2000 and today.

Headspace-GC-MS Method



Gerstel MPS2 headspace sampler-Agilent 6890GC-5973MS

Formation of VOC in Garlic

Alliin (*S*-allyl-L-cysteine sulfoxide)
(mesophyll cells)

S-methyl and *S-trans*-1-propenylcysteine sulfoxides

ALLIINASE (vascular bundle cells;
released upon crushing)

Allyl thiosulfinates

Allicin (diallyl thiosulfinate) (67-81%)

Allyl methyl thiosulfinates (16-26%)

Allyl *trans*-1-propenyl thiosulfinates (4-7%)

spontaneous
hot water

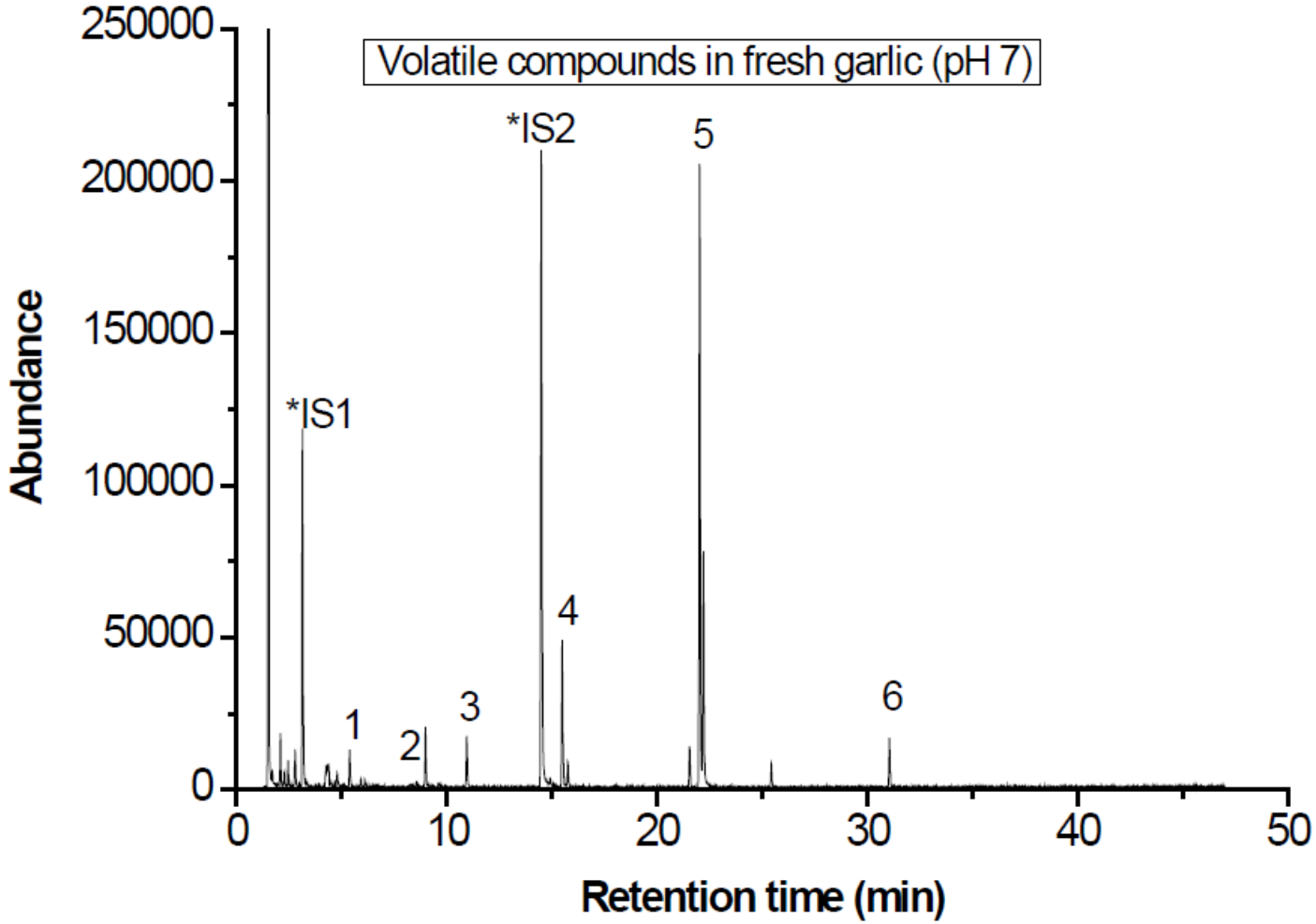
Allyl sulfides

Diallyl sulfides, n=1-3 (83-92%)

Allyl methyl sulfides, n=2-3 (8-17%)

Allyl *trans*-1-propenyl sulfides, n=2-3 (not detected)

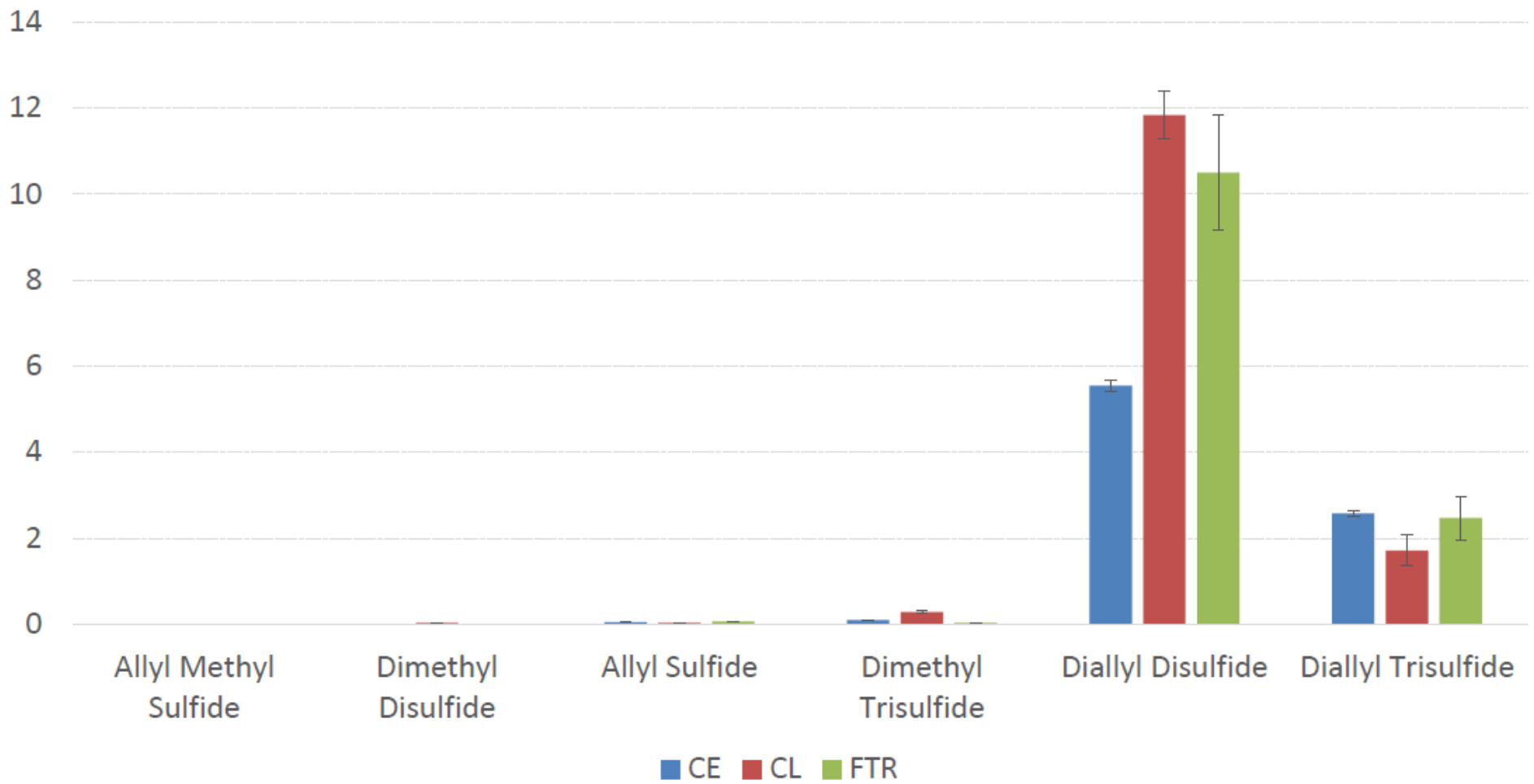
Volatile sulfur compounds in Fresh Garlic



*IS1: Ethyl Methyl Sulfide
*IS2: Isopropyl Disulfide
1: Allyl Methyl Sulfide
2: Dimethyl Disulfide
3: Allyl Sulfide
4: Methyl Allyl Disulfide
5: Diallyl Disulfide
6: Diallyl Trisulfide

*IS represents for Internal standard, which is added manually for quantitative analysis purpose

Fresh Garlic (3 samples)

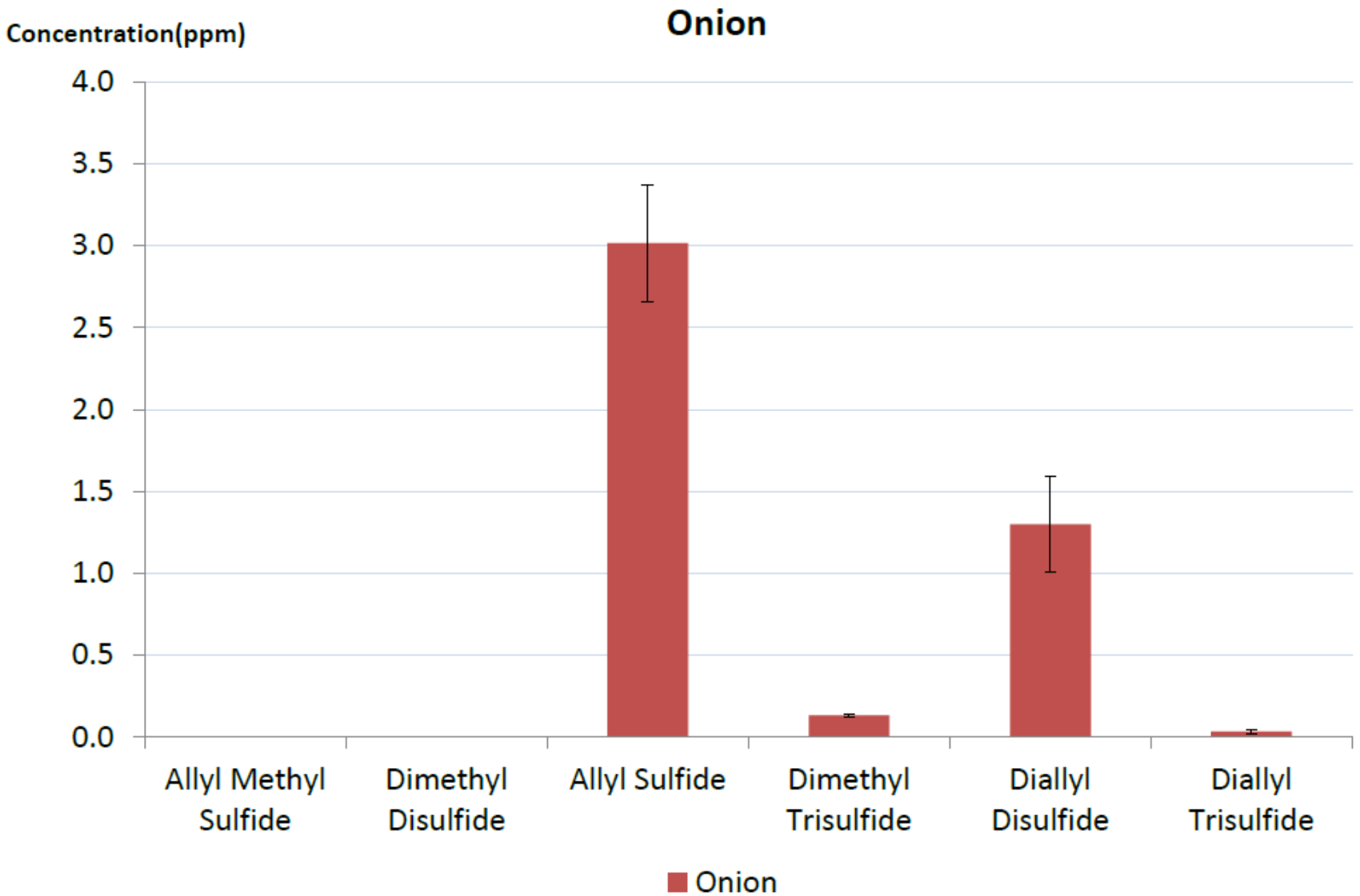


CE: California Early

CL: California Late

FTR: Forked Tree Ranch

fresh onion

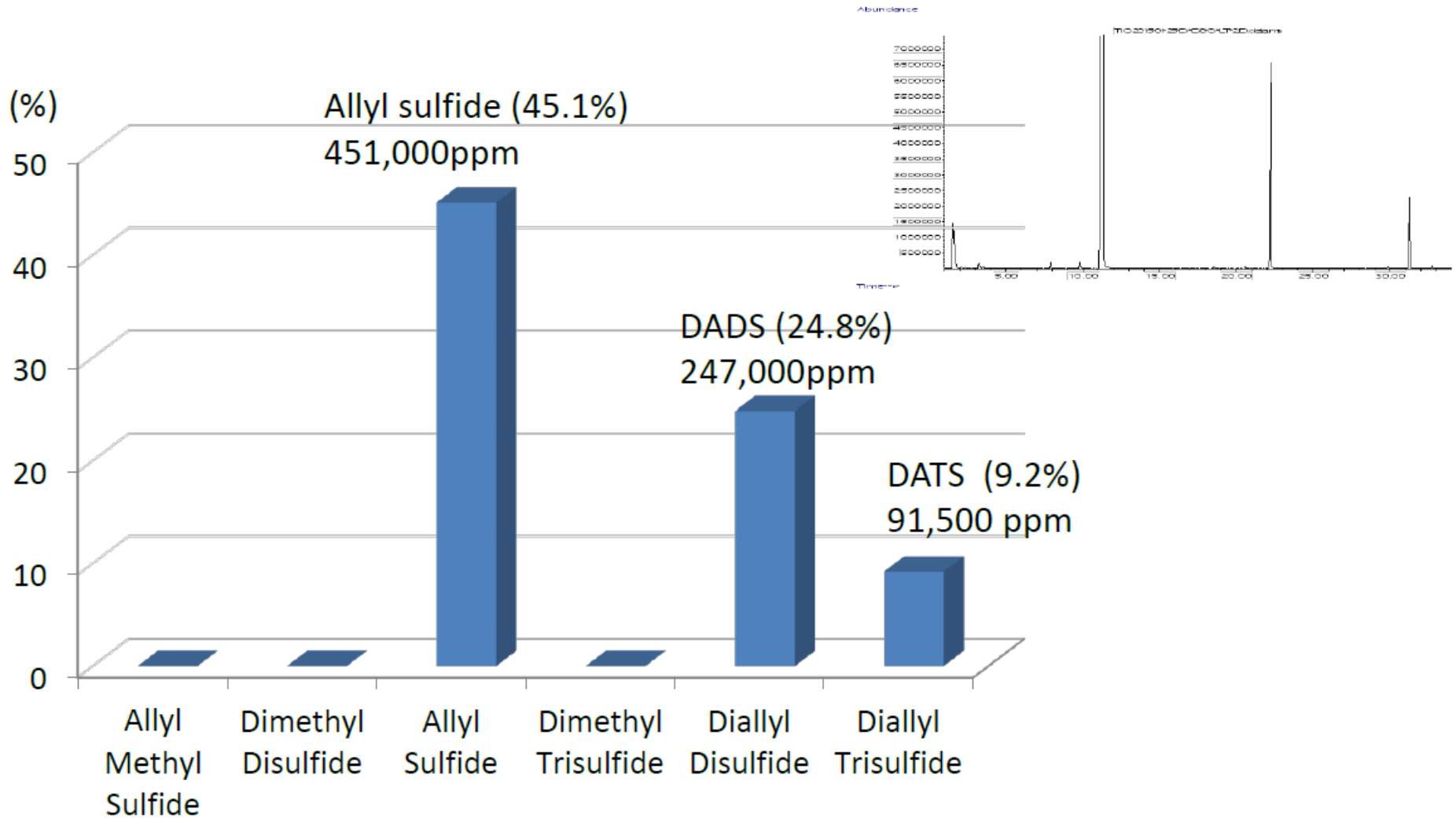

Onion
Concentration(ppm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Allyl Methyl Sulfide
Dimethyl Disulfide
Allyl Sulfide
Dimethyl Trisulfide
Diallyl Disulfide
Diallyl Trisulfide
Onion

Garlic Juice Samples-2016

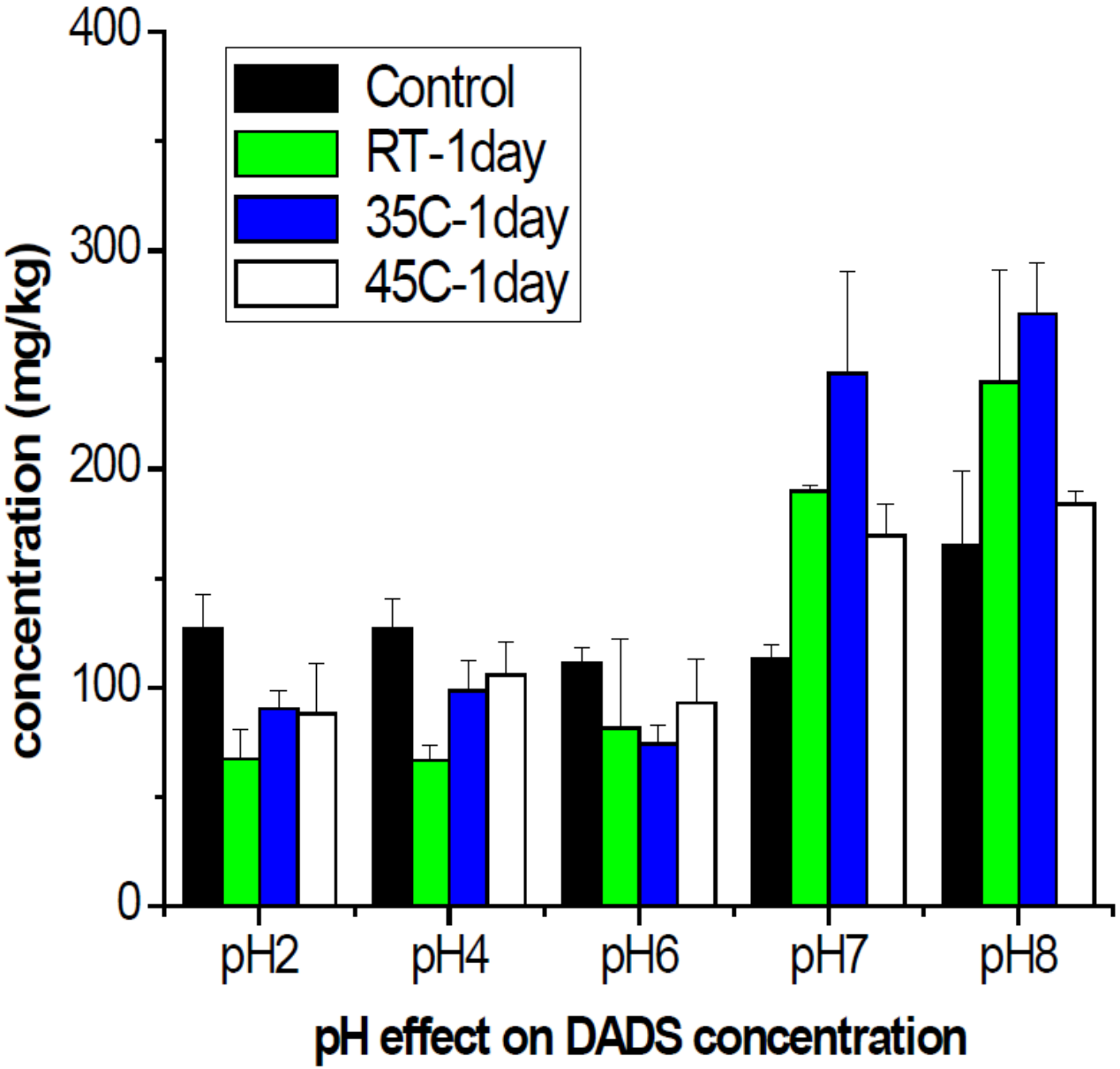
Concentration of sulfur compounds (mg/ kg)

Compounds	GC 1522903	K 032651
Allyl Methyl Disulfide	5.50 ± 0.26	0.57 ± 0.03
Dimethyl Disulfide	0.50 ± 0	0.15 ± 0
Allyl Disulfide	4.13 ± 0.23	0.35 ± 0
Dimethyl Trisulfide	0.32 ± 0.03	0.05 ± 0
Diallyl Disulfide	3.22 ± 0.20	0.12 ± 0.03
Diallyl Trisulfide	0.00 ± 0	0.00 ± 0

Commercial DADS sample



Effect of pH on the formation of DADS in garlic paste



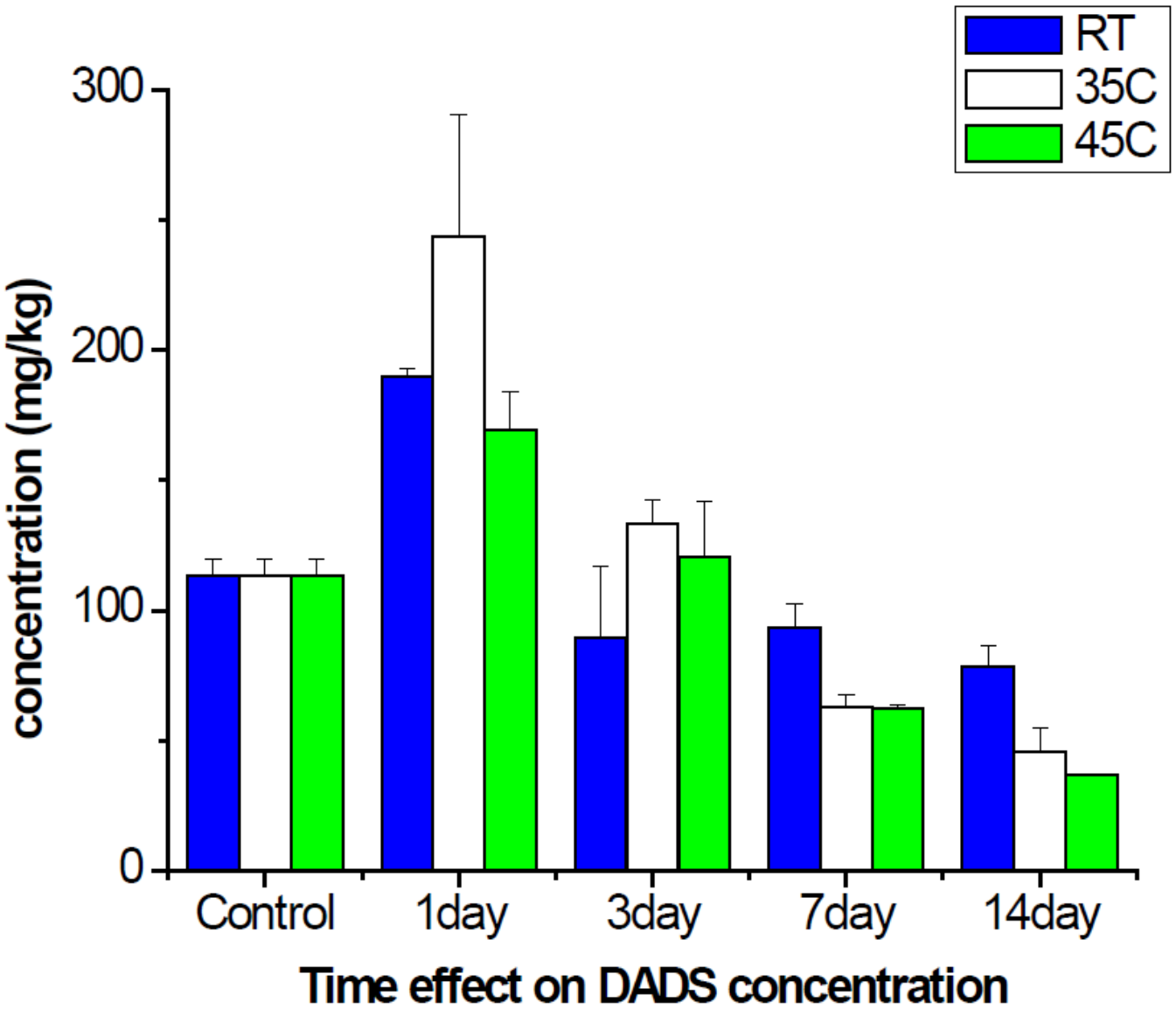
Conclusion:

(1) DADS formation is more favorable at higher pH (i.e. pH 7 and pH 8)

(2) acidic condition will inhibit the alliinase activity,

(3) DADS is also not stable in acidic conditions.

Effect of incubation time on the formation of DADS at pH 7



Conclusion:

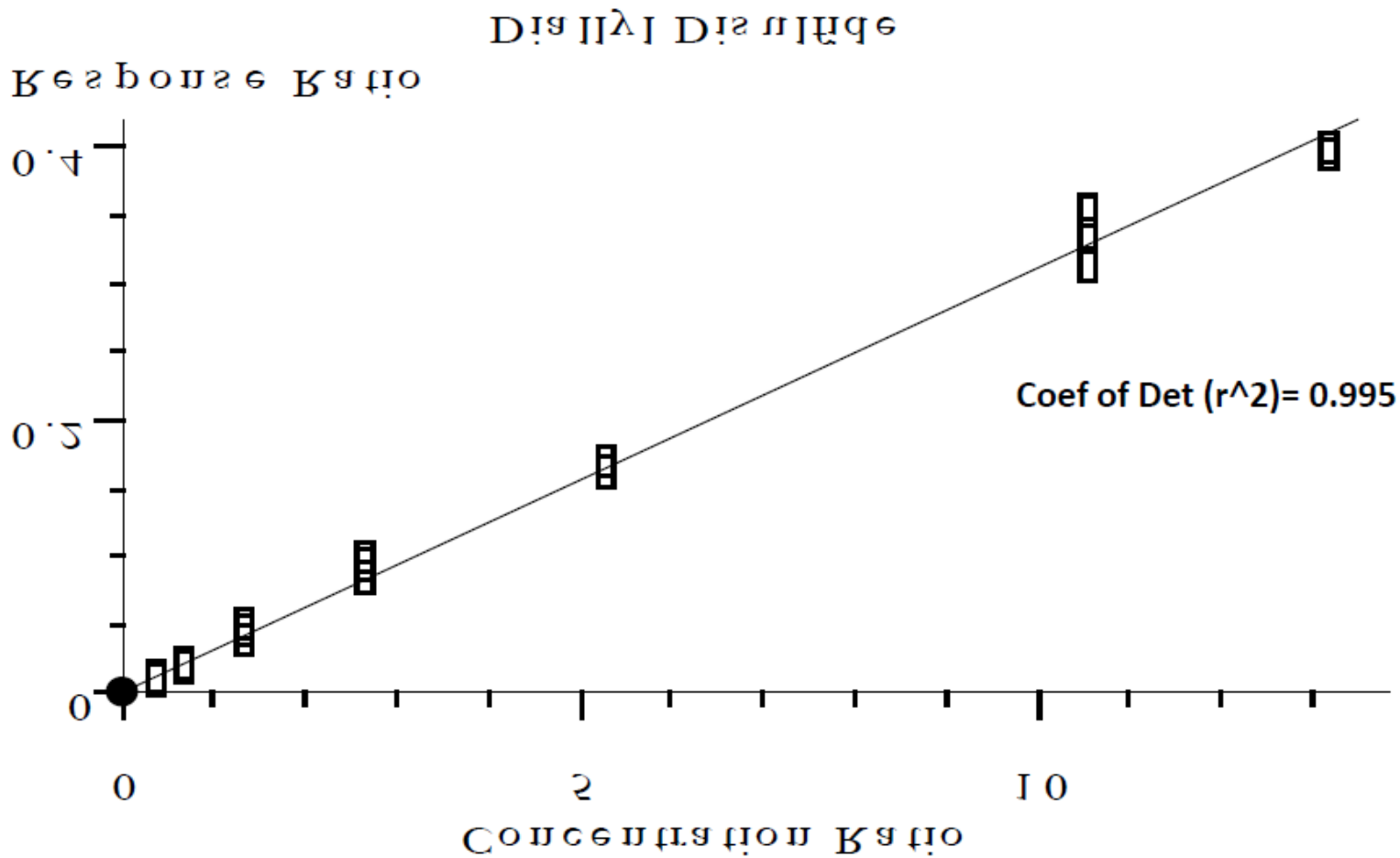
(1) After 1 day incubation, the DADS concentration will increase about 2.5 time compared to no incubation treatment at pH 7

(2) Mild heating (35C) will improve enzyme activity compared to room temperature

Volatile Sulfur Compounds Analysis

- **Method development**
  - **Sample preparation**
    - ***Fresh garlic/onion***: dried with liquid nitrogen, blended into powder → 2g of garlic/onion powder + 6 mL H_2O + 2 mL methanol + 50 µl IS (518 ppm Ethyl Methyl Sulfide, 776 ppm Isopropyl Disulfide) + 2g salt
  - **Instrument analysis**
    - ***Headspace sampling:*** Samples placed into a 20 ml headspace vial; Equilibrated at 50ºC for 50 min; Syringe temp 50° C; Inject 500 µl, 1:5 split
    - ***GC-MS Parameters:*** HP-Wax Column, 30m length, 250 µm diameter, 0.25 µm film thickness; Oven program: 40 ° C, 2 min

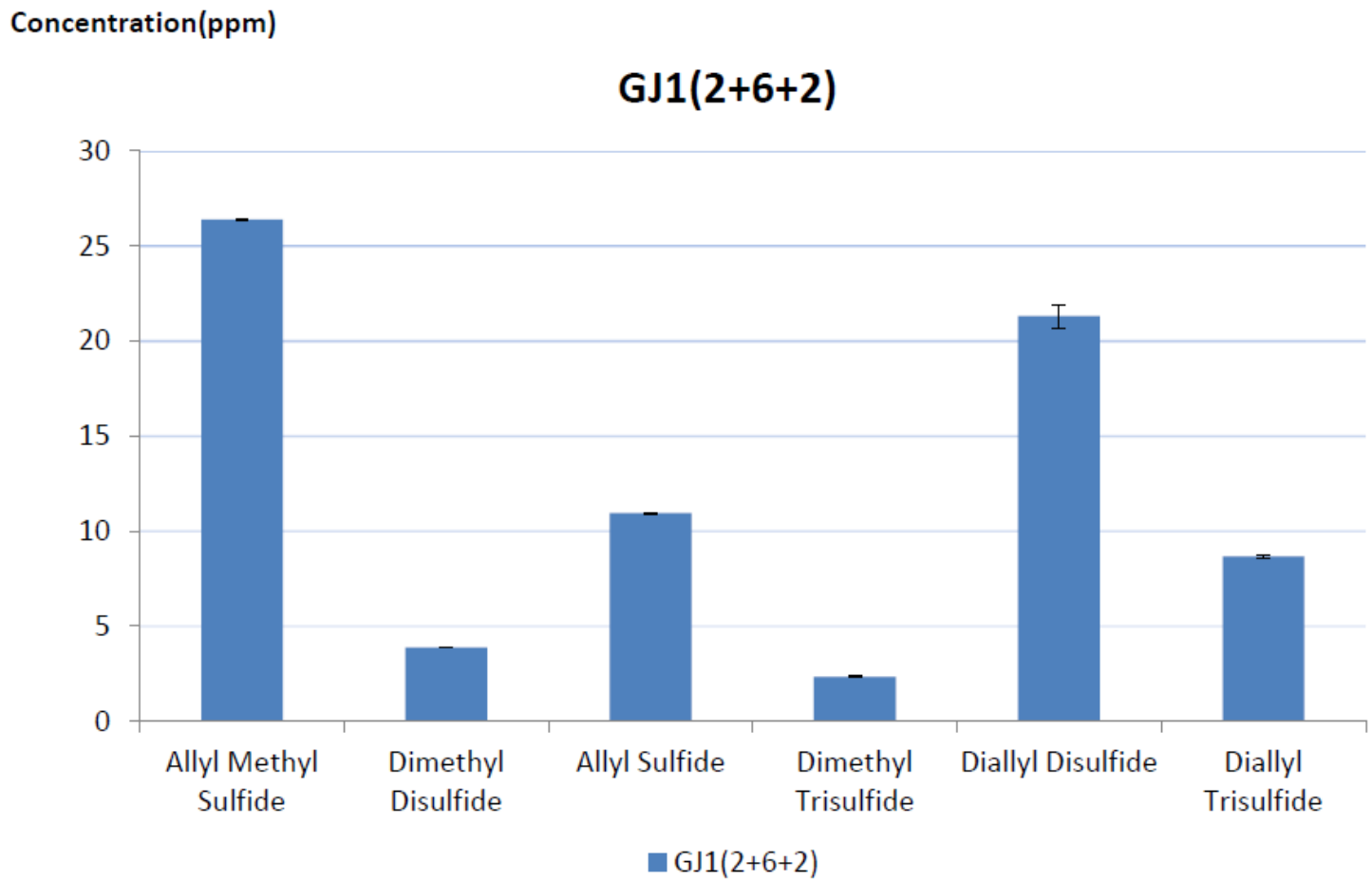
Calibration curve development



Calibration Curve Development

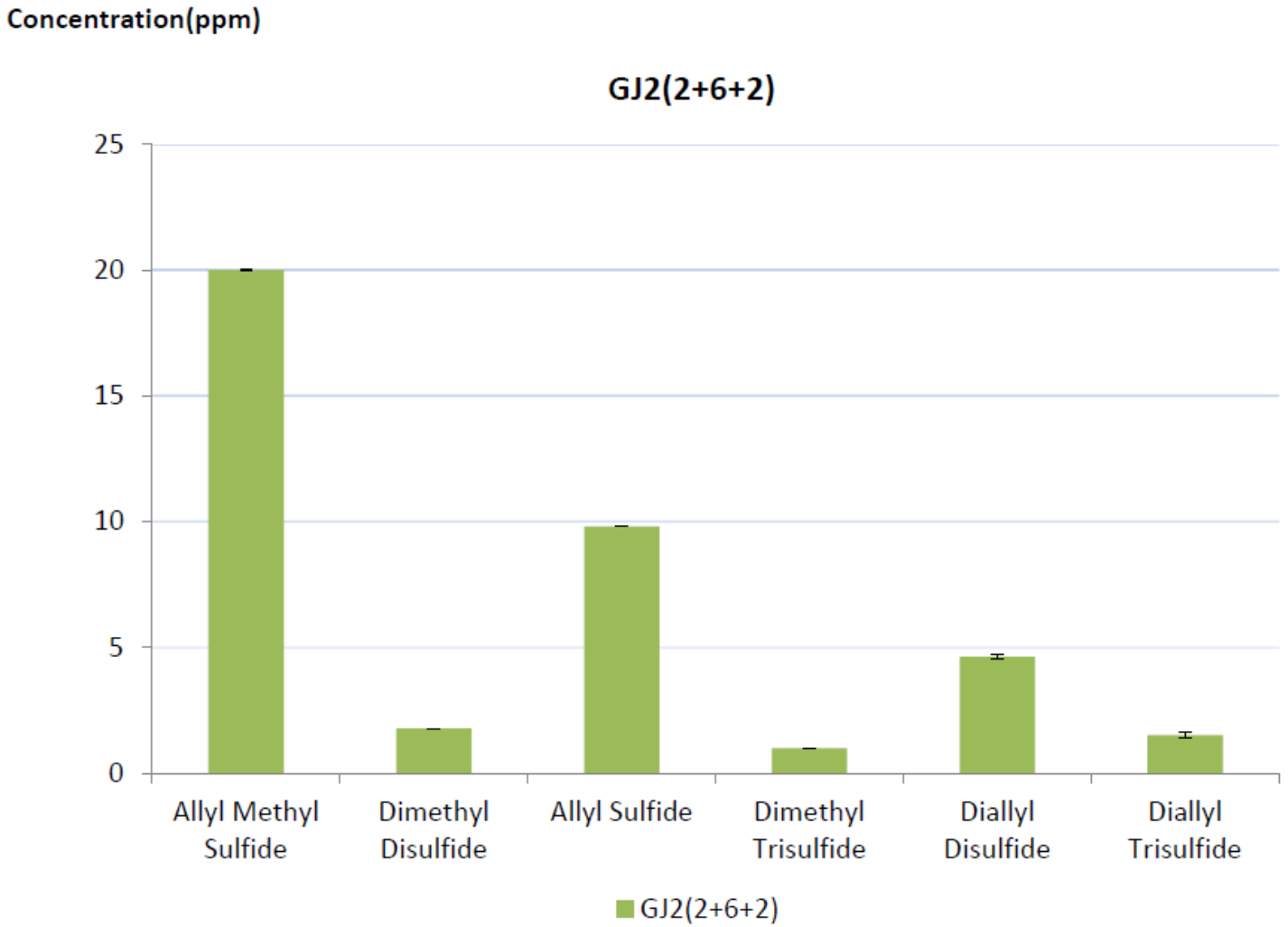
Compound	Average ppm	Stdev	Actual ppm	%recovery	Linear dynamic range (mg/L)	Equation	R2
Allyl Methyl Sulfide	10.2	0.25	10.00	102%	1.25~40	Y=0.9435x	0.998
Dimethyl Disulfide	4.0	0.10	4.00	101%	0.5~16	Y=1.386x	0.998
Allyl Sulfide	4.0	0.14	4.00	100%	0.5~16	Y=0.5307x	0.997
Dimethyl Trisulfide	3.8	0.06	4.00	94%	0.5~16	Y=0.1485x	0.998
Diallyl Disulfide	11.3	0.72	10.00	113%	1.25~20	Y=0.1485x	0.996
Diallyl Trisulfide	2.88	0.27	4.00	72%	0.5~16	Y=0.003145x2 +0.01669x	0.992

Garlic juice 1 (n=3)-2015



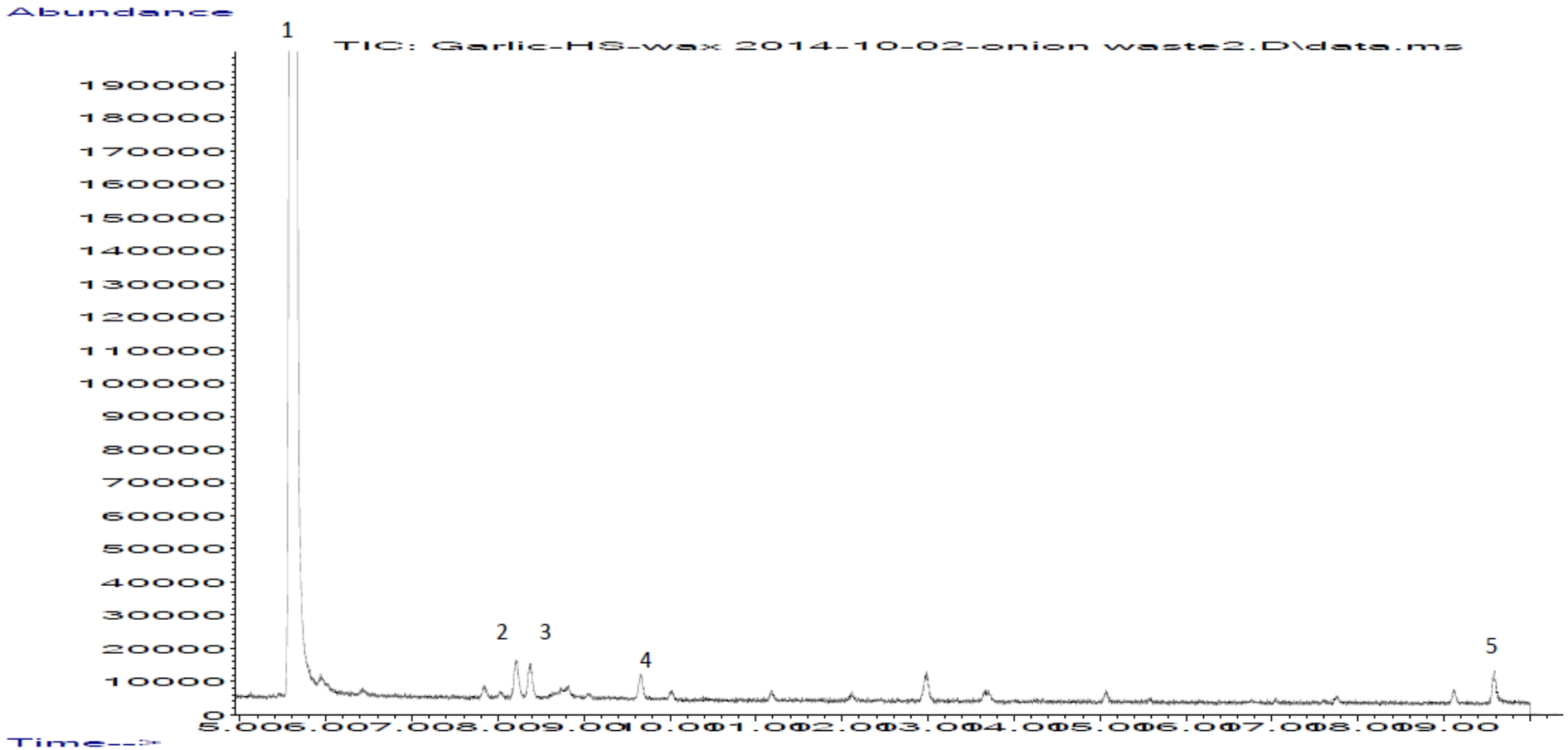
GJ1: LOT # 4555-315 (the Garlic Company, CA)

Garlic juice 2 (n=3)-2015



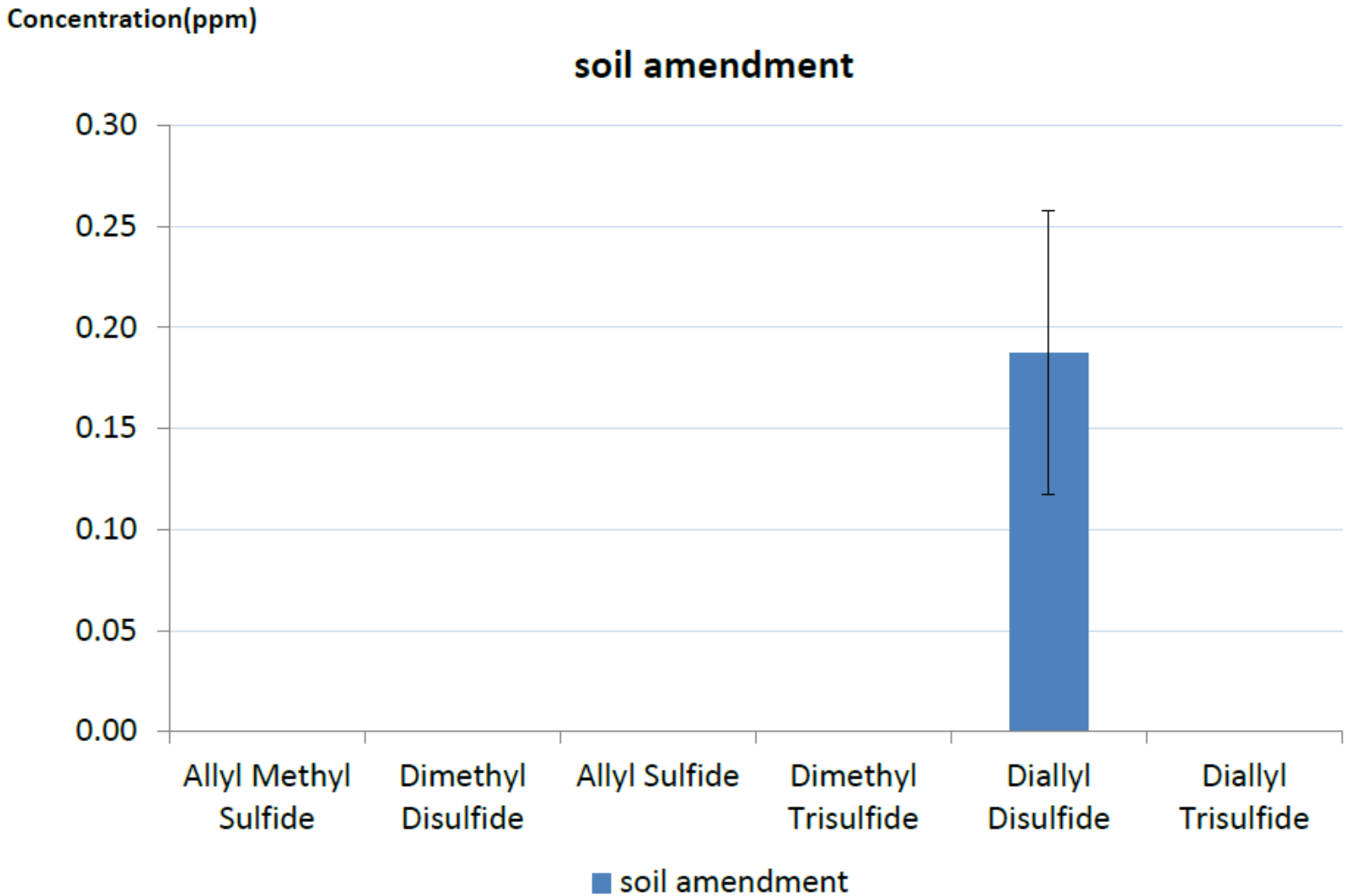
GJ 2: Garlic Juice Crop App, Formula #2, LOT # 4466-315 (the Garlic Company, CA)

Onion Waste



1: Ethanol 2: Propanol 3: S-Methyl thioacetate 4: Isobutyl alcohol 5: Acetic acid

Soil amendment

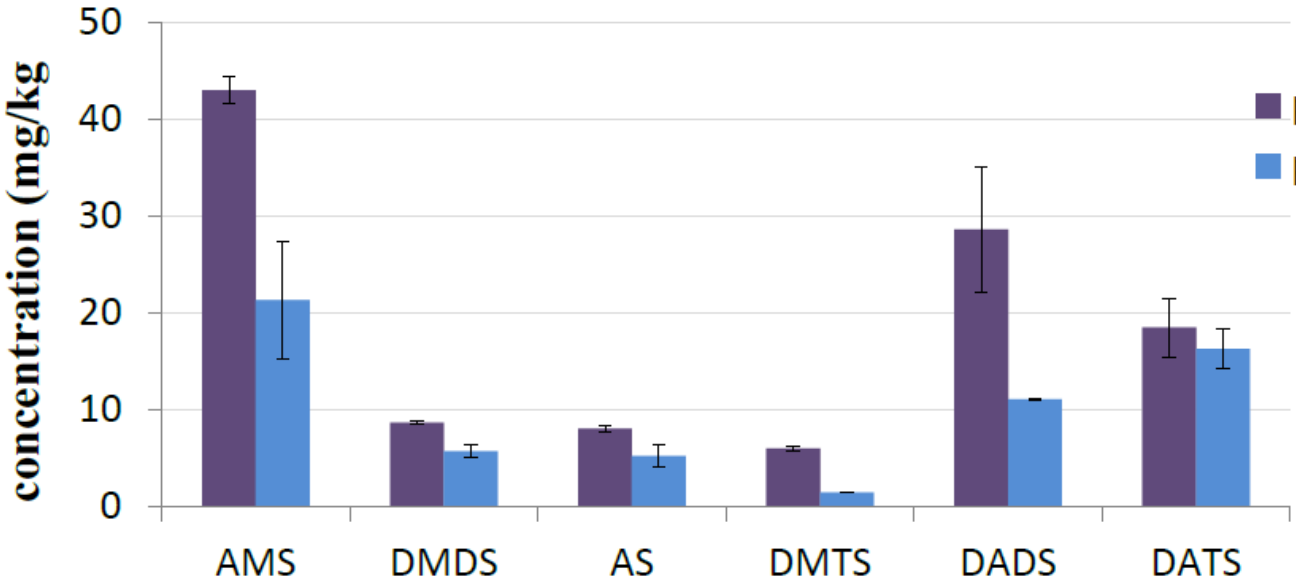


Formation of VOC in fresh garlic

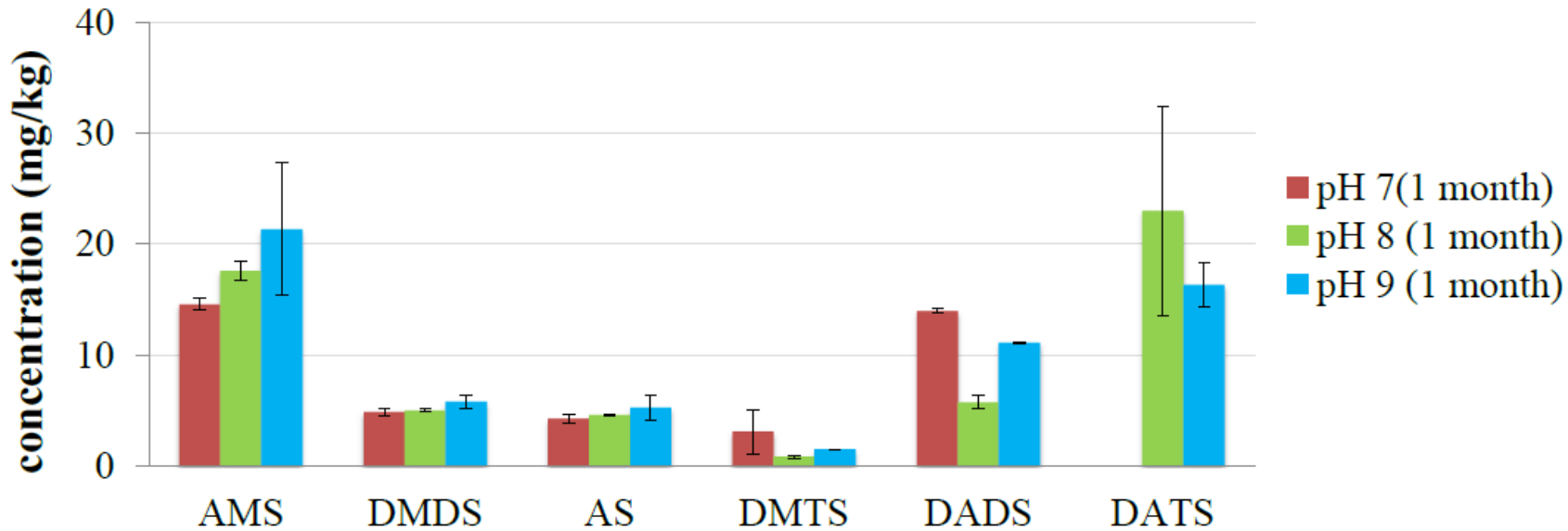
Sample preparation

- Garlic cloves were dried with liquid nitrogen and blended into a powder.
  + 2.0 g of frozen garlic powder
  + 6.0 mL of 0.01 M phosphate buffer
    (pH 2,4,6,7, or 8)
- Samples were incubated:
  -Room temperature, 35° C and 45° C degrees.
  -GC/MS analysis at 5 hours, 1 day, 3, 7, 14 days.
- Preparation for analysis:
  + small stir bar
  + 2.0 mL methanol (MeOH)
  + 50 µL internal standard (538 ppm ethyl methyl sulfide, 760 ppm isopropyl disulfide.
  + 2.0 g salt

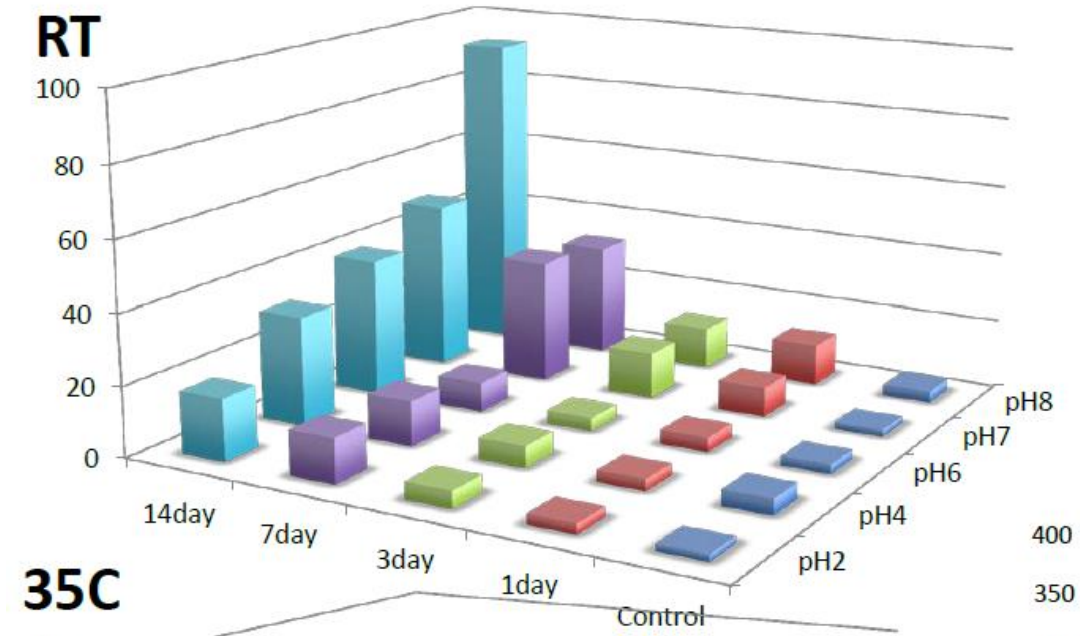
Concentration of sulfur compounds in home made garlic juice during storage

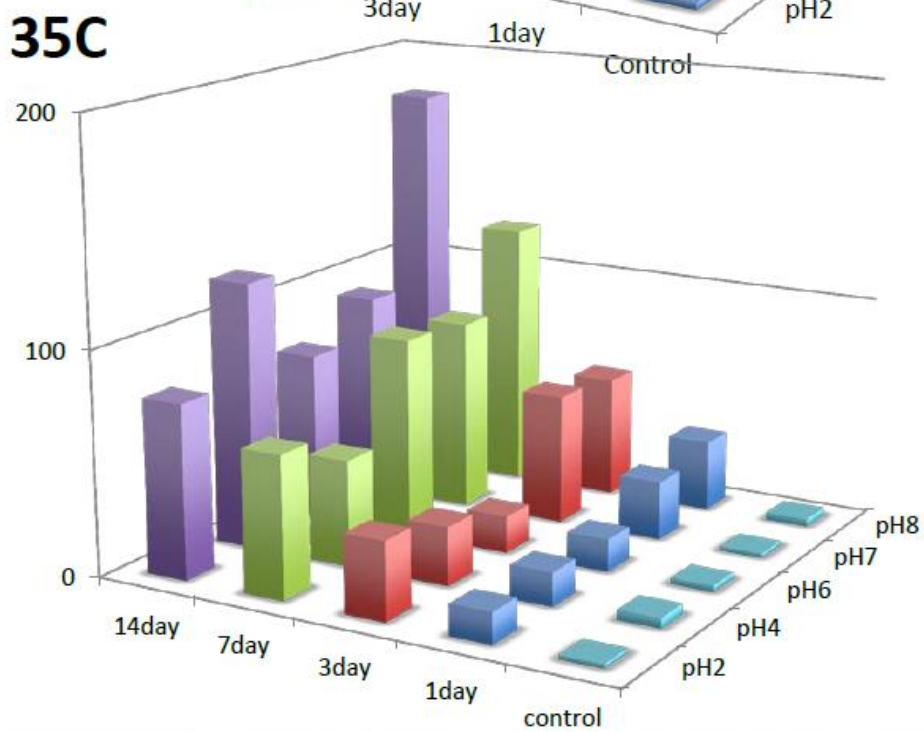


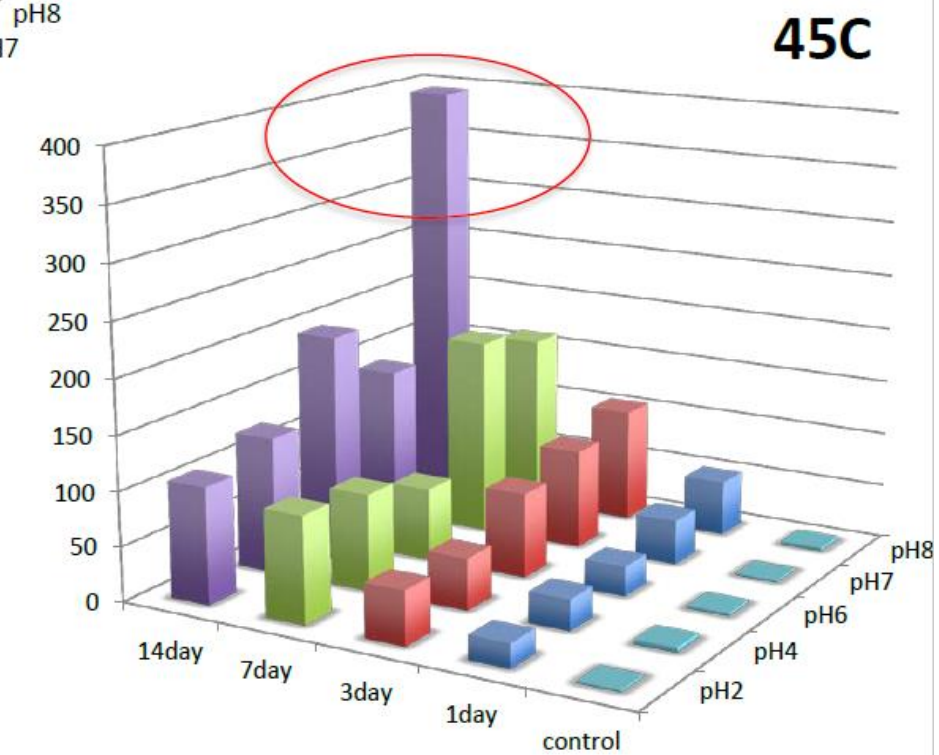
AMS: Allyl Methyl Sulfide,
DMDS: Dimethyl Disulfide,
AS: Allyl Sulfide,
DMTS: Dimethyl Trisulfide,
DATS: Diallyl Trisulfide,
DADS: Diallyl Disulfide



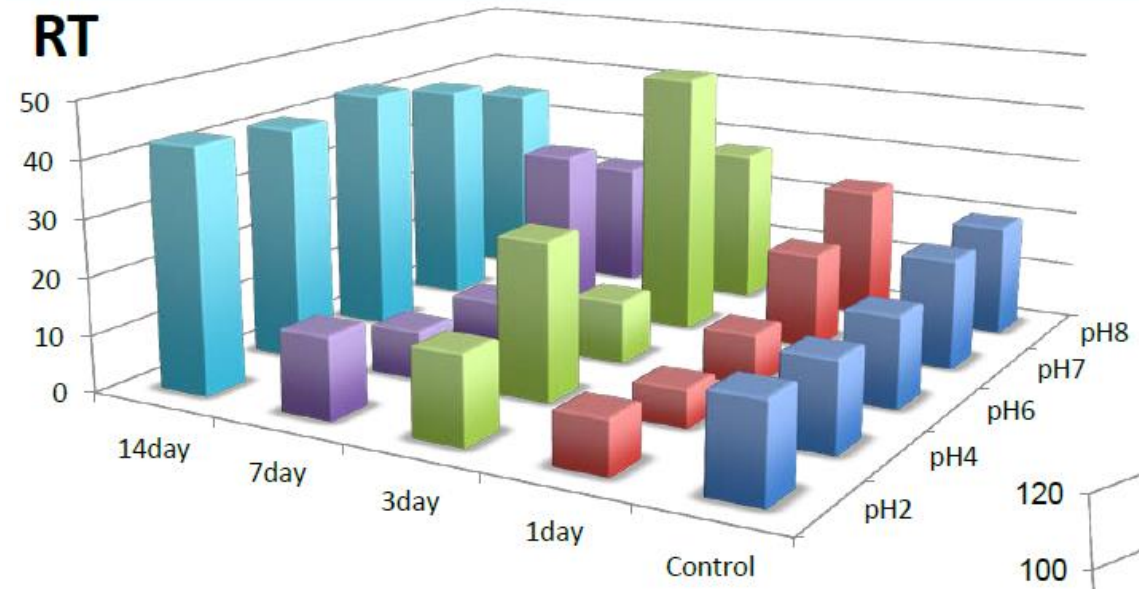
Other compounds- Allyl Sulfide

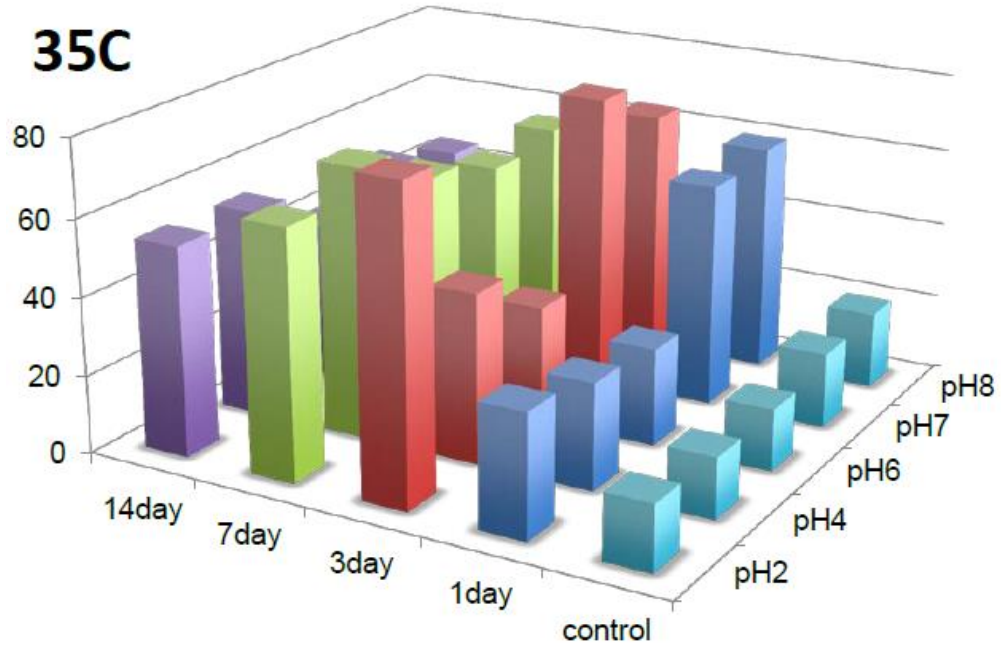


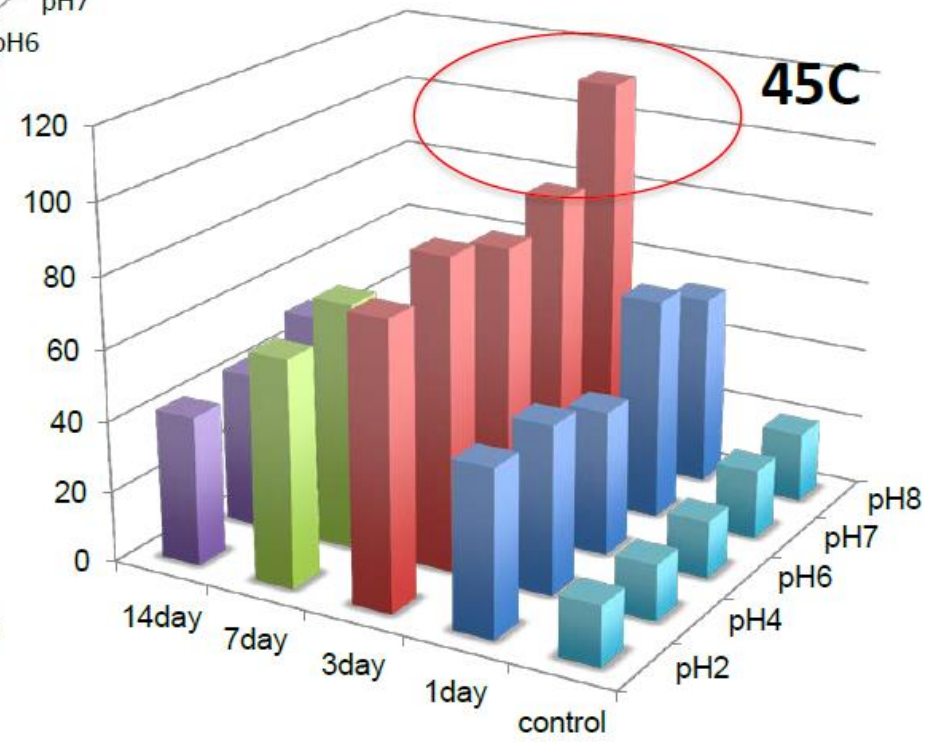




Other compounds- Diallyl Trisulfide







Limits of the Sky

- Dynamic conversions of various forms of volatile sulfur compounds
- Can achieve 250 ppm of DADS, 10-100x of the garlic juice products
- All forms of volatile sulfur compounds were about 1000 ppm
- 1000x less than the commercial DADS

Homemade garlic juice (revised protocol)

Sample preparation

- Garlic cloves were dried with liquid nitrogen, blended into powder.
  - -50 g of frozen garlic powder
  - -100g of 0.05 M phosphate buffer (pH 7, 8, 9)
- Samples were incubated:
  - -35° C water bath, gentle shaking, 1day.
  - -Centrifuge at 4° C, 7000rpm,10min
- Save the supernatant in a plastic tube and keep in 4 °C refrigerator (Garlic Juice)
- Instrument analysis
  - - HS-GC-FID
  - - 50 µL internal standard (538 ppm ethyl methyl sulfide, 760 ppm isopropyl disulfide).
  - - 2ml Garlic Juice+ 6ml Salt Water+2ml MeOH + 2g Salt