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

Dr. Fred Crowe

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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

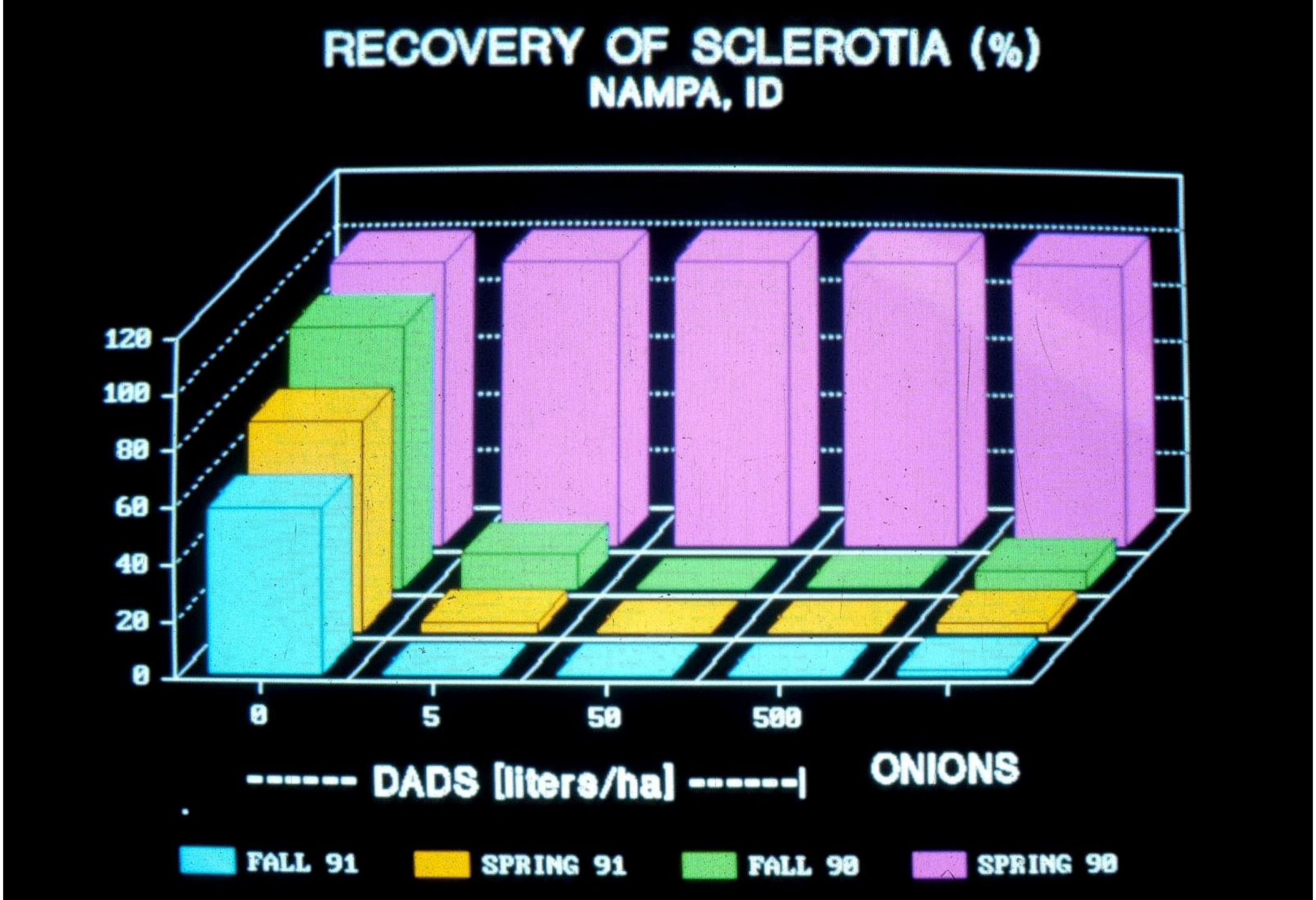
- Garlic fresh 6k acres
- Garlic dehydration 20k acres
- Garlic seed 4k acres

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

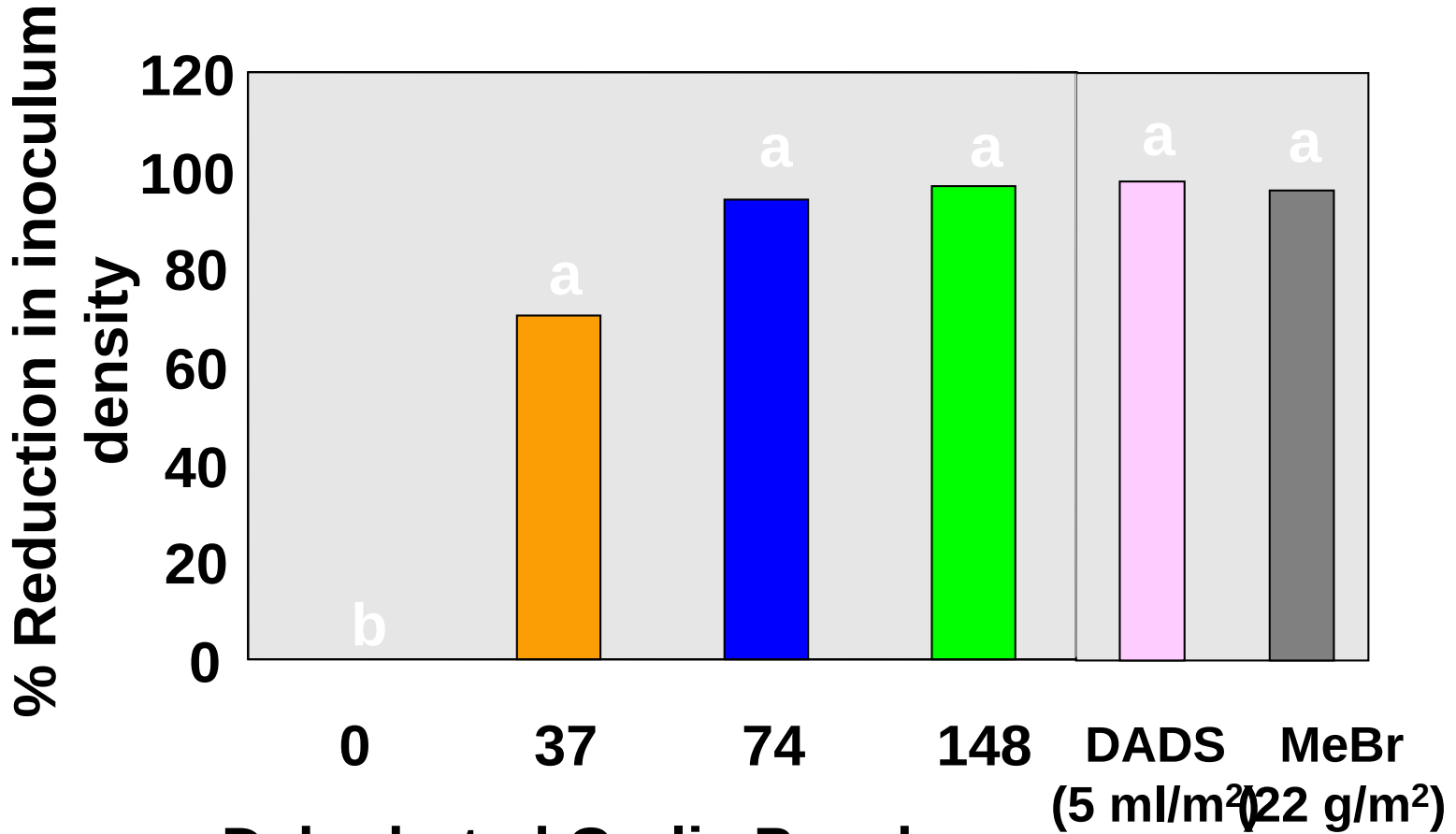
- 1985-2005: Stimulated germination (also included $$ from 3 other onion regions and Phillips Petroleum, later UAP)
  - Crowe, F.J., J. Debons, E. Redondo, T. Darnell, D. McGrath, J. Laborde, P. Koepsell, and M. Thornton. 1990. "Use of germination synthetic stimulants of sclerotial germination as a method 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

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- e-mail: robertehn@sbcglobal.net

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- 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:

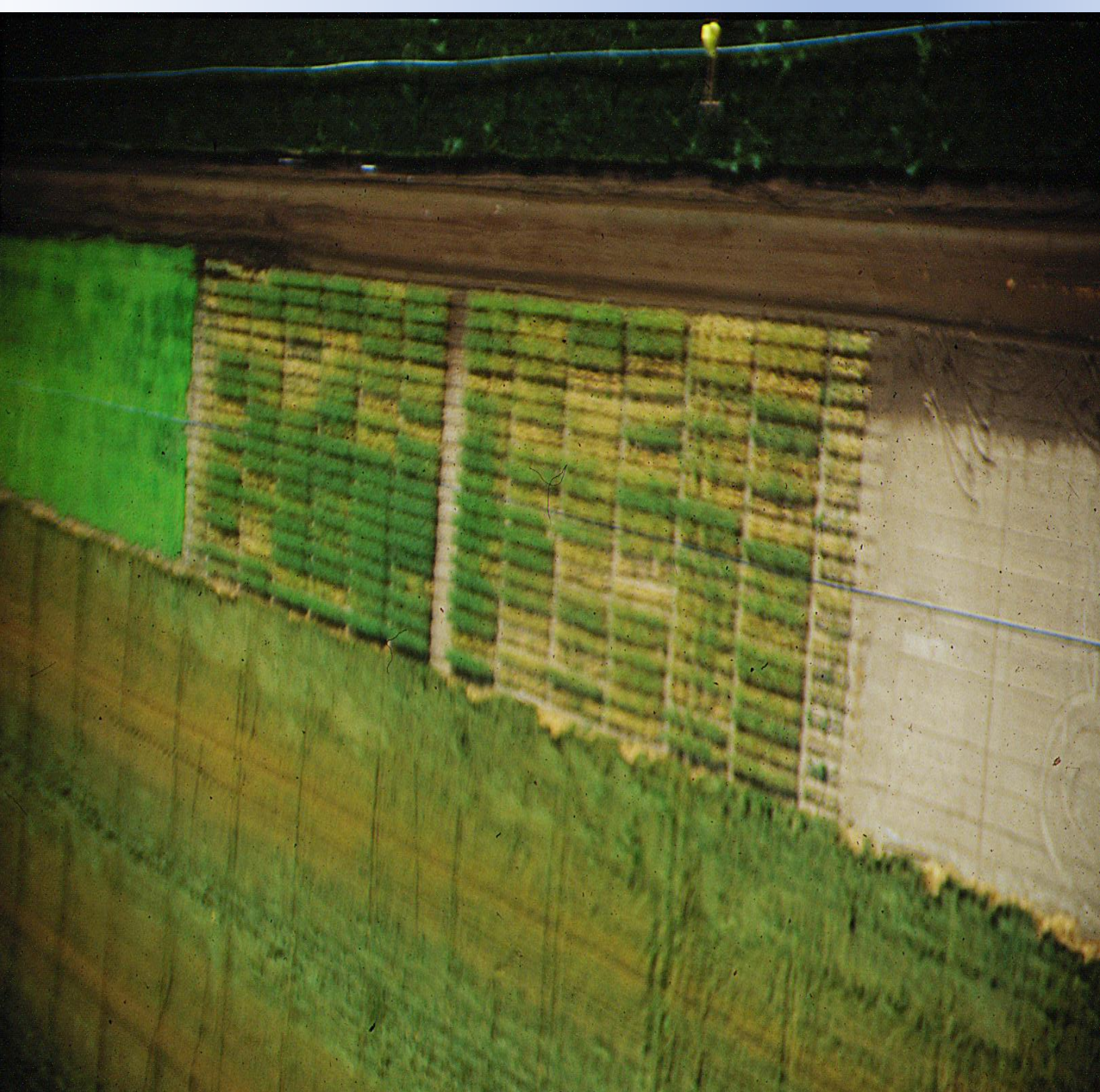
Beds: 36-40”

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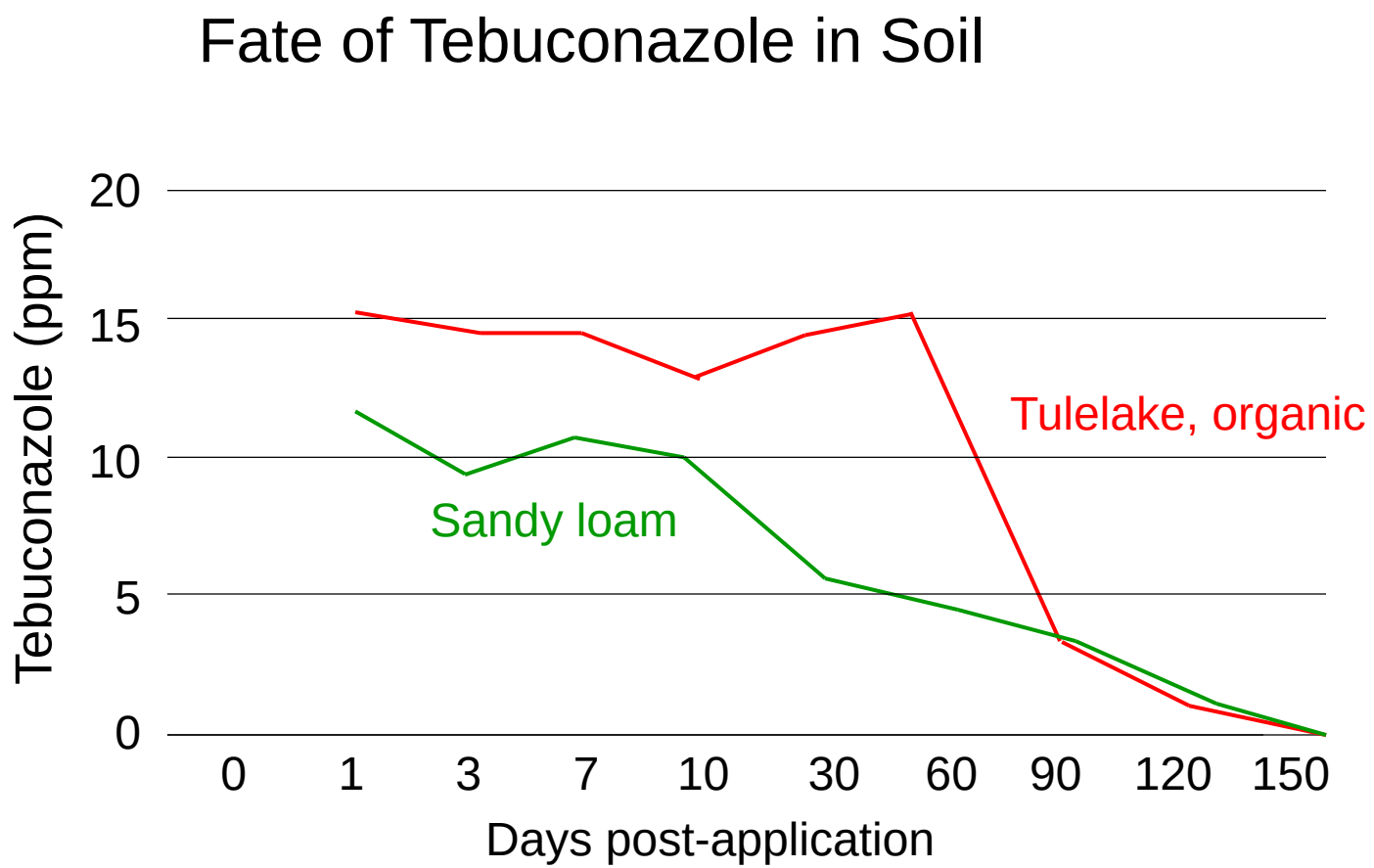
IPM potatoes, onions, grain,….. MS in weed science. Particularly good with chemical application and planting techniques. The WR team leader on testing of fungicides and germination stimulants on onion.

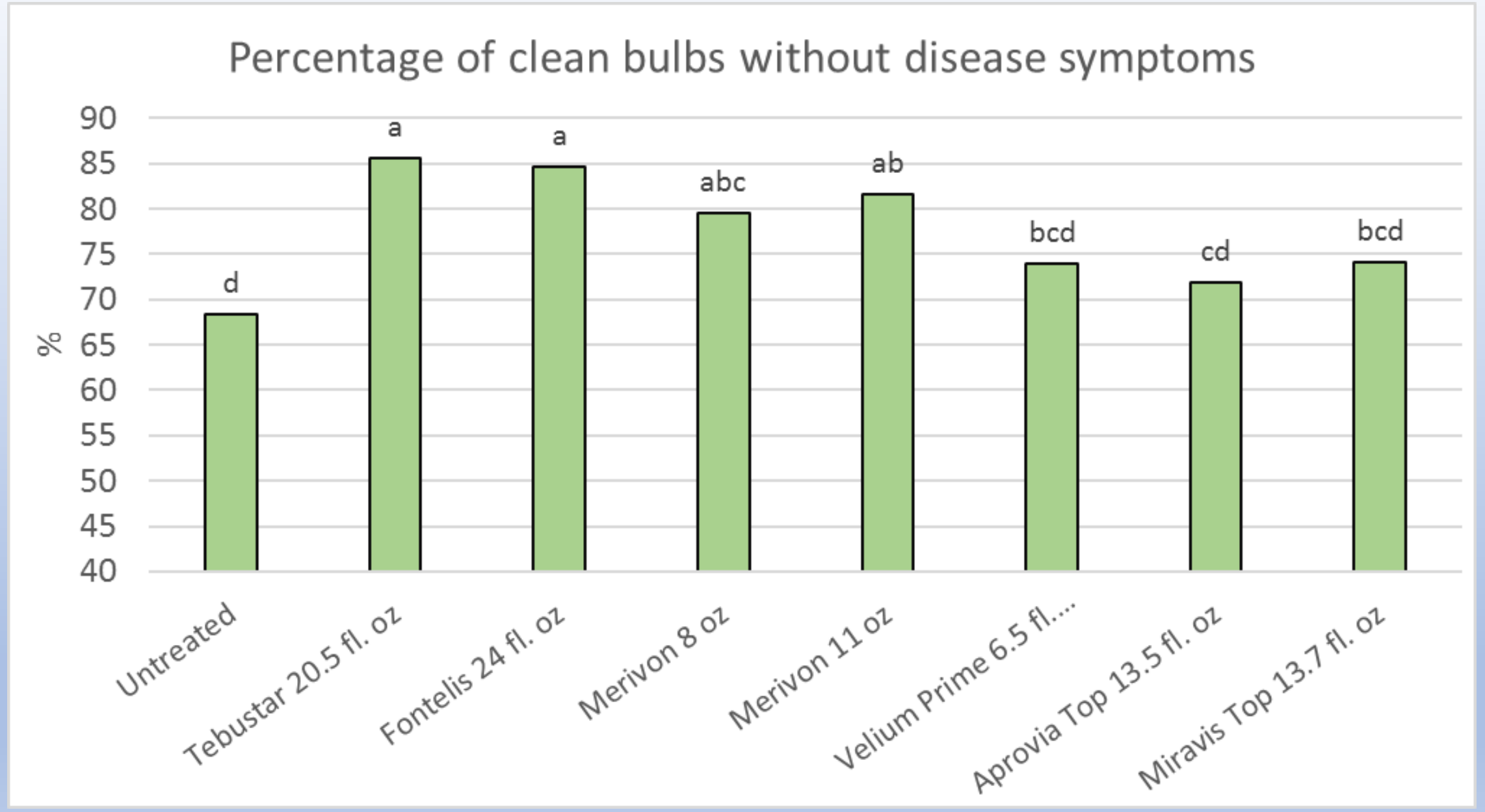
- Tebuconazole (Tebustar) and penthiopyrad (Fontelis) were most effective in CA testing
- Fungicides work best when sclerotia populations are low and conditions are not optimal for rapid disease development

- 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

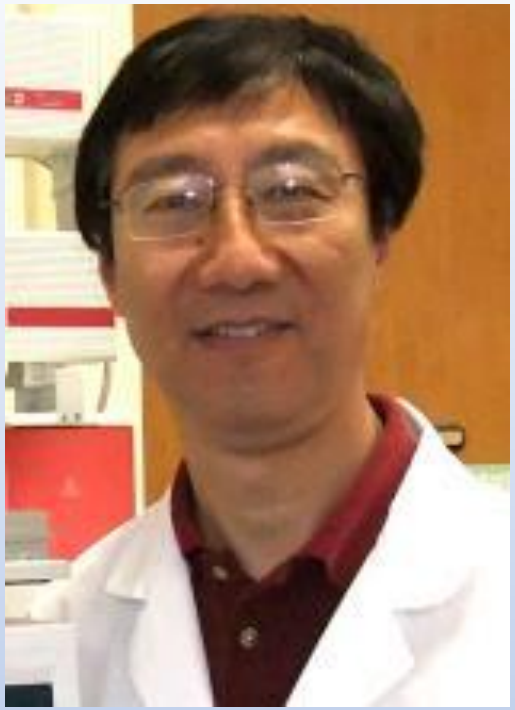
Pathology projects on peppermint (verticillium wilt), grass seed (ergot), and Allium (white rot). Has not accomplished much yet re AWR, but by backing up field efforts of AWR activities in Calif, he has developed a good, basic understanding WR biology and techniques.

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.

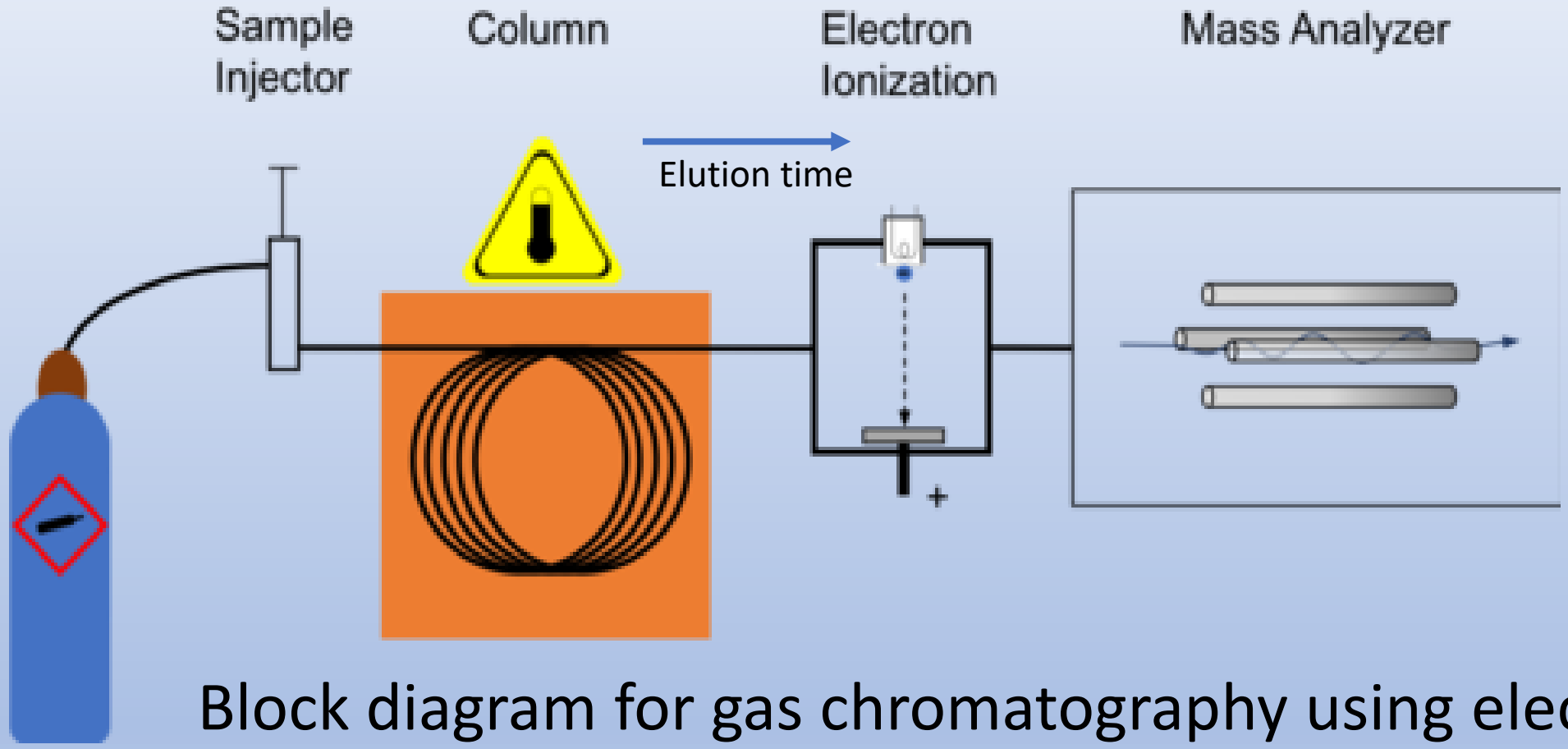
Objectives

- 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



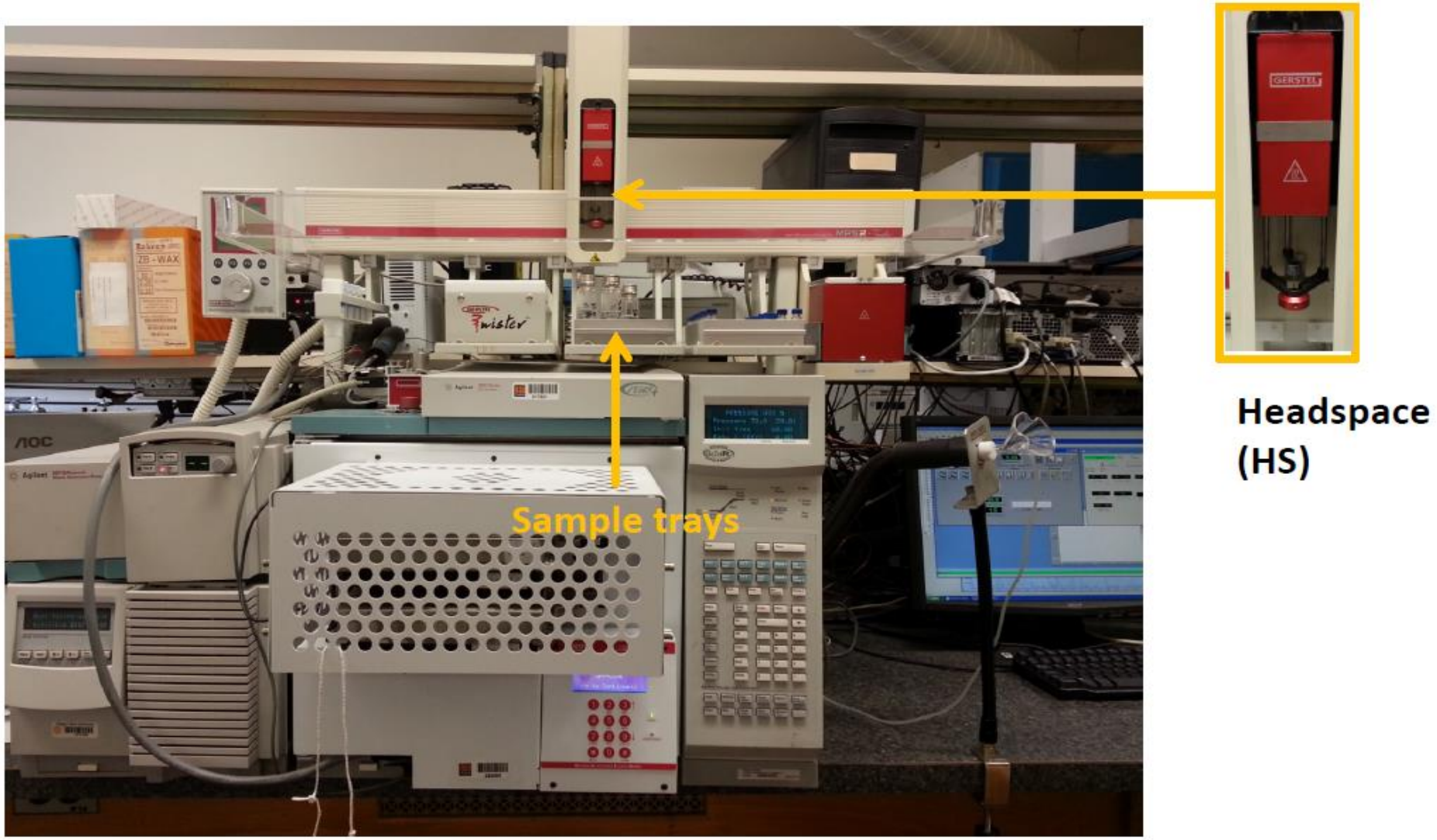
Block diagram for gas chromatography using electron ionization for collecting mass spectrum

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

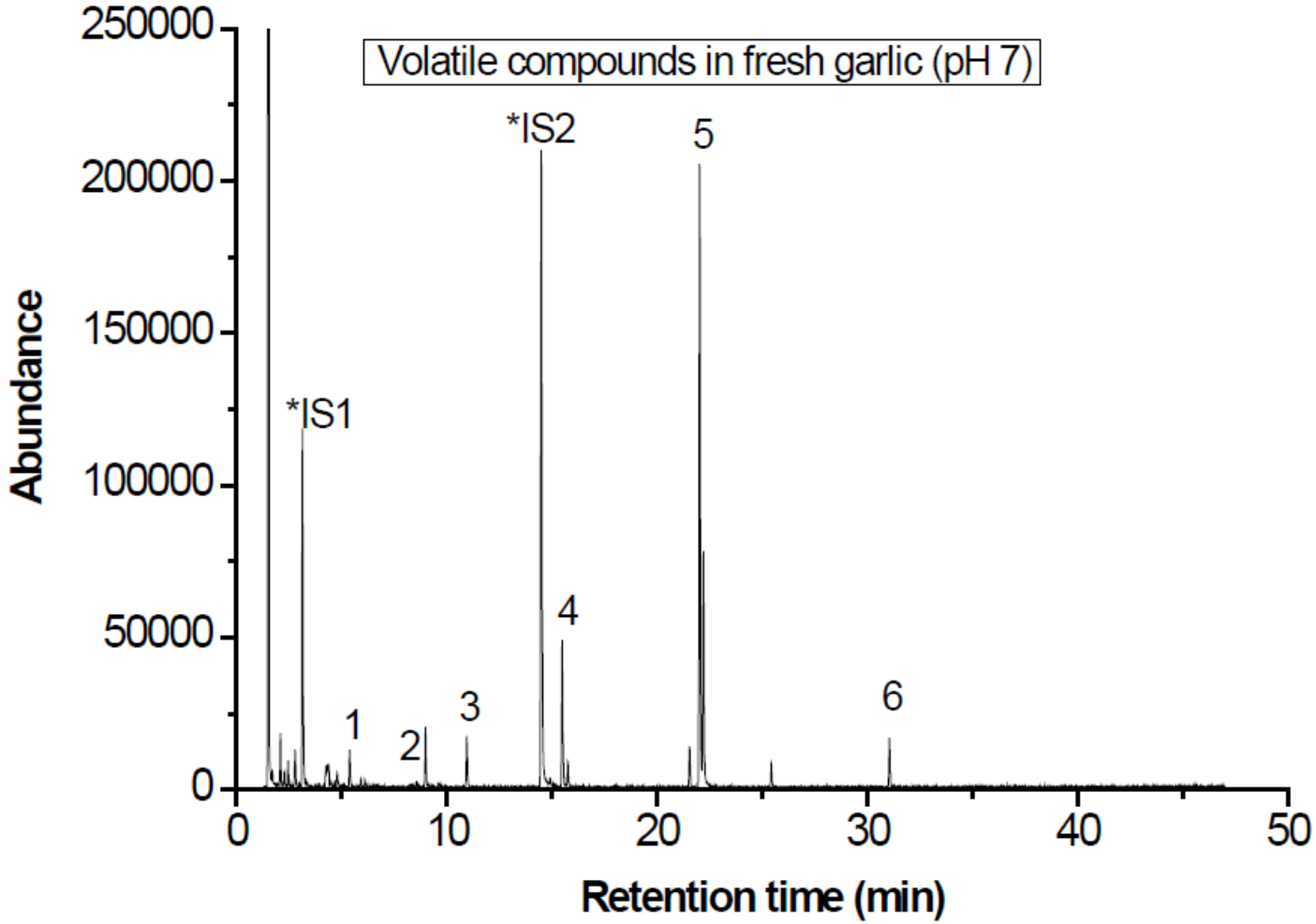
Allyl sulfides

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)

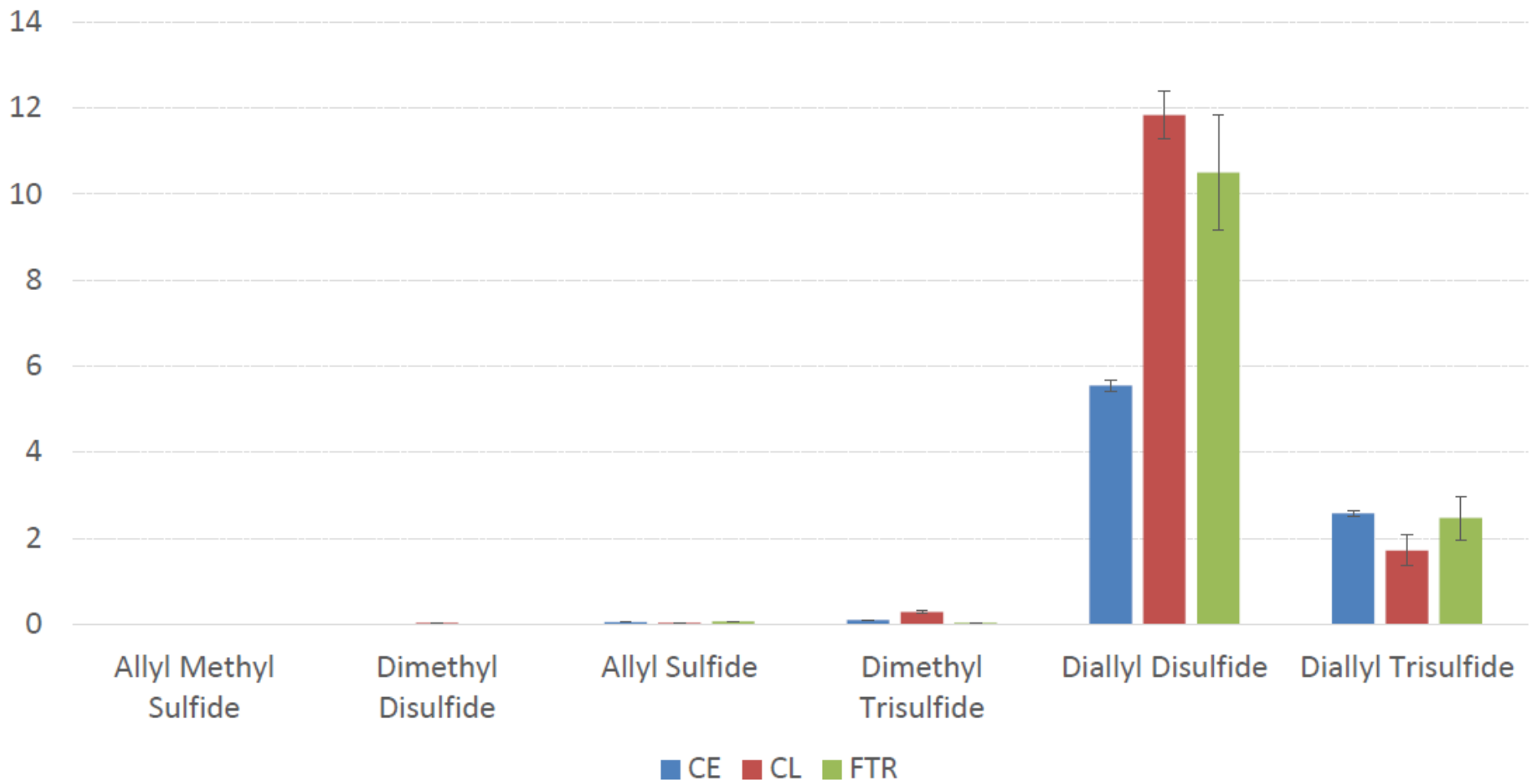
Volatile sulfur compounds in Fresh Garlic



*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)

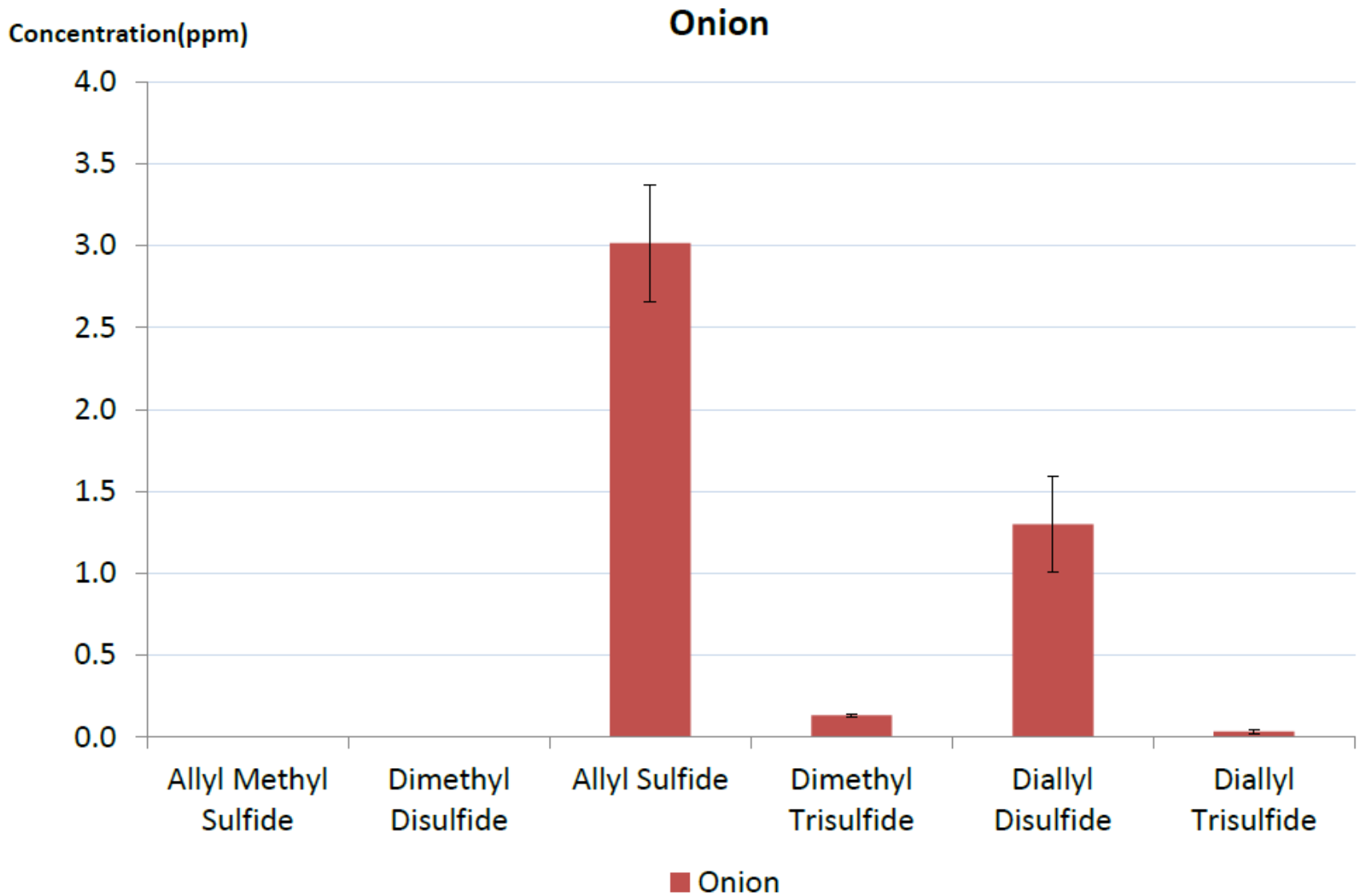


CE: California Early

CL: California Late

FTR: Forked Tree Ranch

fresh onion

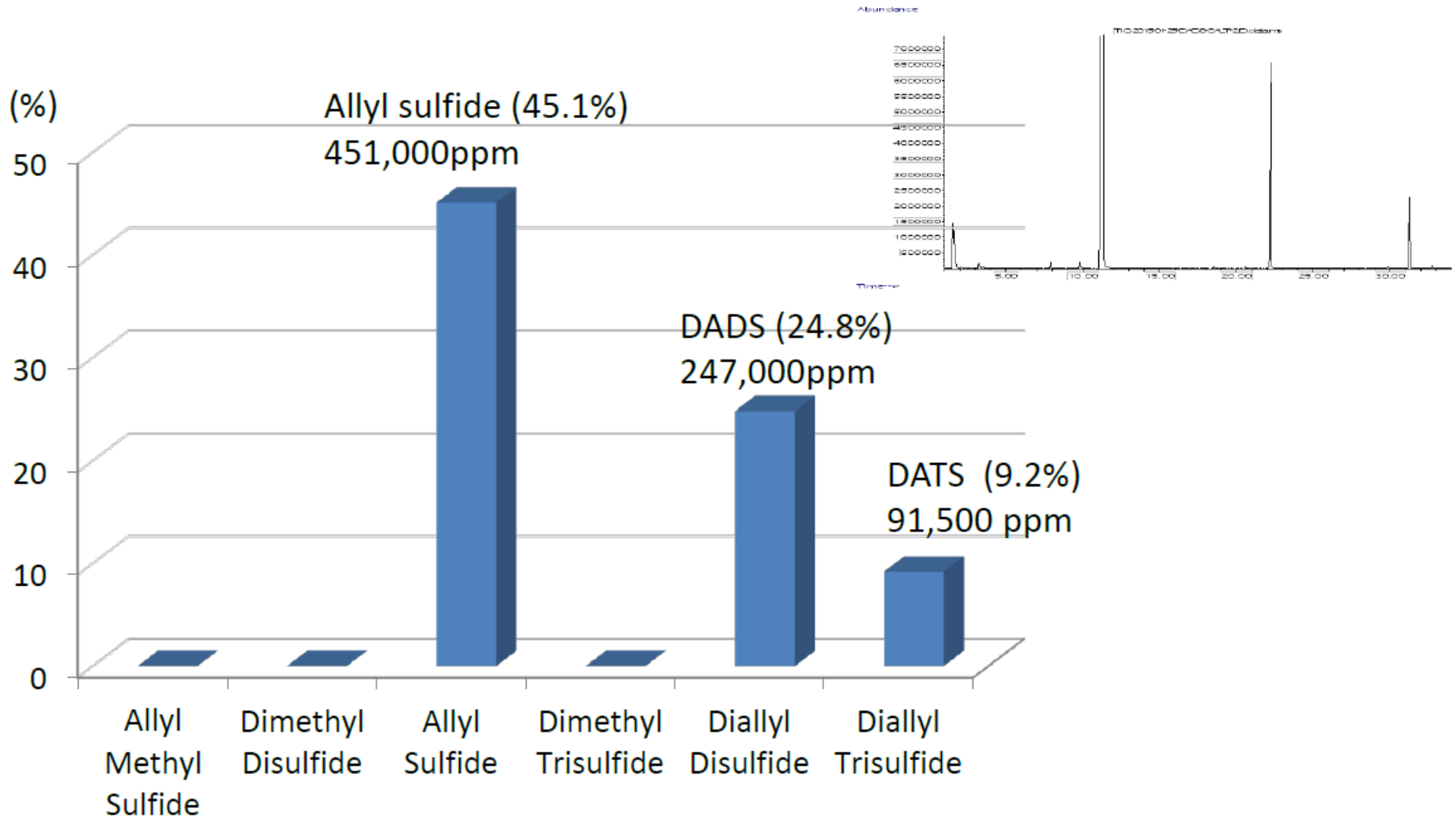

Onion
Concentration(ppm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Allyl Methyl Sulfide
Dimethyl Disulfide
Allyl Sulfide
Dimethyl Trisulfide
Diallyl Disulfide
Diallyl Trisulfide
Onion

Garlic Juice Samples-2016

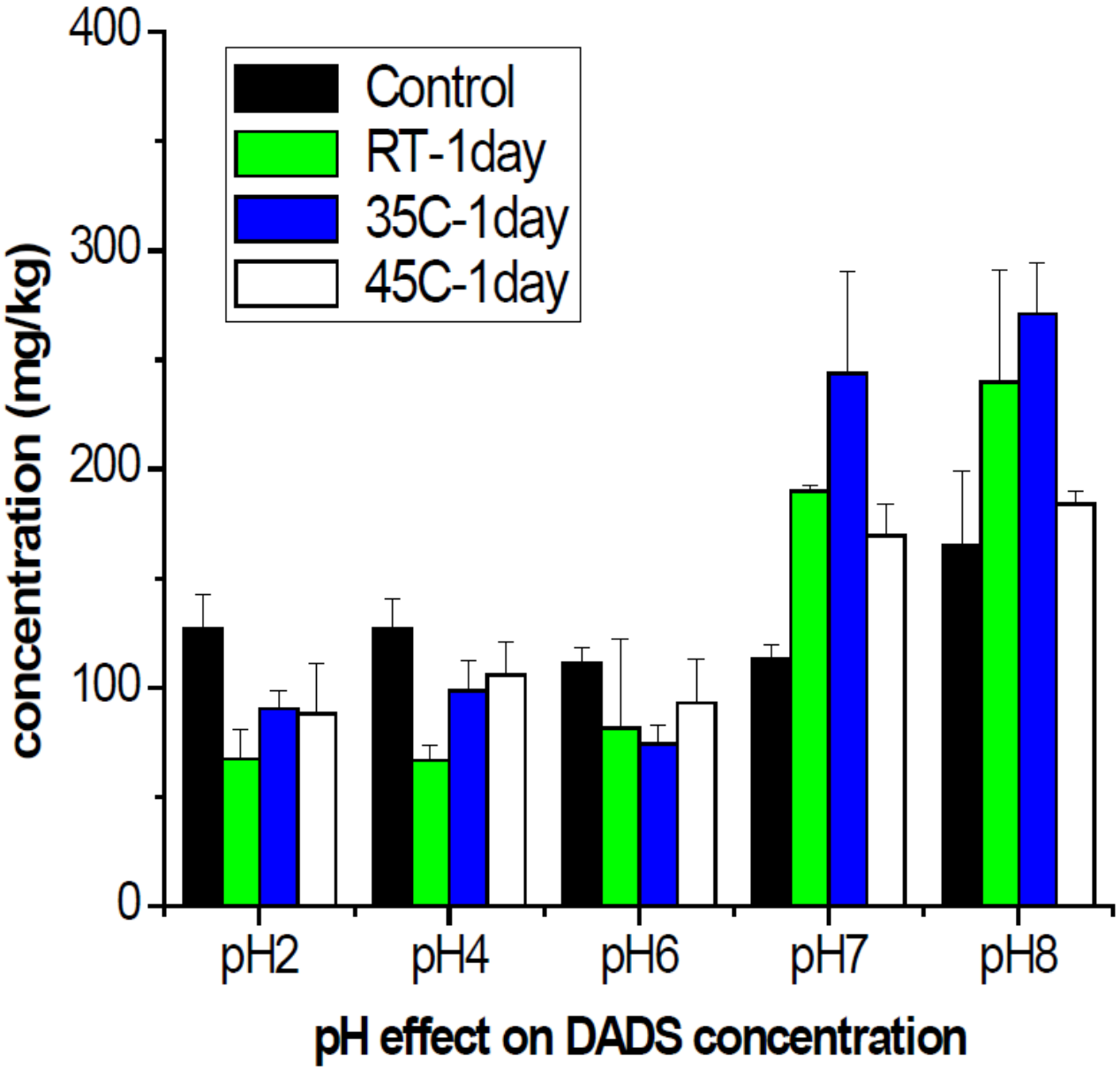
Concentration of sulfur compounds (mg/ kg)

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



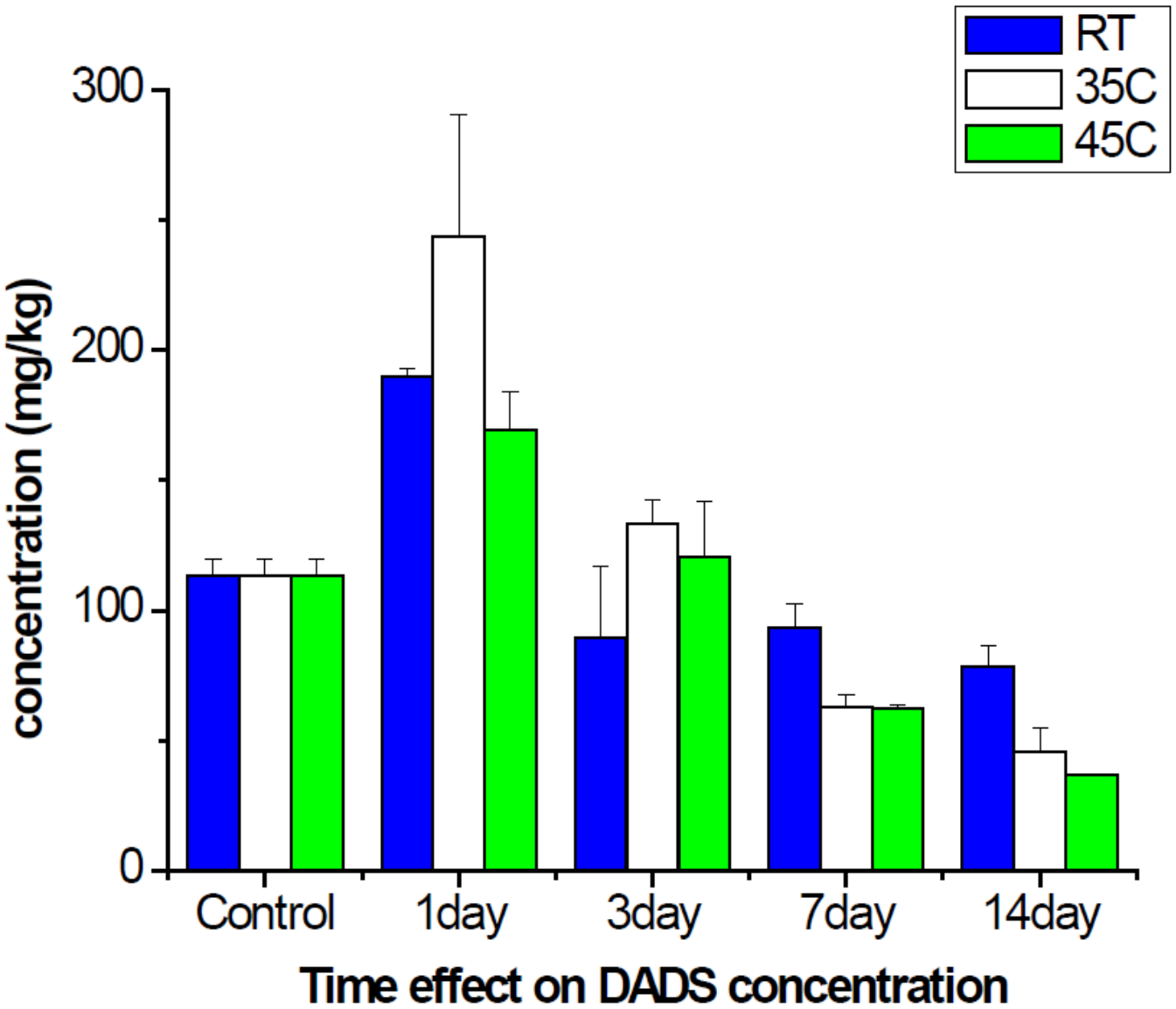
Conclusion:

(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:

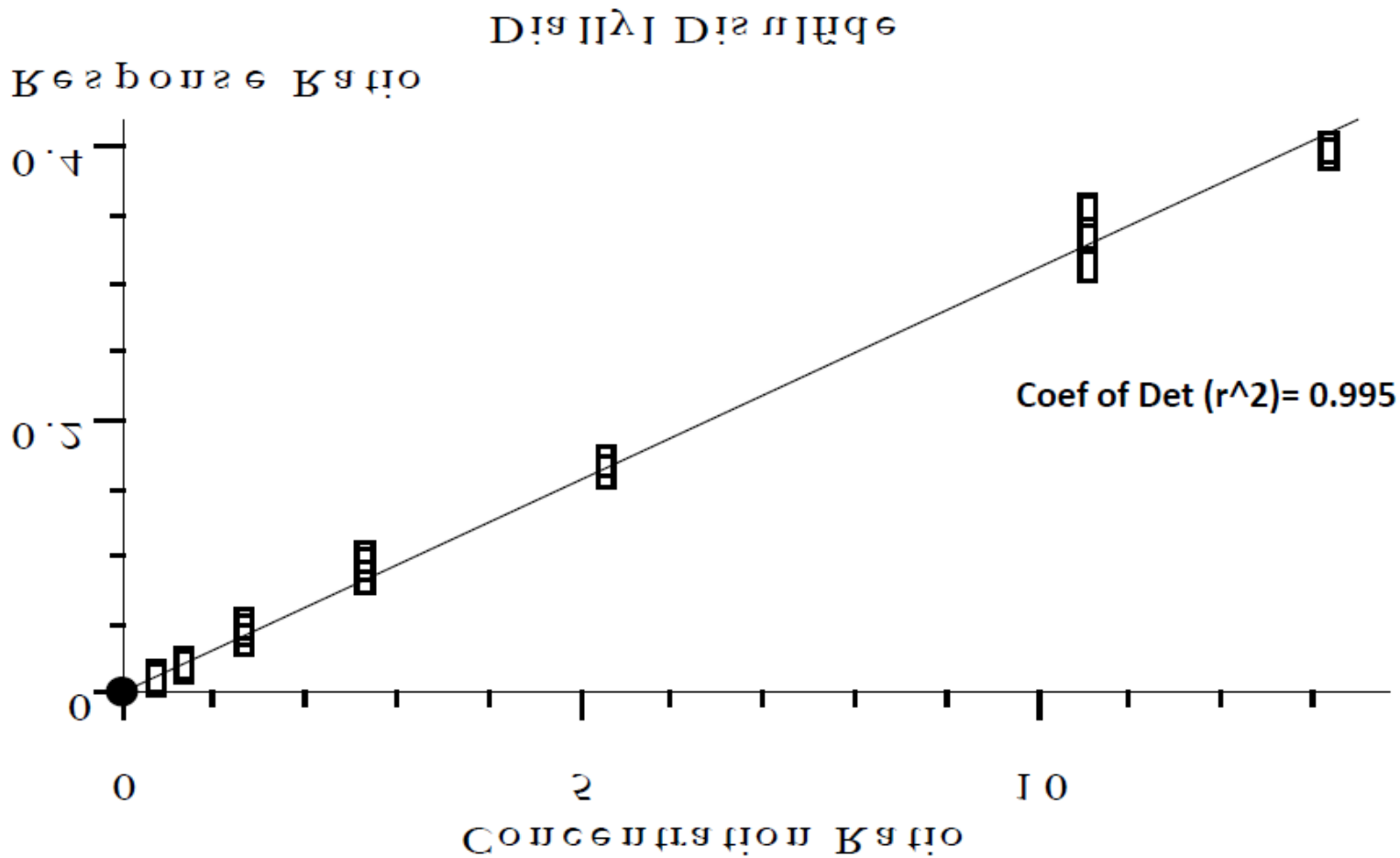
(1) After 1 day incubation, the DADS concentration will increase about 2.5 time compared to no incubation treatment at pH 7

(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

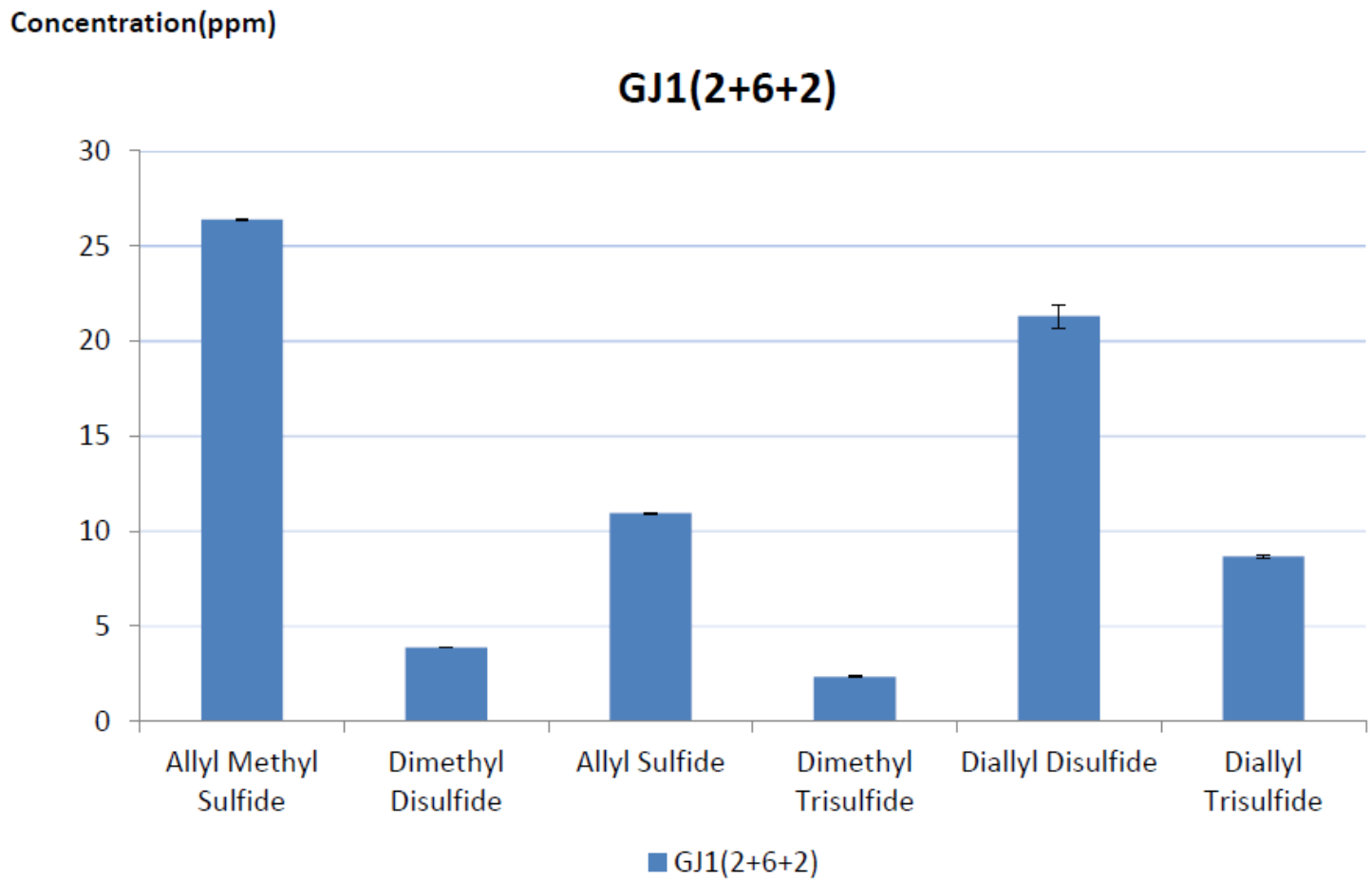
Calibration curve development



Calibration Curve Development

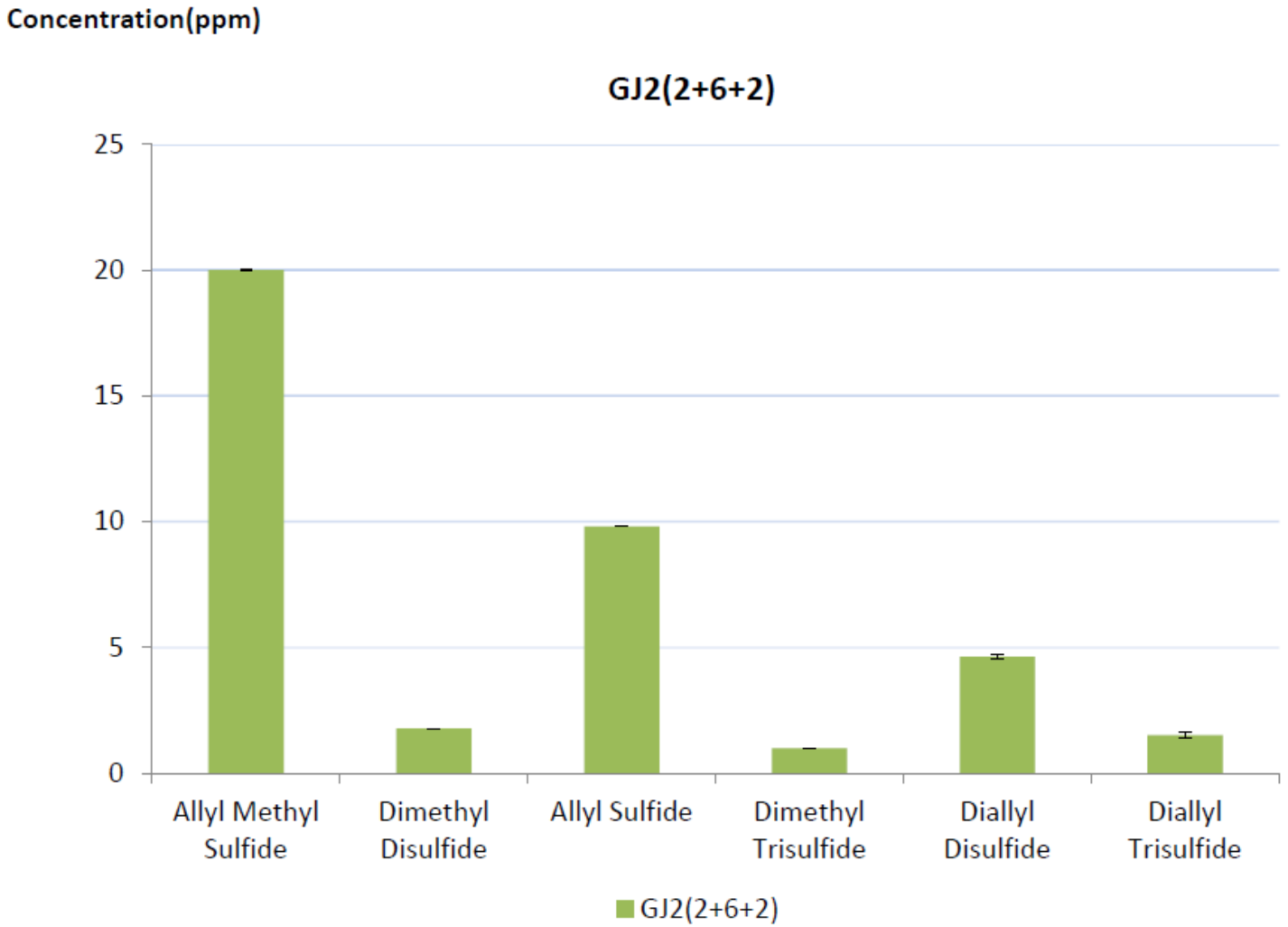
Compound	Average ppm	Stdev	Actual ppm	%recovery	Linear dynamic range (mg/L)	Equation	R2
Allyl Methyl Sulfide	10.2	0.25	10.00	102%	1.25~40	Y=0.9435x	0.998
Dimethyl Disulfide	4.0	0.10	4.00	101%	0.5~16	Y=1.386x	0.998
Allyl Sulfide	4.0	0.14	4.00	100%	0.5~16	Y=0.5307x	0.997
Dimethyl Trisulfide	3.8	0.06	4.00	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



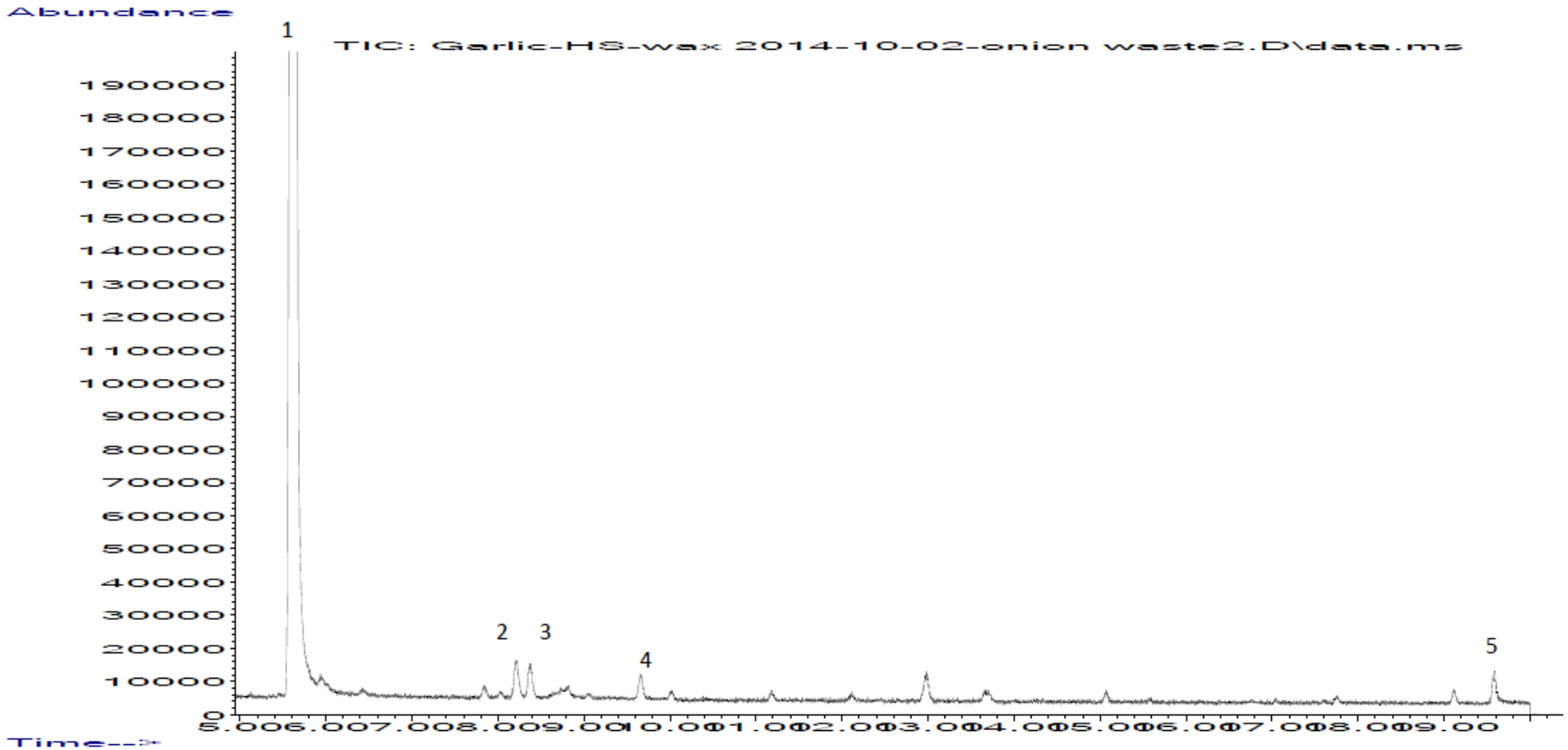
GJ1: LOT # 4555-315 (the Garlic Company, CA)

Garlic juice 2 (n=3)-2015



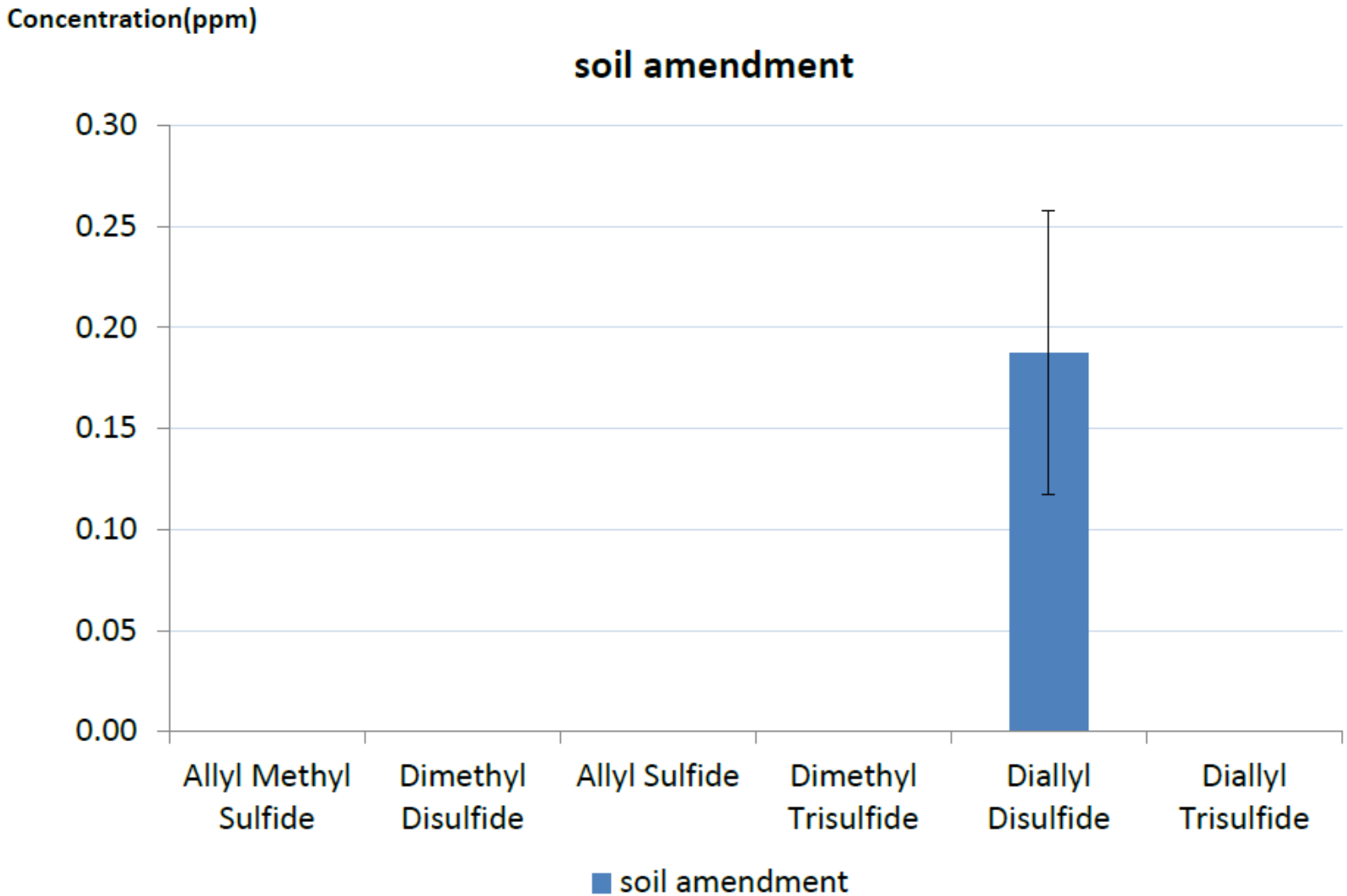
GJ 2: Garlic Juice Crop App, Formula #2, LOT # 4466-315 (the Garlic Company, CA)

Onion Waste



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

Soil amendment

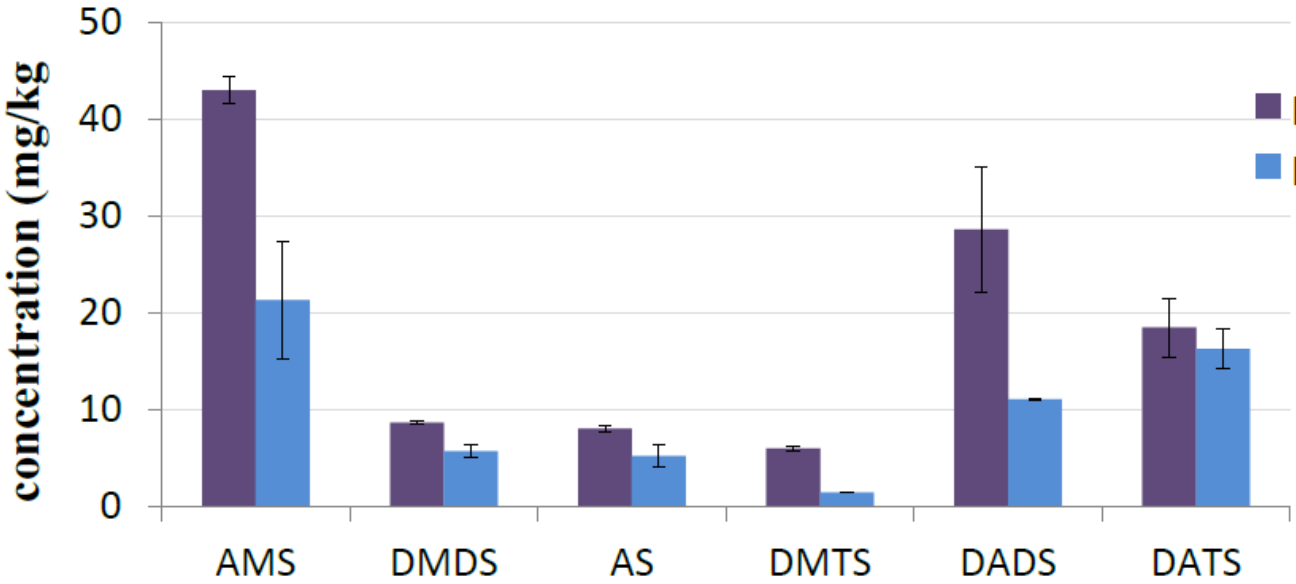


Formation of VOC in fresh garlic

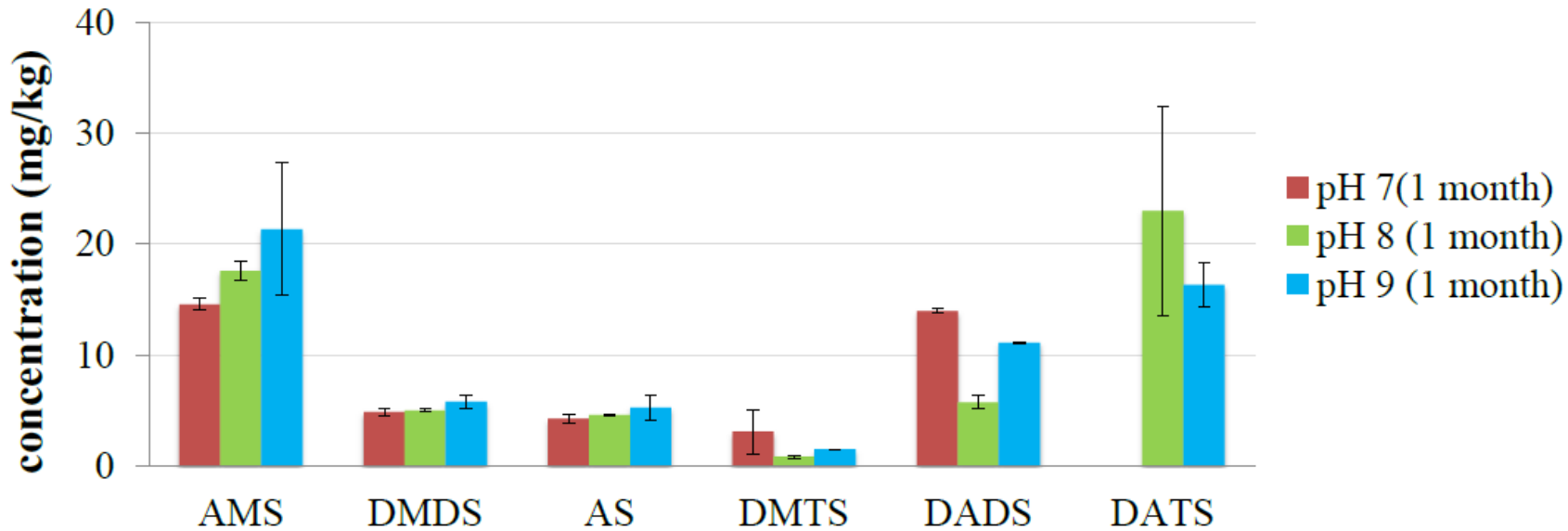
Sample preparation

- 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

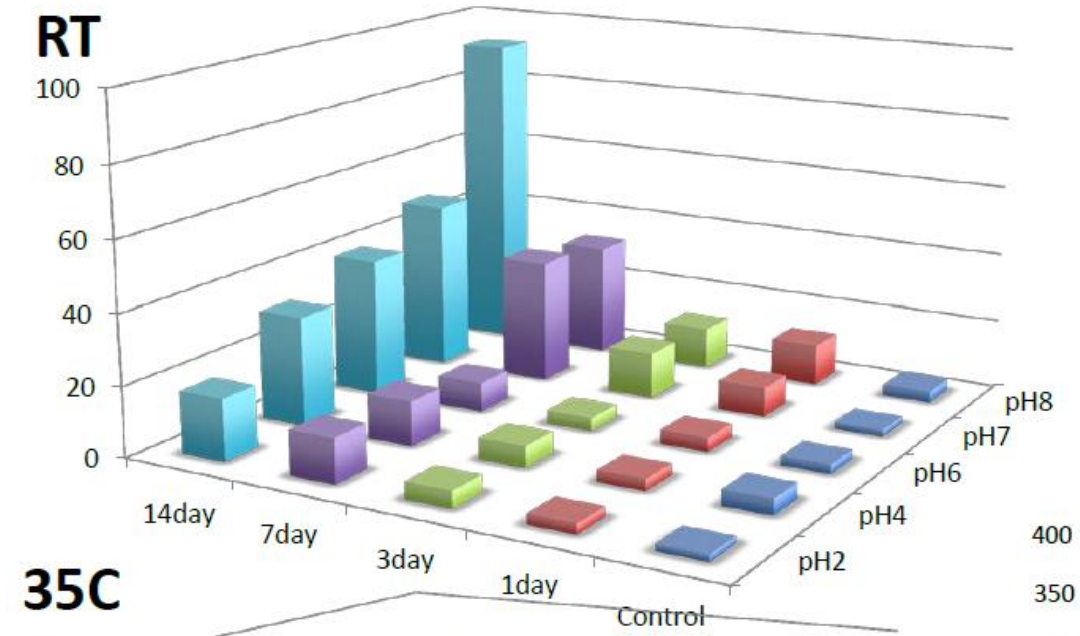
Concentration of sulfur compounds in home made garlic juice during storage

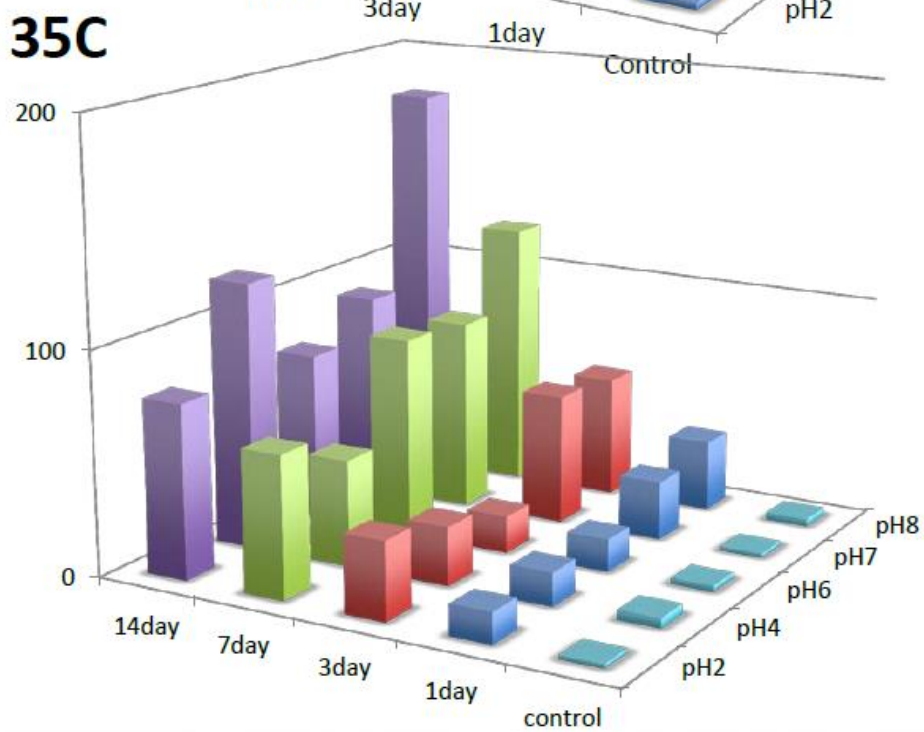


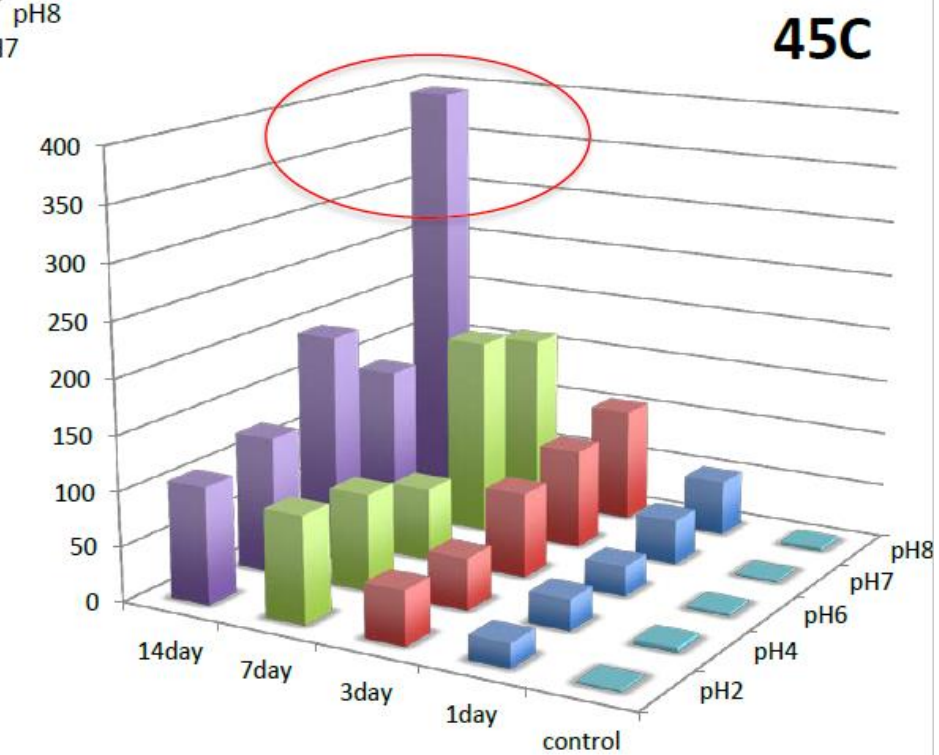
AMS: Allyl Methyl Sulfide,
DMDS: Dimethyl Disulfide,
AS: Allyl Sulfide,
DMTS: Dimethyl Trisulfide,
DATS: Diallyl Trisulfide,
DADS: Diallyl Disulfide



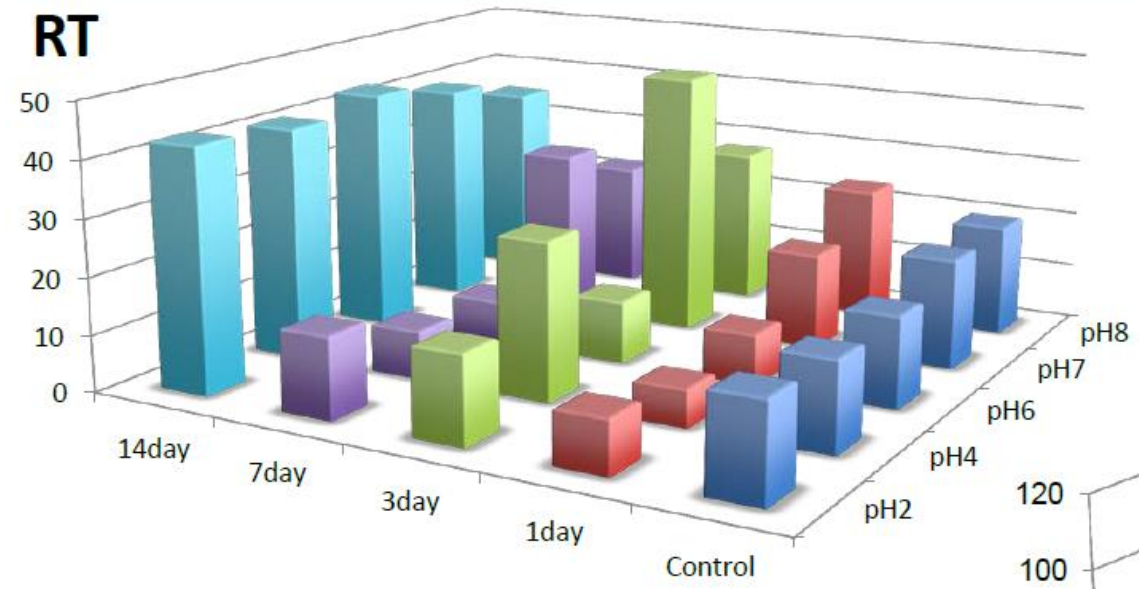
Other compounds- Allyl Sulfide

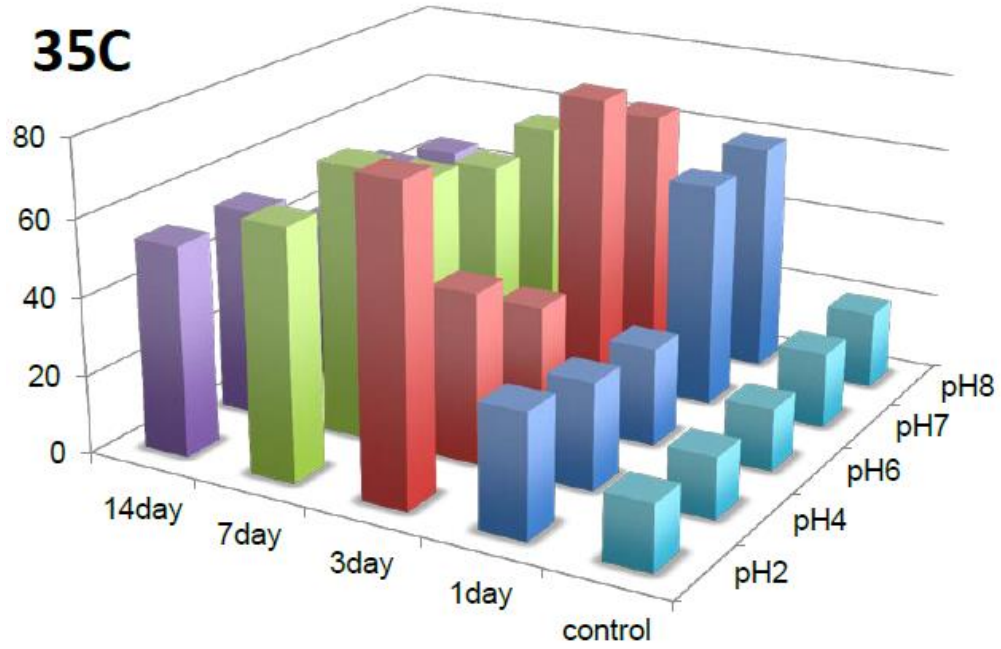


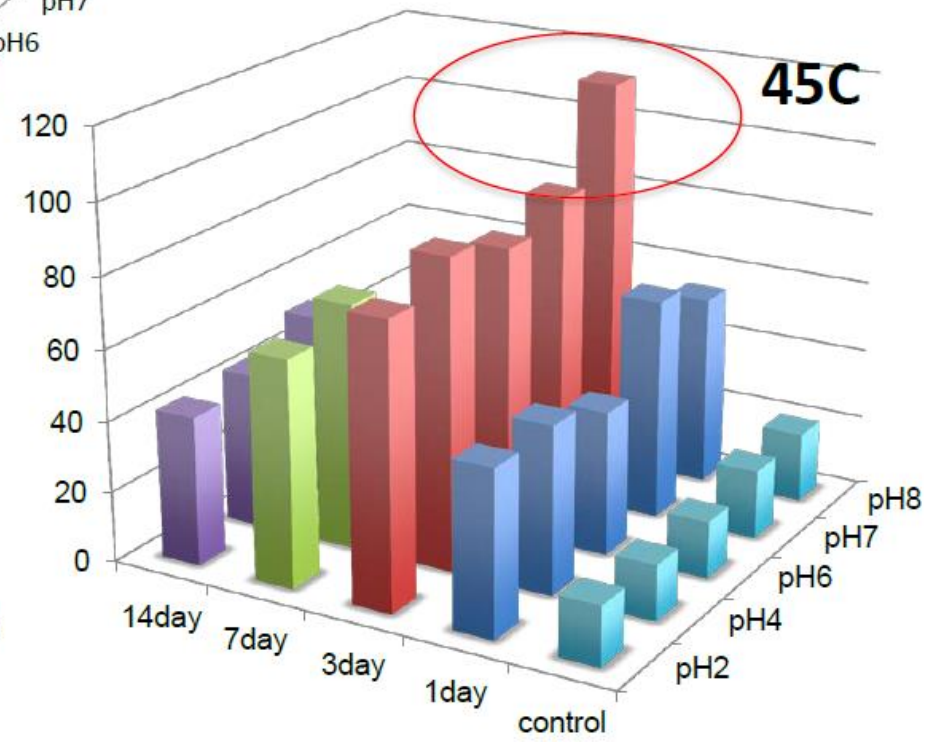




Other compounds- Diallyl Trisulfide







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