Mechanisms of Broccoli-Mediated Soilborne Pathogen and Disease Suppression in Vegetable Cropping Systems

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Focus

- Genesis of the idea
- Proof of concept
- Mechanisms








Background

- Extensive surveys revealed the high degree of susceptibility of cauliflower and near-immunity of broccoli to Verticillium wilt.
- *Verticillium dahliae* isolates from crucifer crops were weakly pathogenic on broccoli in greenhouse tests, and others were non-pathogenic.
- Dry crucifer residues are better than fresh residue.

Reaction of broccoli to *Verticillium dahliae* from various hosts

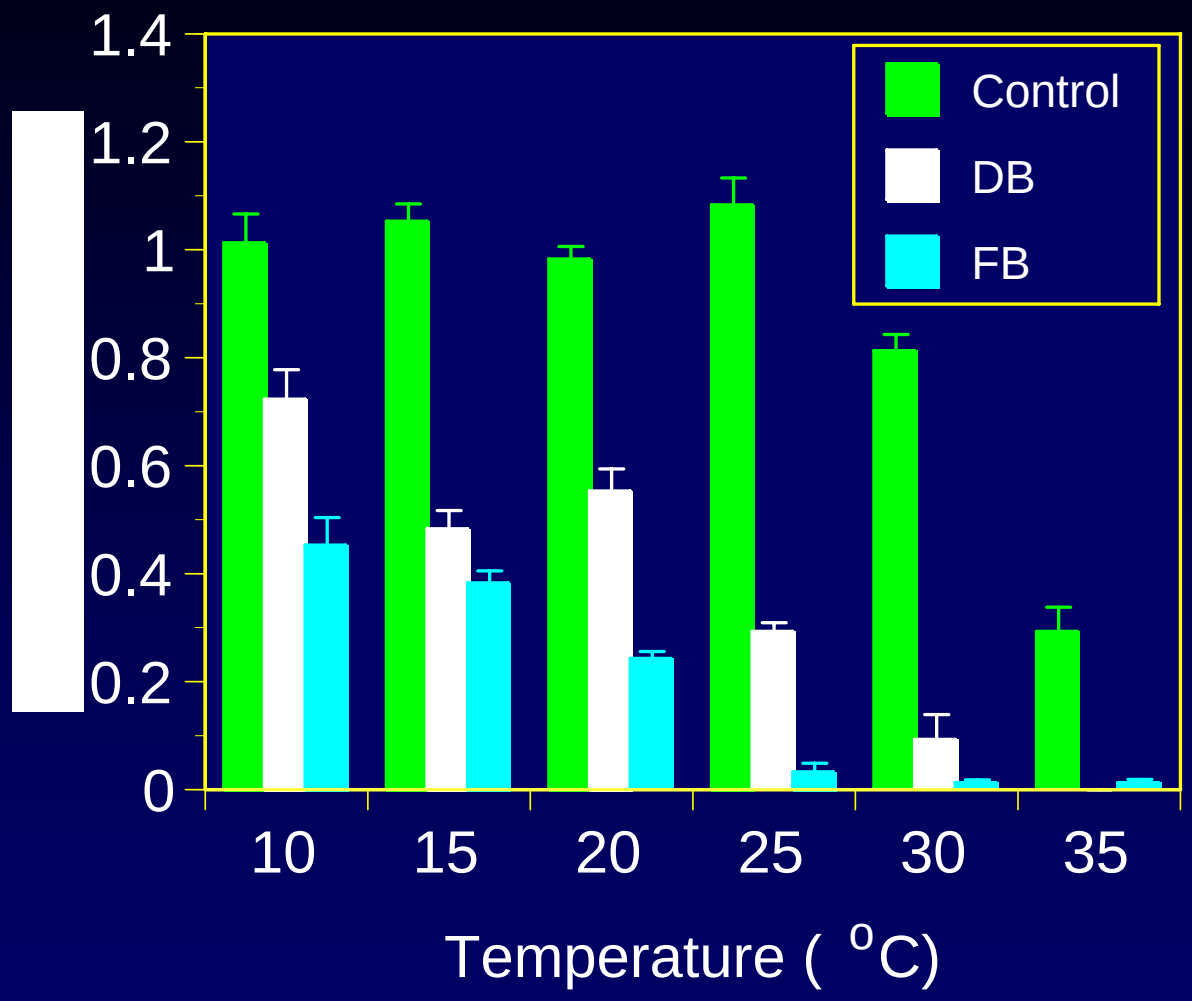
Isolate	Disease severity	Dry weight (g) Root	Shoot
Cauliflower	1	2.31	4.07
Chili pepper	0	2.57	3.90
Artichoke	1	2.28	3.58
Cabbage	1	2.18	4.34
Lettuce	0	2.52	4.02
Strawberry	0	2.41	3.60
Bell pepper	0	2.50	4.23
Eggplant	0	2.57	3.61
Tomato	1	2.14	4.06
Watermelon	0	1.90	3.75
Mint	1	2.29	4.22
Potato	1	2.79	3.62
Cotton	0	3.03	4.17
Alfalfa (*albo-atrum*)	0	2.61	3.97
Control	0	2.71	4.42
LSD ($P \leq 0.05$)		0.55	0.58
Cauliflower	5	1.80	2.25

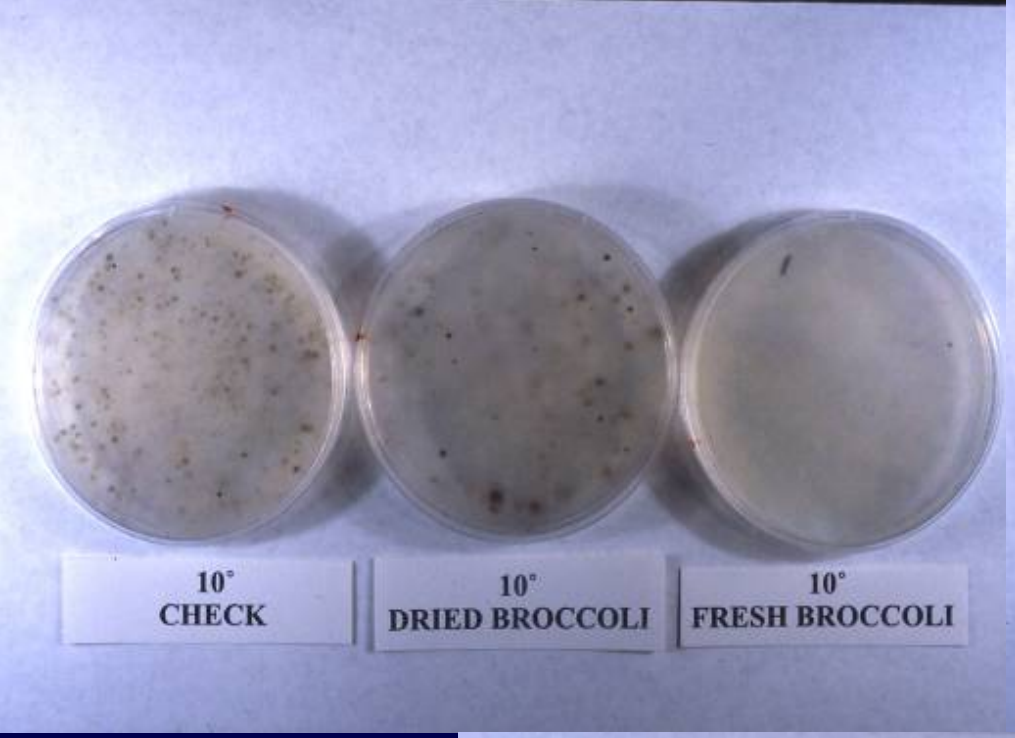
Questions

- When should the broccoli crop be planted and residue incorporated?
- Is there a specific temperature at which propagule reduction by broccoli is maximized?
- Is dry broccoli better than fresh broccoli?

Methods

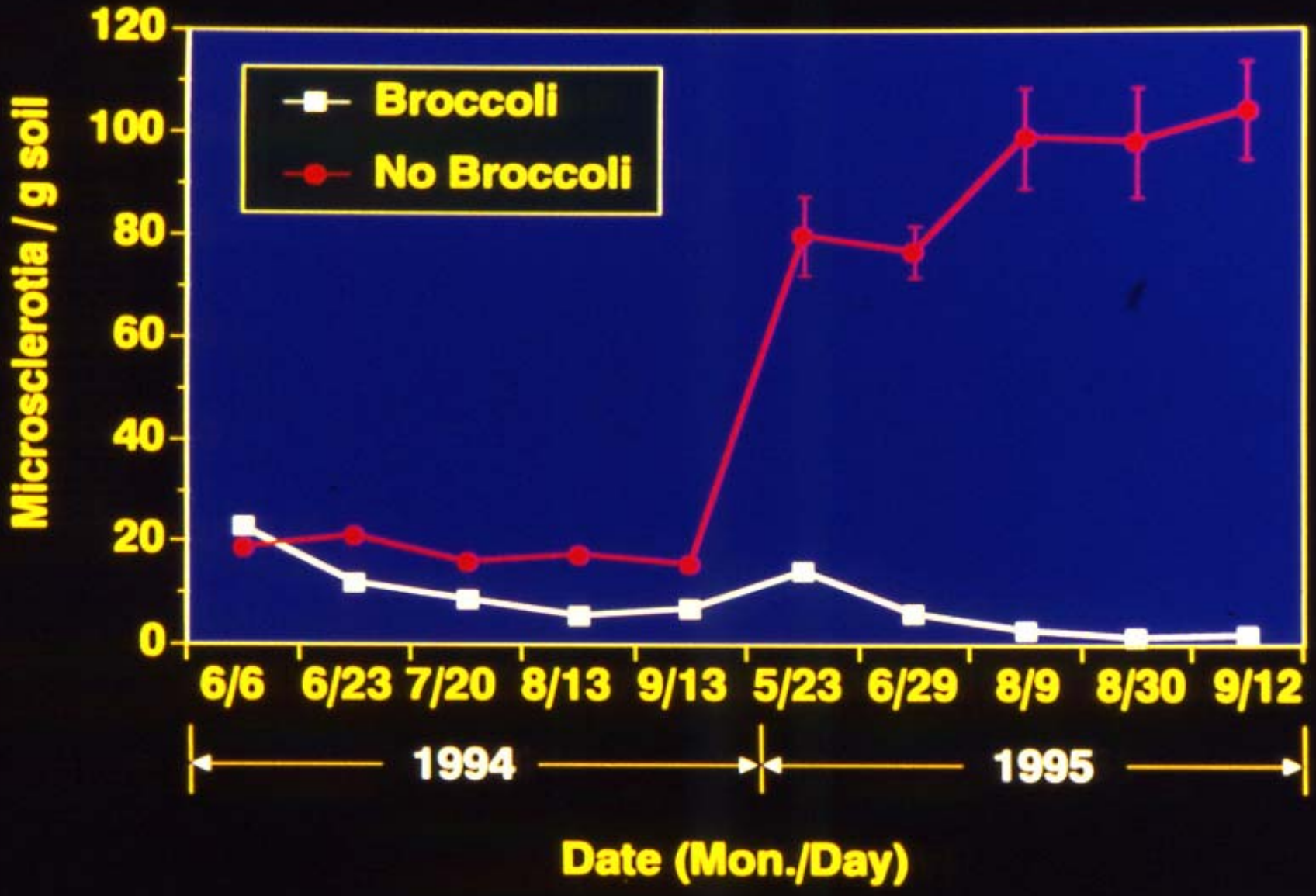
- Soil: Fields 1 & 2
- Treatments: Fresh (8%), dry, and no broccoli in 25 g dry soil.
- Incubation: 10, 15, 20, 25, 30, & 35 C
- Assay: After 45 days incubation using the Anderson sampler technique.
- In a parallel set of experiments, soil sampled after 15, 30, and 45 days incubation and assayed.


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
DB
FB
10
15
20
25
30
35
Temperature (°C)

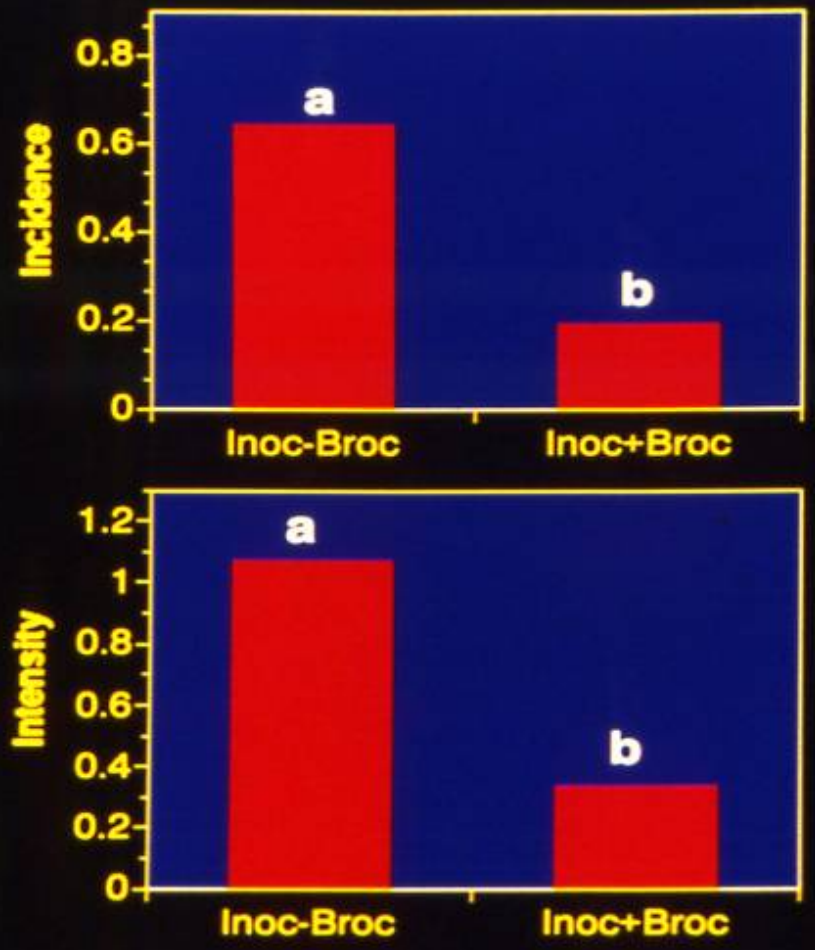

10°
CHECK
10°
DRIED BROCCOLI
10°
FRESH BROCCOLI


15°
CHECK
15°
DRIED BROCCOLI
15°
FRESH BROCCOLI


20°
CHECK
20°
DRIED BROCCOLI
20°
FRESH BROCCOLI


Broccoli
No Broccoli
Microsclerotia / g soil
120
100
80
60
40
20
0
6/6
6/23
7/20
8/13
9/13
5/23
6/29
8/9
8/30
9/12
1994
1995
Date (Mon./Day)

Formation of microsclerotia on roots



Are all broccoli varieties same?

Other Applications of this Research

- Efficacy of Broccoli - Strawberry Rotation
- Conventional versus organic production systems
- Reductions in *Sclerotinia minor* sclerotia and lettuce drop incidence.

LLLL

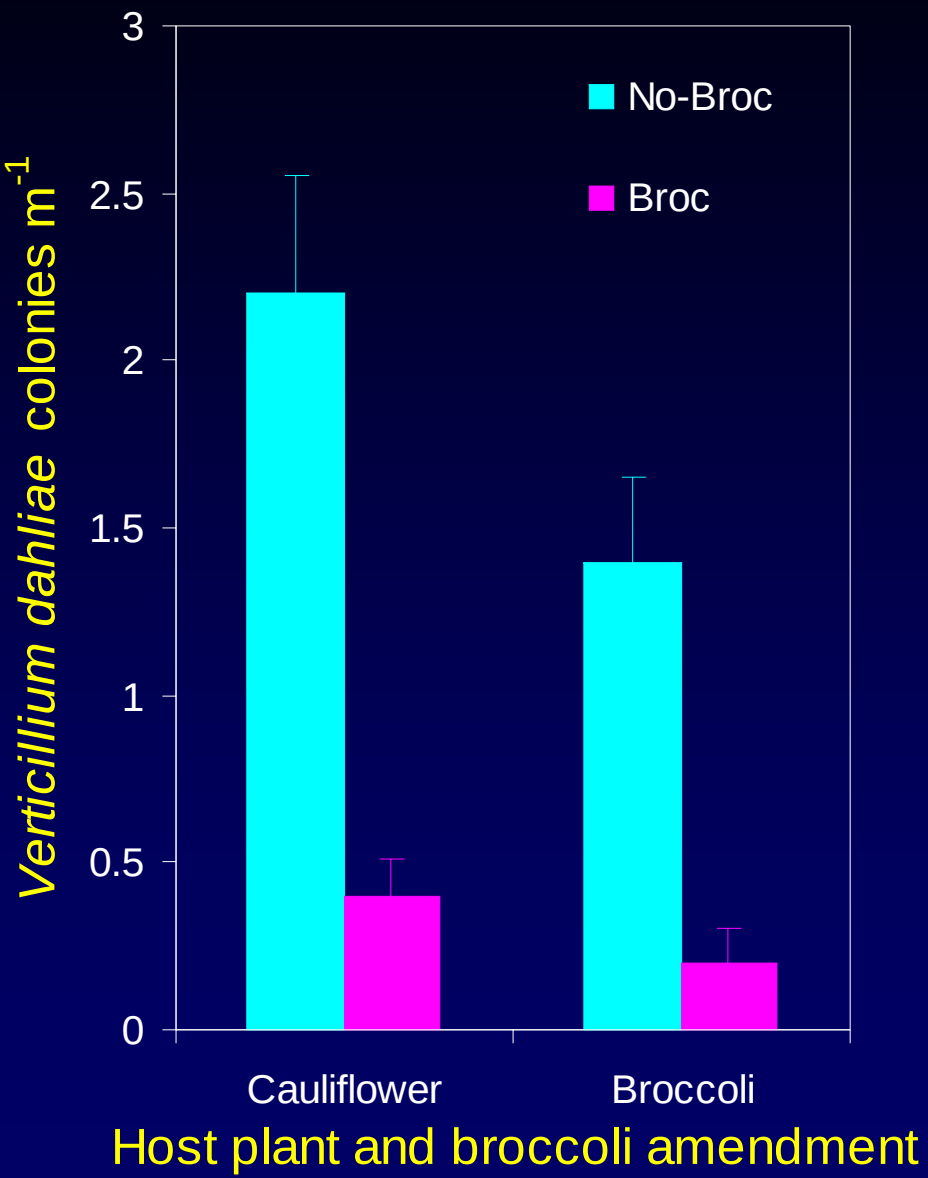
BLBL

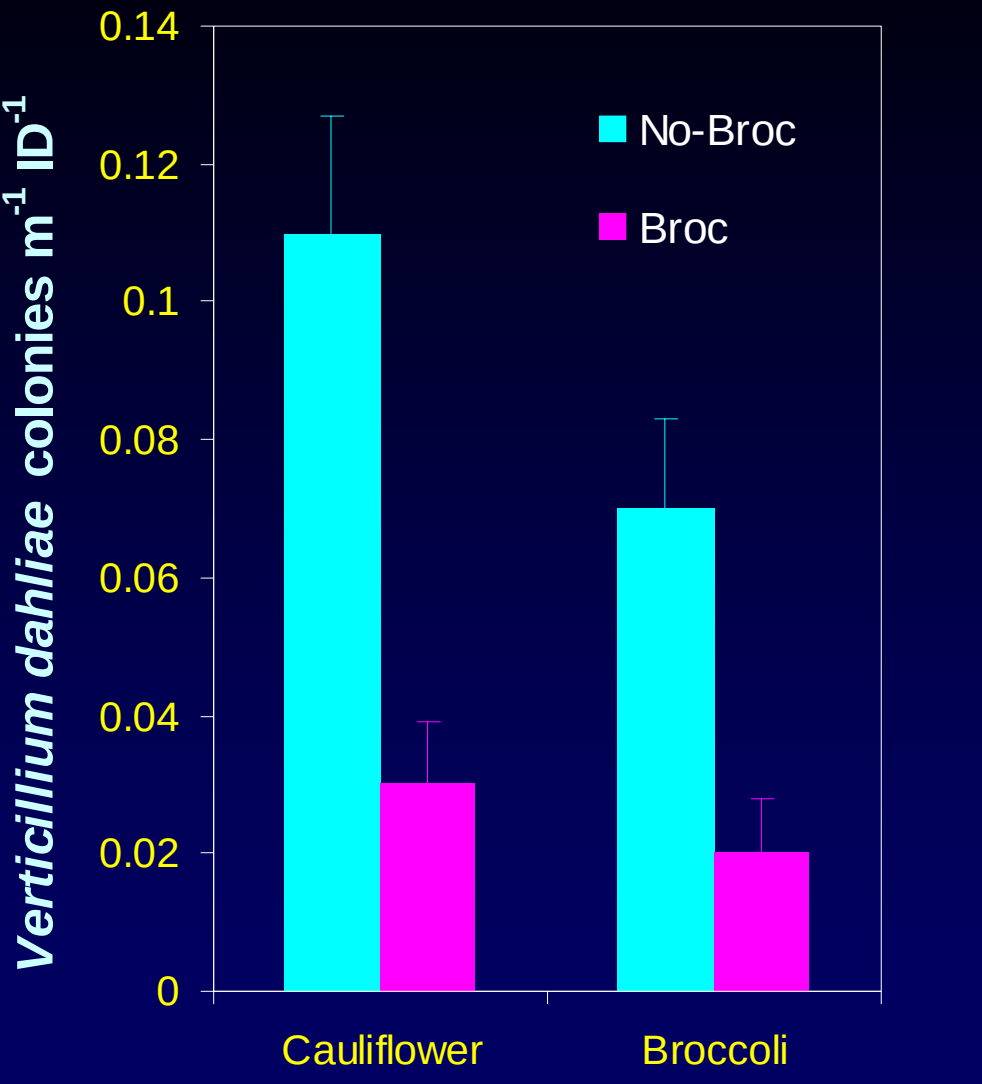
Potential Mechanisms

- Why is broccoli resistant to *V. dahliae* infection?
- How does broccoli result in attrition of propagules?
  - Chemical
  - Microbial
    - Specific to pigmented propagules?

Colonization of Root Cortex

- Broccoli and Cauliflower
- High and low inoculum density soils.
- Broccoli-amended and unamended.
- Periodic root sampling.
  - Immunohistochemical staining assay

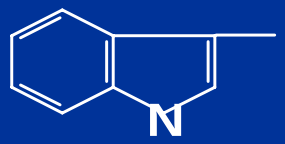

3
2.5
2
1.5
1
0.5
0
No-Broc
Broc
Verticillium dahliae colonies m-1
Cauliflower
Broccoli
Host plant and broccoli amendment


0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Verticillium dahliae colonies m-1 ID-1
No-Broc
Broc
Cauliflower
Broccoli
Host plant and broccoli amendment

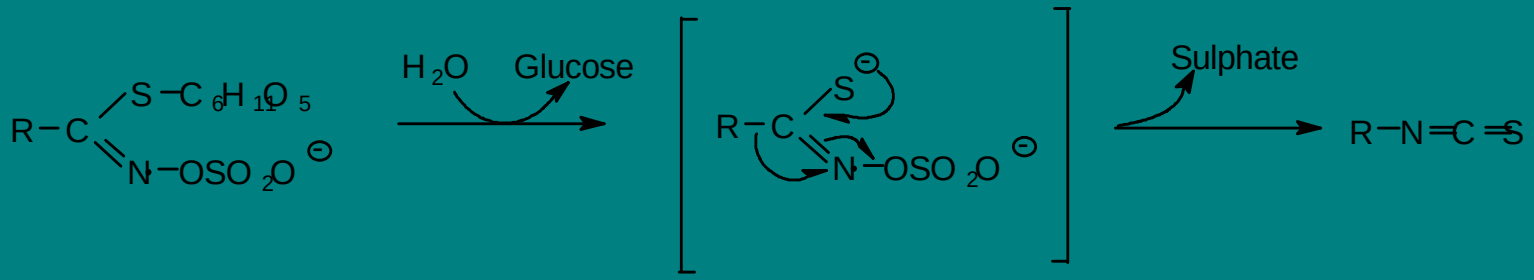
Broccoli

Cauliflower

Major Classes of Glucosinolates

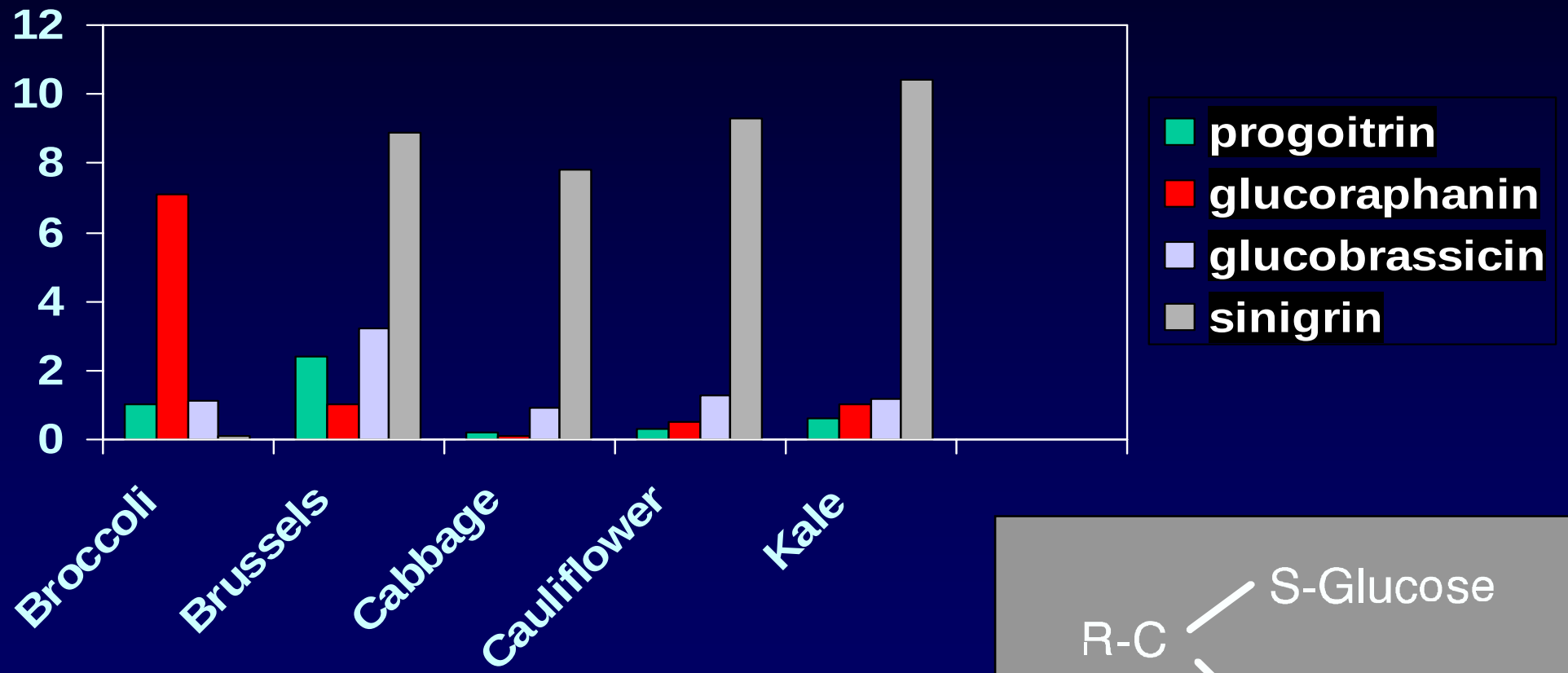
$CH_3 - (CH_2)_n - R$	**alkyl-**
$CH_2 = CH - (CH_2)_n - R$	**alkenyl-**
$CH_2 = CH - CHOH - (CH_2)_n - R$	**β-OH alkenyl-**
$CH_3 - SO_x - (CH_2)_n - R$	**methylthioalkyl-**
	**indolylmethyl-**
	**aryl-**
	**benzoyloxy-**

Glucosinolates breakdown to isothiocyanates through the action of **Myrosinase**



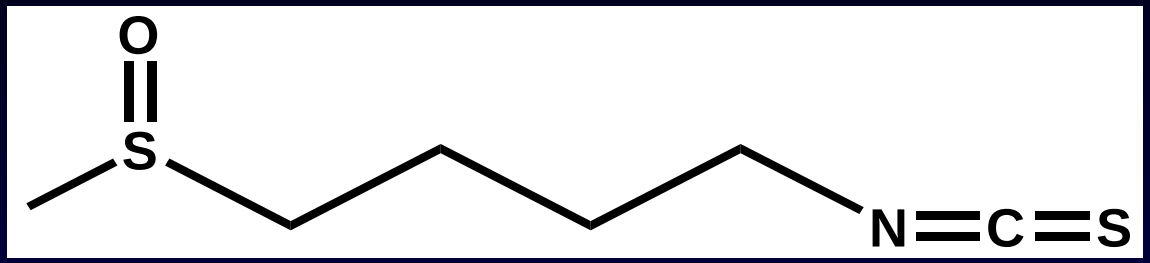
This can occur in the plant,
and possibly in an animal's mouth or gut.

Glucosinolate variation among Cruciferous vegetables



Jeffery, U. Illinois

Sulforaphane (SF)



- Anticarcinogenic hydrolysis product of *Glucoraphanin*
- Increases detoxification enzymes that remove carcinogens
- Potent upregulator of Quinone Reductase, in vitro

Summary of cover crop biomass and equivalent metam sodium content

Treatment	Biomass (T/A)		N (lb/A)		Metam sodium Eq (gal/A) – 2005
	2004	2005	2004	2005	
Broccoli	2.08	1.84	132.8	71.0	0.11
White Mustard	2.51	1.78	194.8	130.8	2.28
Indian Mustard	2.23	1.33	199.7	120.5	1.69

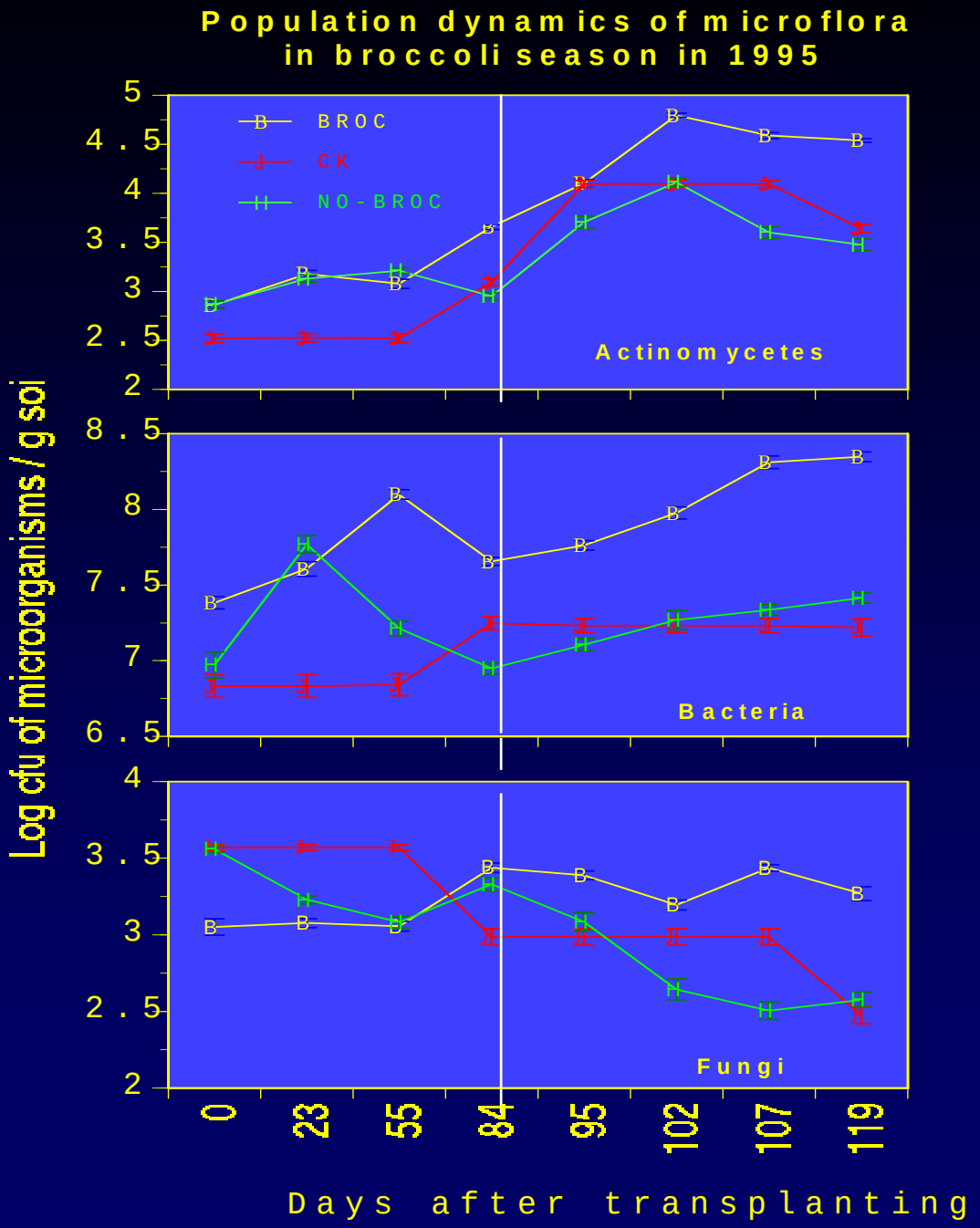
Labelled rates of metam sodium 30 -75 gal/A

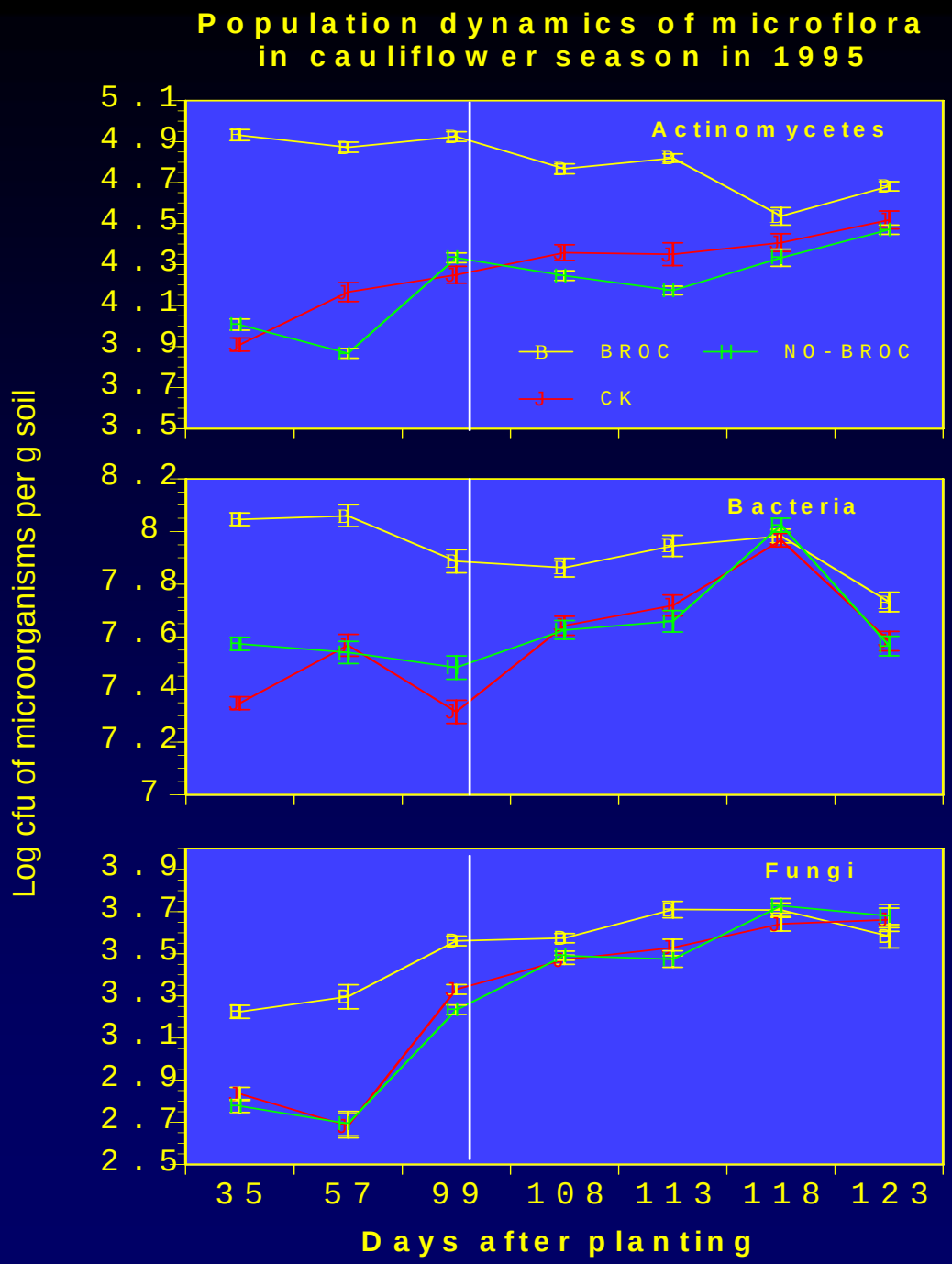
Soil Microbiological Changes

Significant differences between broccoli and cauliflower.

Broccoli residue incorporation results in 100-fold increases in bacteria and 1000-fold increase in actinomycetes.

Not only these changes are quantitative but also qualitative. The diversity of these groups is highest in broccoli-amended soils compared with cauliflower-residue amended soils.


Population dynamics of microflora
in broccoli season in 1995
BROC
CK
NO-BROC
Actinomycetes
Bacteria
Fungi
Log cfu of microorganisms / g soi
Days after transplanting
0
23
55
84
95
102
107
119


Population dynamics of microflora
in cauliflower season in 1995
Actinomycetes
Bacteria
Fungi
BROC
NO-BROC
CK
Log cfu of microorganisms per g soil
Days after planting
35
57
99
108
113
118
123

Bacterial diversity - Broccoli

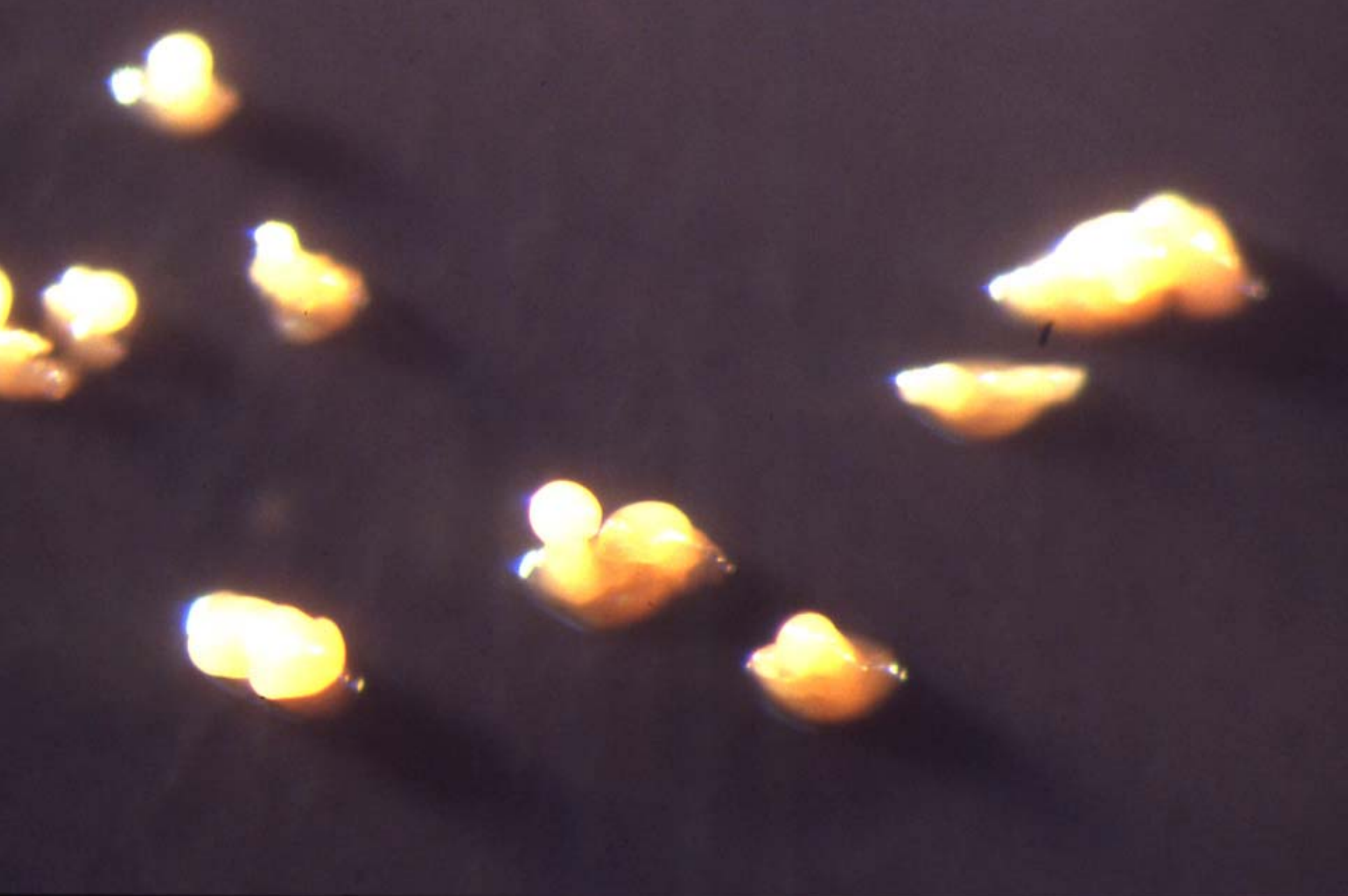
- *Arthrobacter saperdae*
- *A. barkeri*
- *A. oxydans*
- *A. ramosus*
- *A. viscosus*
- *Aureobacterium esteraromaticum*
- *Bacillus circulans*
- *B. atrocyaneus*
- *B. brevis*
- *B. lentus*
- *B. pantothenticus*
- *B. psychrophilus*
- *B. pumilus*
- *Micrococcus lylae*
- *M. kristinae*
- *M. roseus*
- *Pseudomonas florescens*
- *Stephylococcos epidermis*
- *S. cohnii*
- *Stenotrophomonas maltophilia*

Bacterial diversity – non-broccoli

- *Bacillus chlororaphis*
- *B. laterosprus*
- *B. linens*
- *B. psychrophilus*
- *Curtobacterium inologenes*
- *C. flaccumfaciens*
- *Micrococcus halobius*
- *Pseudomonas putida*

Myxobacteria

- Gram negative, gliding bacteria
- Produce swarms on nutrient poor media
- Produce characteristic fruiting bodies with dormant myxospores
- Lytic activity on microorganisms and nematodes



Myxobacteria

- *Myxococcus coralloides*
- *M. fulvus*
- *M. virescens*
- *M. xanthus* (2)
- *M. stipitatus*
- *M. flavascense*

Melanin

- Dark pigment molecule composed of polymers with a complex structure closely related to plant lignins
- Occurs in microorganisms, animals, protozoa and plants
- Melanin is closely associated with cell wall components

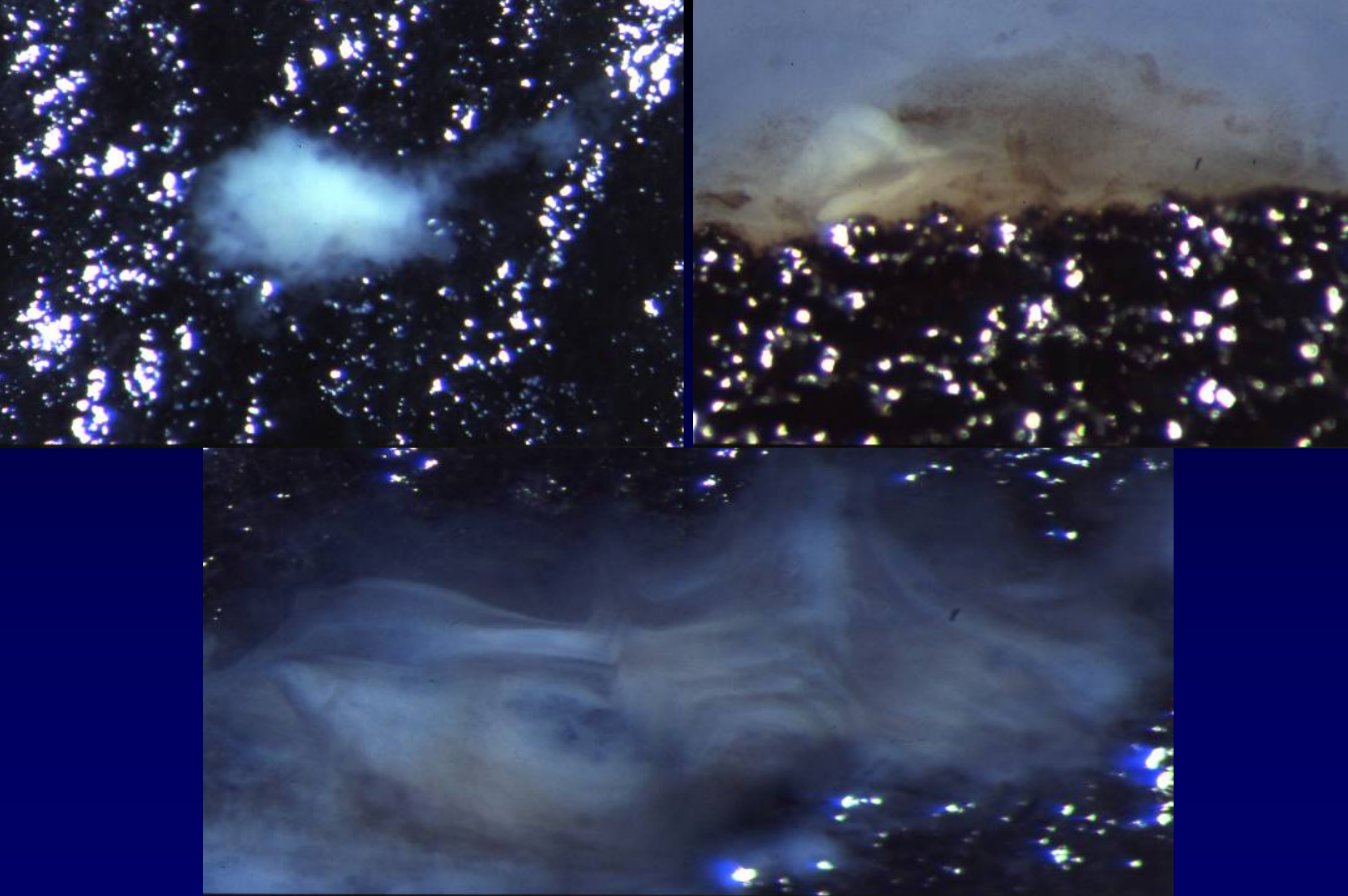
Benefits of Melanin

- Abiotic Stress

  UV radiation, temperature extremes, desiccation, free radicals, metal toxicity
- Biotic Stress

  Soil microbial/host plant origin hydrolytic enzymes and free radicals
- Pathogenicity

  Appressorial penetration, virulence factor?



Control plot (minus Broccoli)

Plot rotated with Broccoli

Research Team

- Chang-Lin Xiao
- Li Liu
- Ashley Bell
- Ravi Bhat
- Kateel Shetty
- Judy Hubbard
- Steve Koike
- Qingming Qin
- Gary Vallad

- Cauliflower growers
- USDA-NRICGP
- USDA-CSREES
- USDA-SARE
- CA-DPR
- CA Lettuce Research Board
- CA Strawberry Commission
- UCIPM

Transcriptional Regulation by the glucosinolate Hydrolysis Product sulforaphane (SF) :

Quinone Reductase

Glutathione S-transferase

Glucuronosyl transferase

Aldehyde dehydrogenase

ARE: 5'-TGACnnnGC-3'

Antioxidant Response Element