Microbial endophytes and TSNA accumulation: some preliminary results

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Introduction

TSNA accumulation affected by:

- nitrogen fertilization
- conversion
- topping
- spacing
- harvest maturity
- storage conditions
- curing environment

TSNA accumulation reduced by

- lower humidity
- lower temperature
- increased air movement
  - to detriment of quality
  - restrict activity of endophytes

Evidence for bacterial endophytes in TSNA accumulation:

- Antibiotics
  - no effect on nitrite or TSNA accumulation in whole leaf
  - did have effect in leaf slurries
- Bacteria isolated from surface sterilized leaf
- Surface sterilization before curing not successful

- Direct contact of leaves with soil at cutting made no difference to TSNA

- Potential nitrite scavengers tested with some success *
  - ongoing
  - ferulic acid use patented

- Nitrate reduction is anaerobic reaction
  - bacteria in decomposing cells in low oxygen conditions
- No TSNA in leaves from greenhouse grown plants
  - until sprayed with soil suspension
- Most bacteria isolated during curing also endophytes of wide range plants

Endophyte-mediated NNN Formation

Burley leaf

nitroso-nornicotine (NNN)

methyl group

$+ CH_2$

Conversion

$$NO_3^- + 2H^+ \longrightarrow NO_x + H_2O + O^-$$

Bacterial endophytes in tobacco

Conclusions vary by study*

- Predominant species
- Denitrifying ability of any one species

- 19 genera identified*
  - All ubiquitous
    - » contaminate any time in field or barn

Range of endophytes in other crops ….

Alabama ….

sweet corn

cotton

36 genera identified

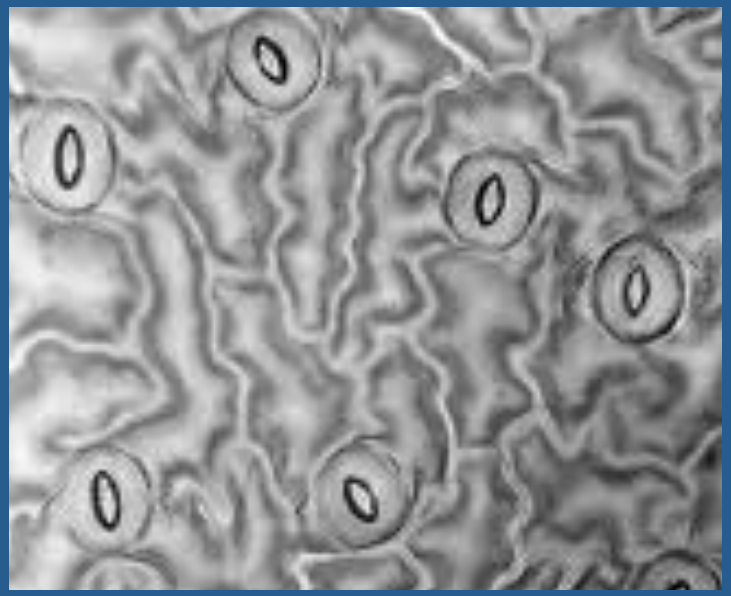
28 d after inoculation, endophytic bacteria found in:

- roots
- stems
- around stomata
- intercellular spaces
- xylem of leaves

grass and wheat
endophyte traced into:
roots
vascular tissue
intercellular spaces .....

..... of tobacco
in gnotobiotic conditions

… colonized
through
stomata



…… are colonized through seed

Endophytes introduced

more successfully into ..

……. by soaking seed before sowing

than by drenching soil media

…. combination of species or strains affects extent of colonization

Endophyte populations change through growing season …..

Differences in endophyte populations between sites (local, regional or continental) .....

Difference in endophyte populations between varieties …….

Possible to manipulate endophyte populations in tobacco?

= Biological control of TSNA ?

- bacteria isolated from curing leaf screened for ability to denitrify or metabolize TSNAs*
- application of bacteria to root zone 15 days after transplanting reduced TSNA in cured leaf
  – by up to 81%*

Therefore, for tobacco:

- When is plant colonized?
- What is inoculum source?
- Does spectrum vary between:
  - geographic areas
  - varieties
  - cropping practices
- What species responsible for nitrate reduction during curing?

Objective:

Test the effect on TSNAs
of applying a suspension of field soil
to greenhouse-grown plants

Premise:

Previous work - no TSNA accumulated in glasshouse-grown plants until sprayed with suspension of field soil

Method:

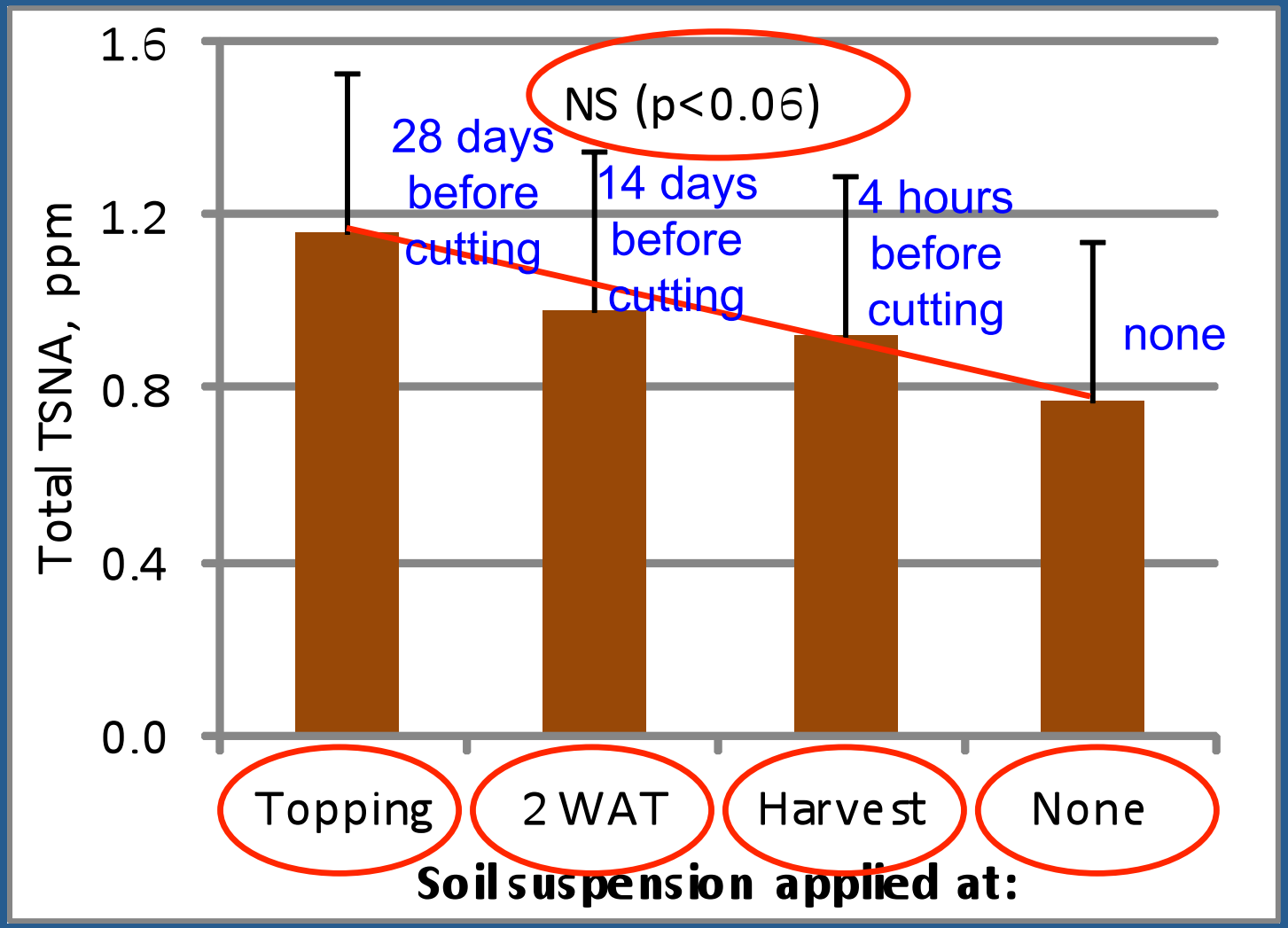
- Plants grown in pots with soilless media in glasshouse on campus far removed from field
- Soil suspension applied at
  - topping
  - 14 days after topping
  - day of harvest
- One set of each treatment cured in
  - glasshouse on campus
  - barn with other tobacco

Cured leaf samples:

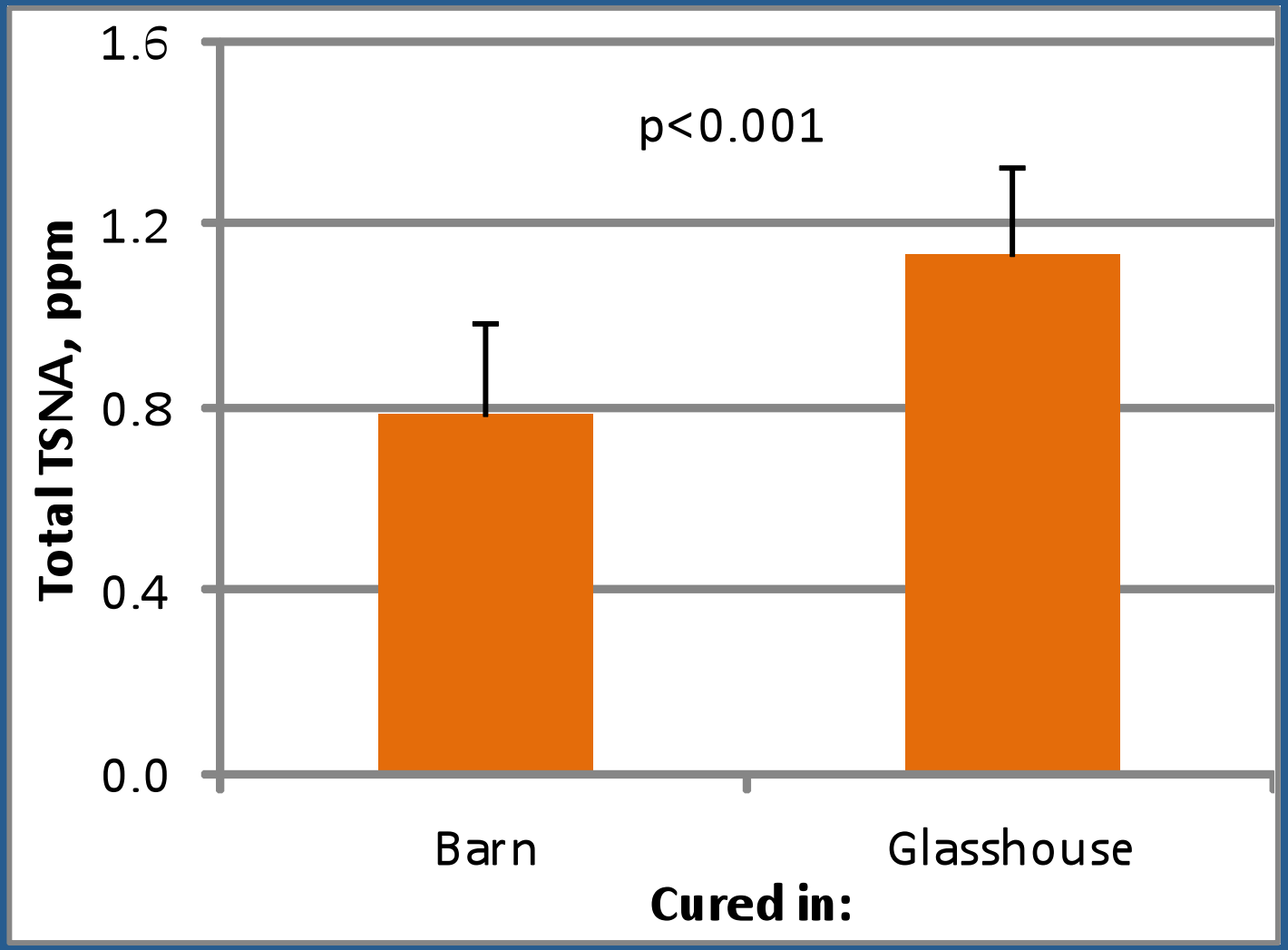
- Each leaf split in half along midrib
- Freeze dried
- One set each for
  - TSNA
  - microbial metagenomic analysis
    - nitrate reductase probe

RESULTS

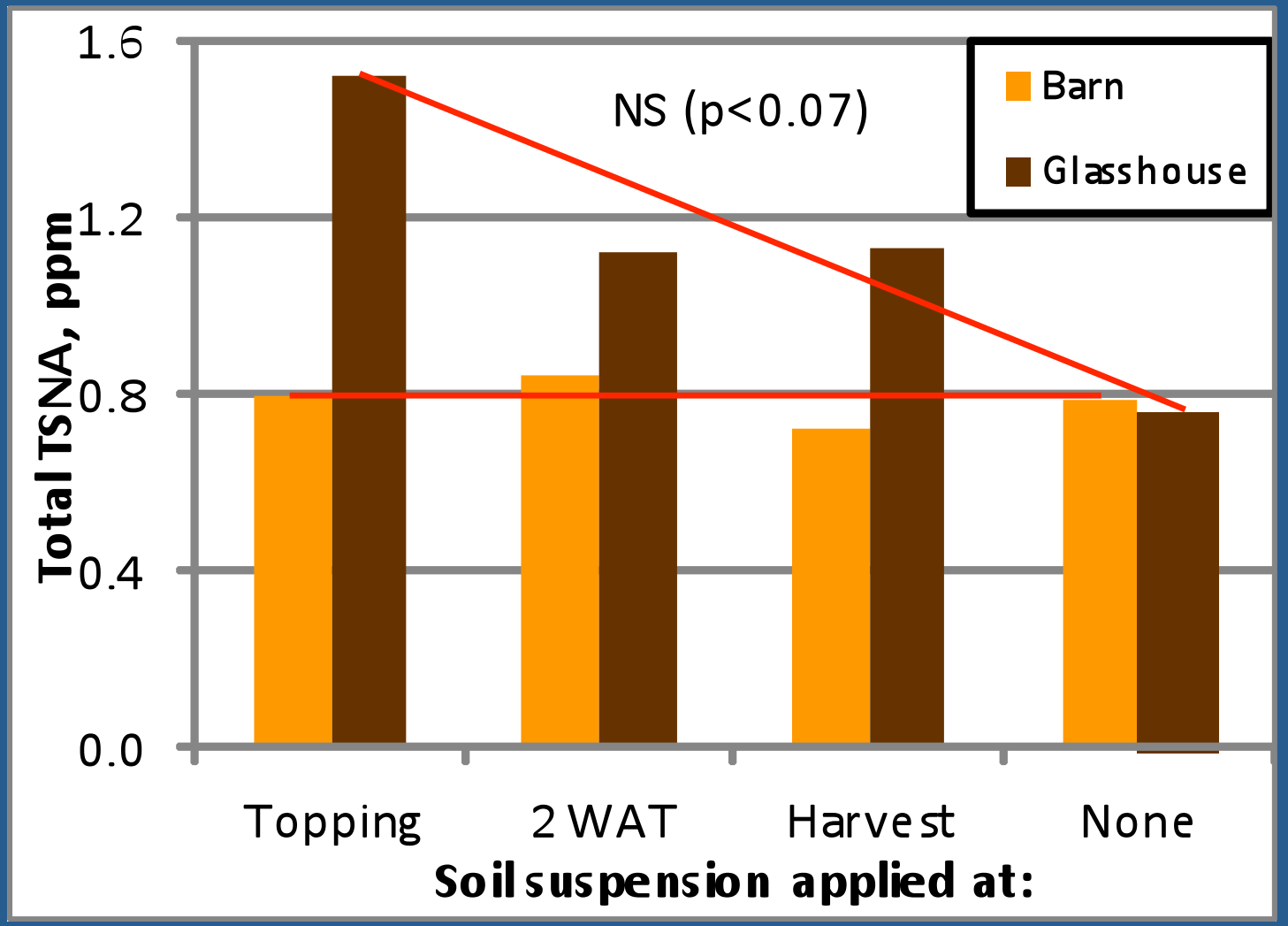
Total TSNA (barn + glasshouse)



Total TSNA, all applications



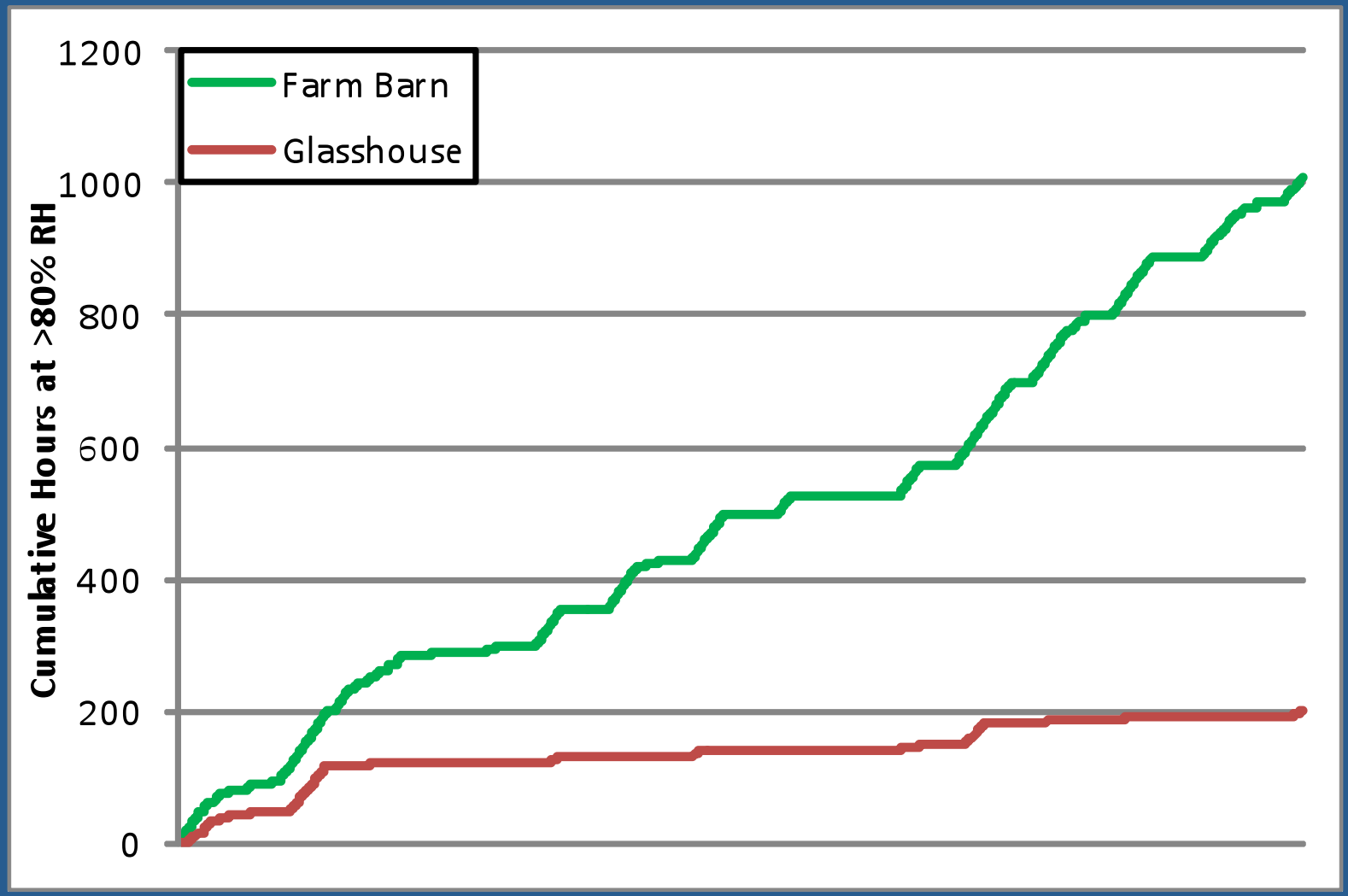
Total TSNA



Curing Conditions – Temperature

Cumulative hours >70°F

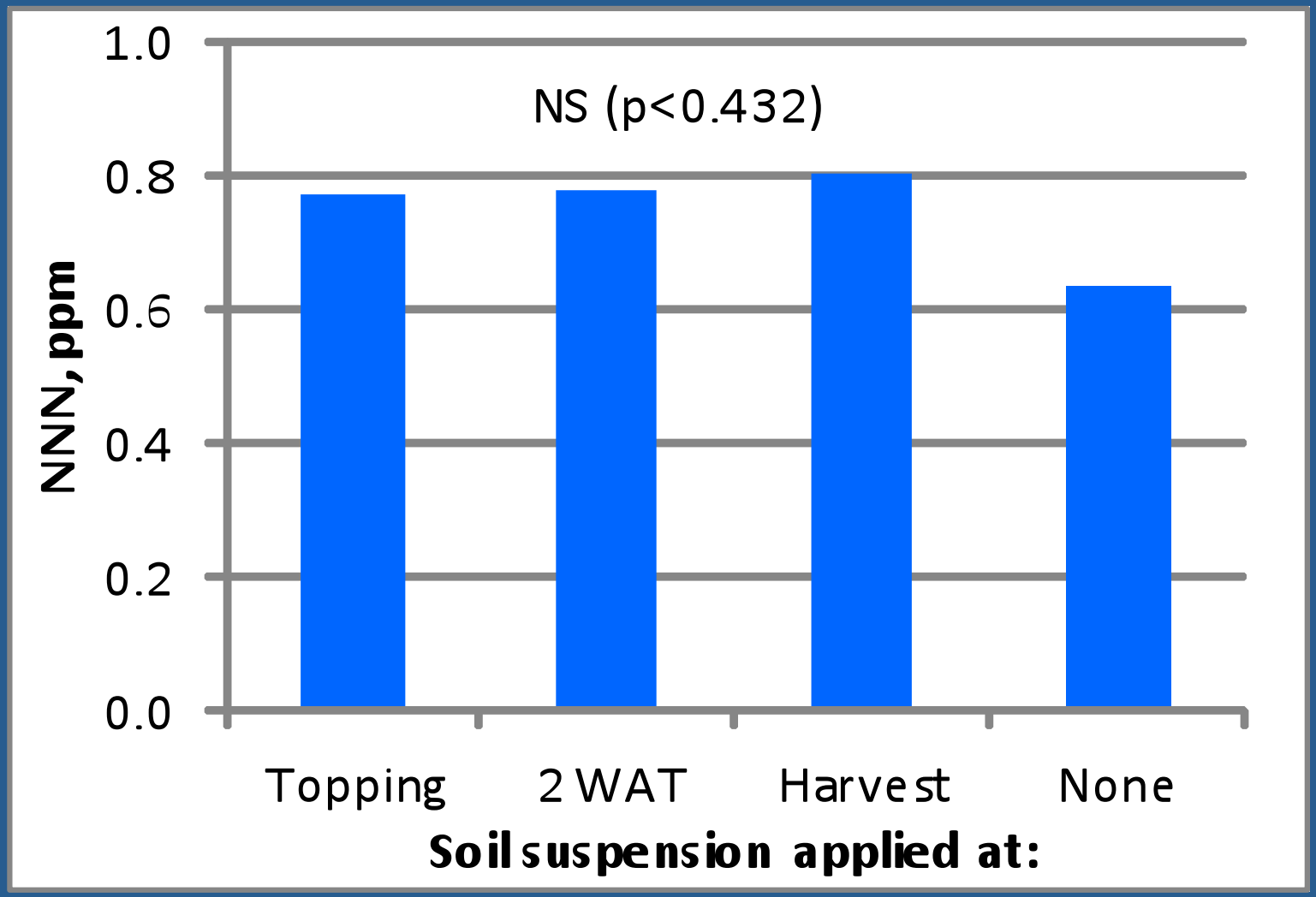
Curing Conditions – Humidity
Cumulative hours >80%



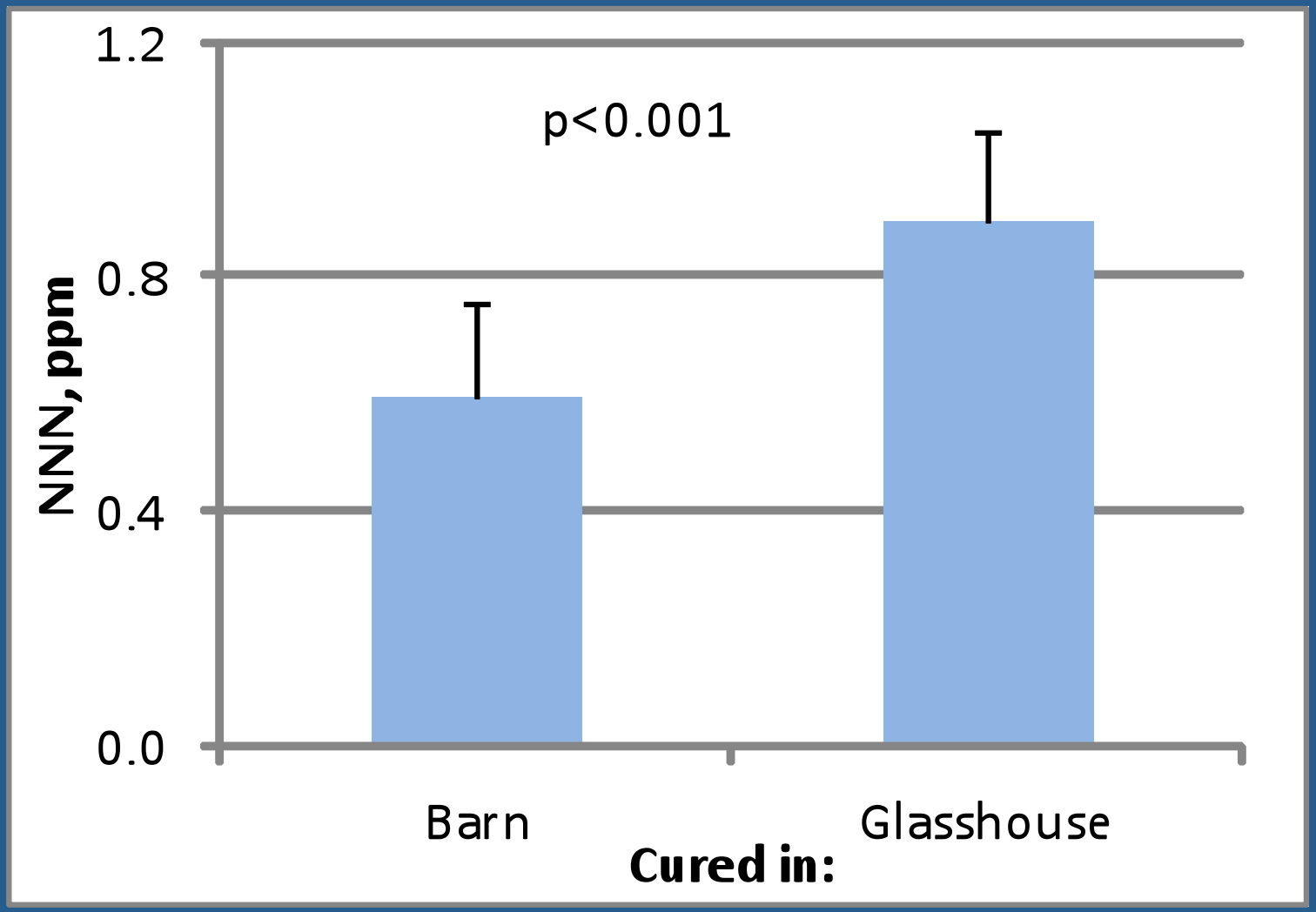
Arithmetic summary of curing conditions

% of curing time at:	Cure location	
	Glasshouse	Farm Barn
Temp >70F	86	5
RH > 80%	8	40

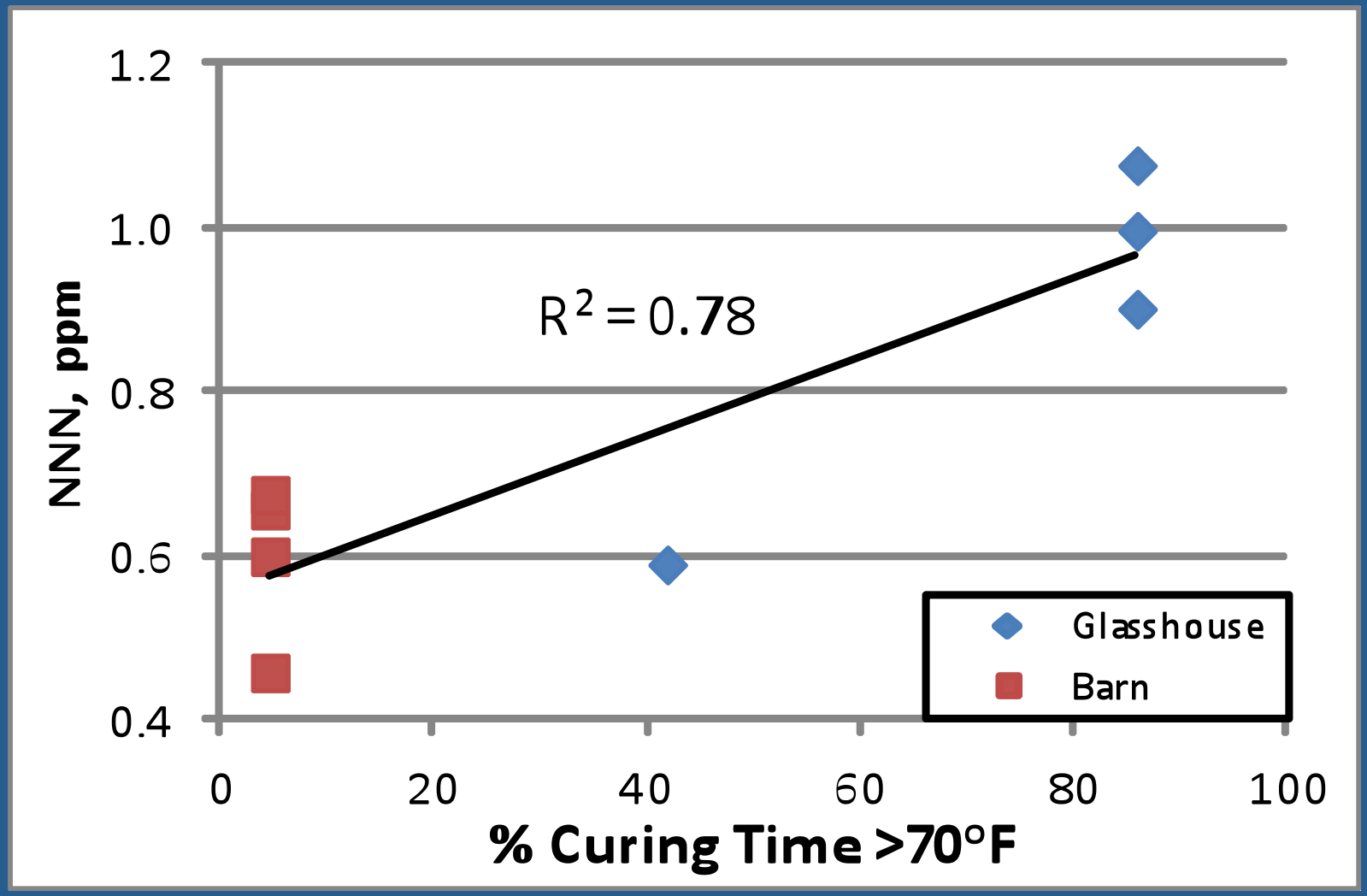
NNN, barn + glasshouse



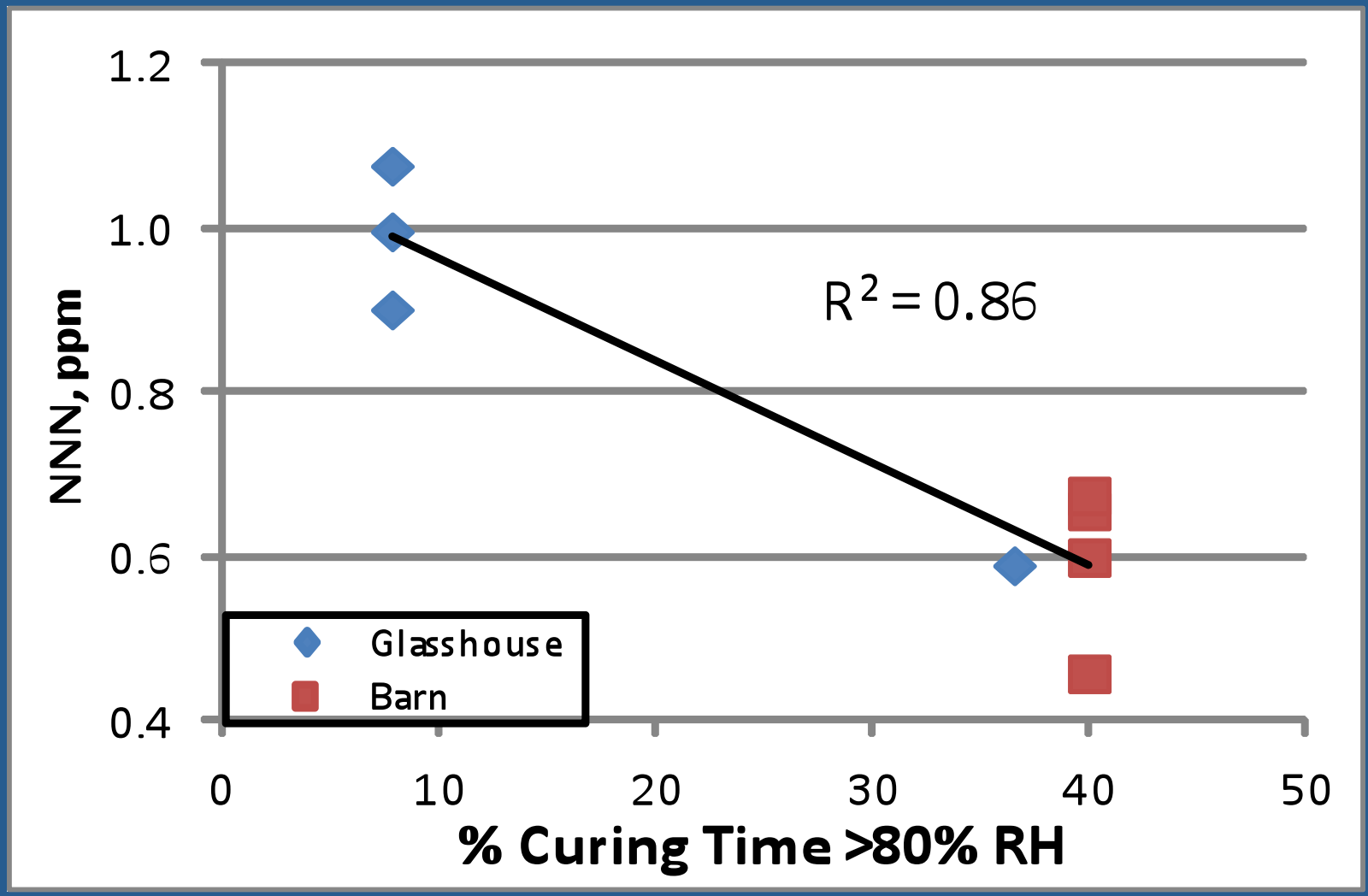
NNN, all applications



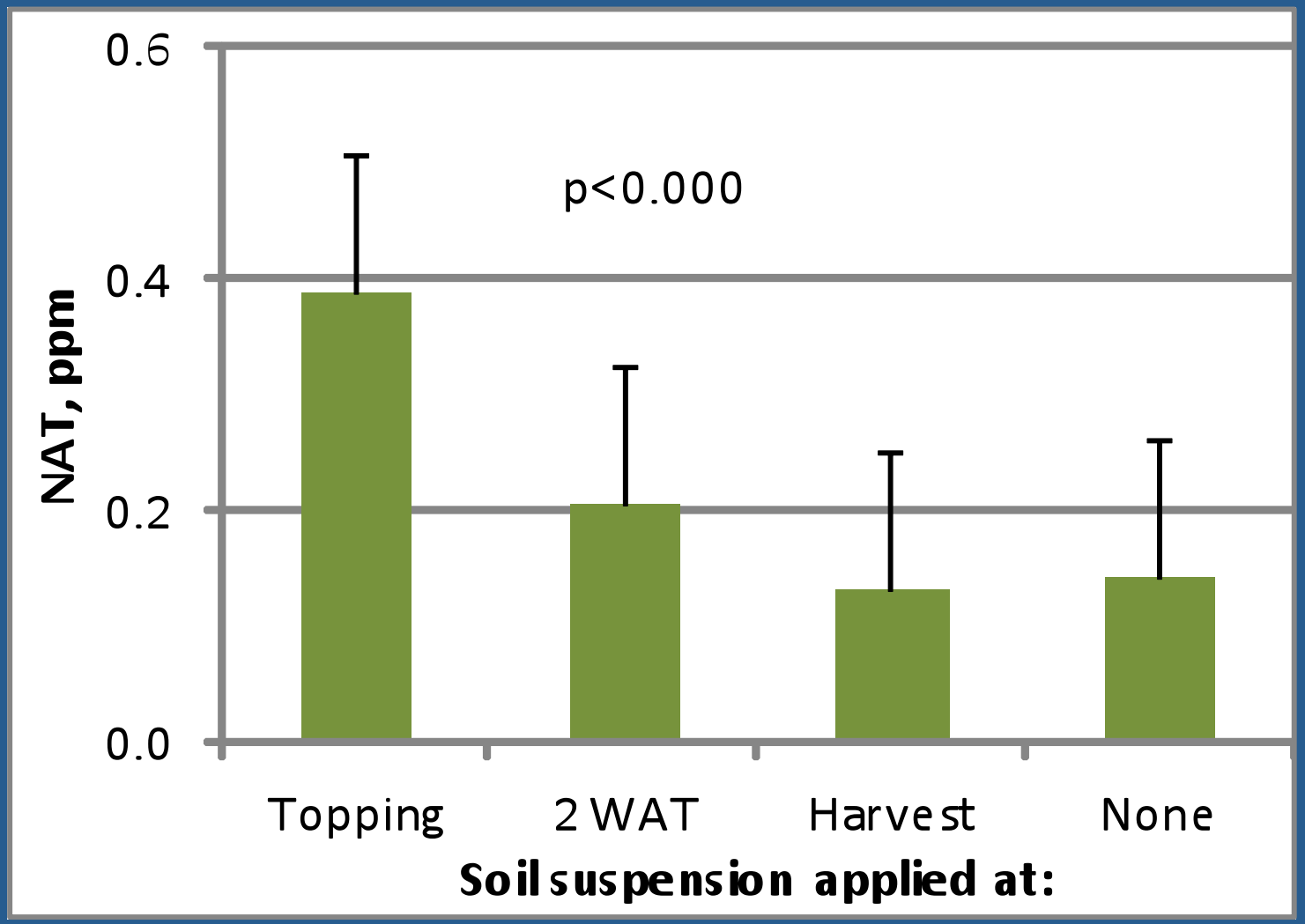
Curing temperature and NNN



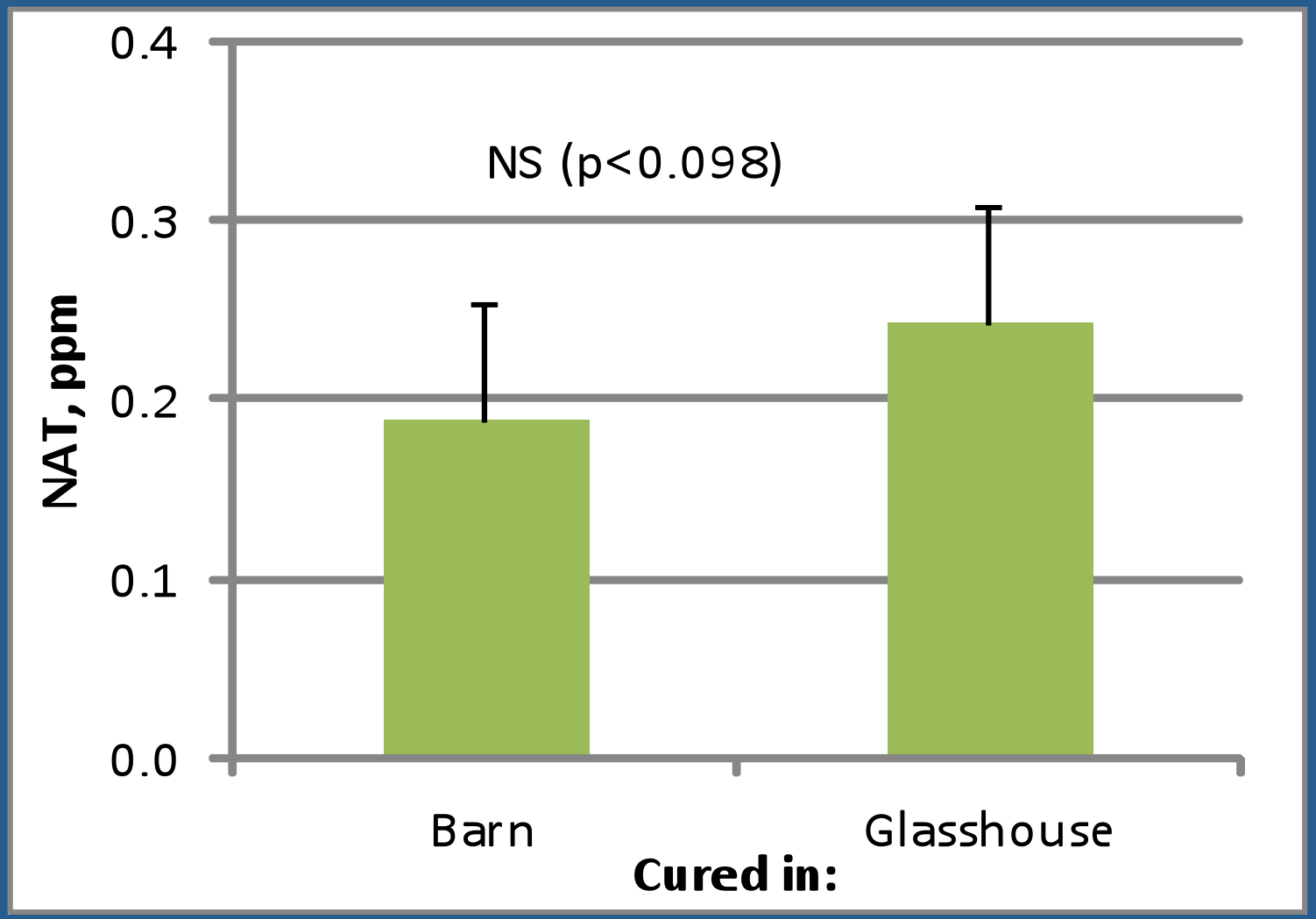
Curing humidity and NNN



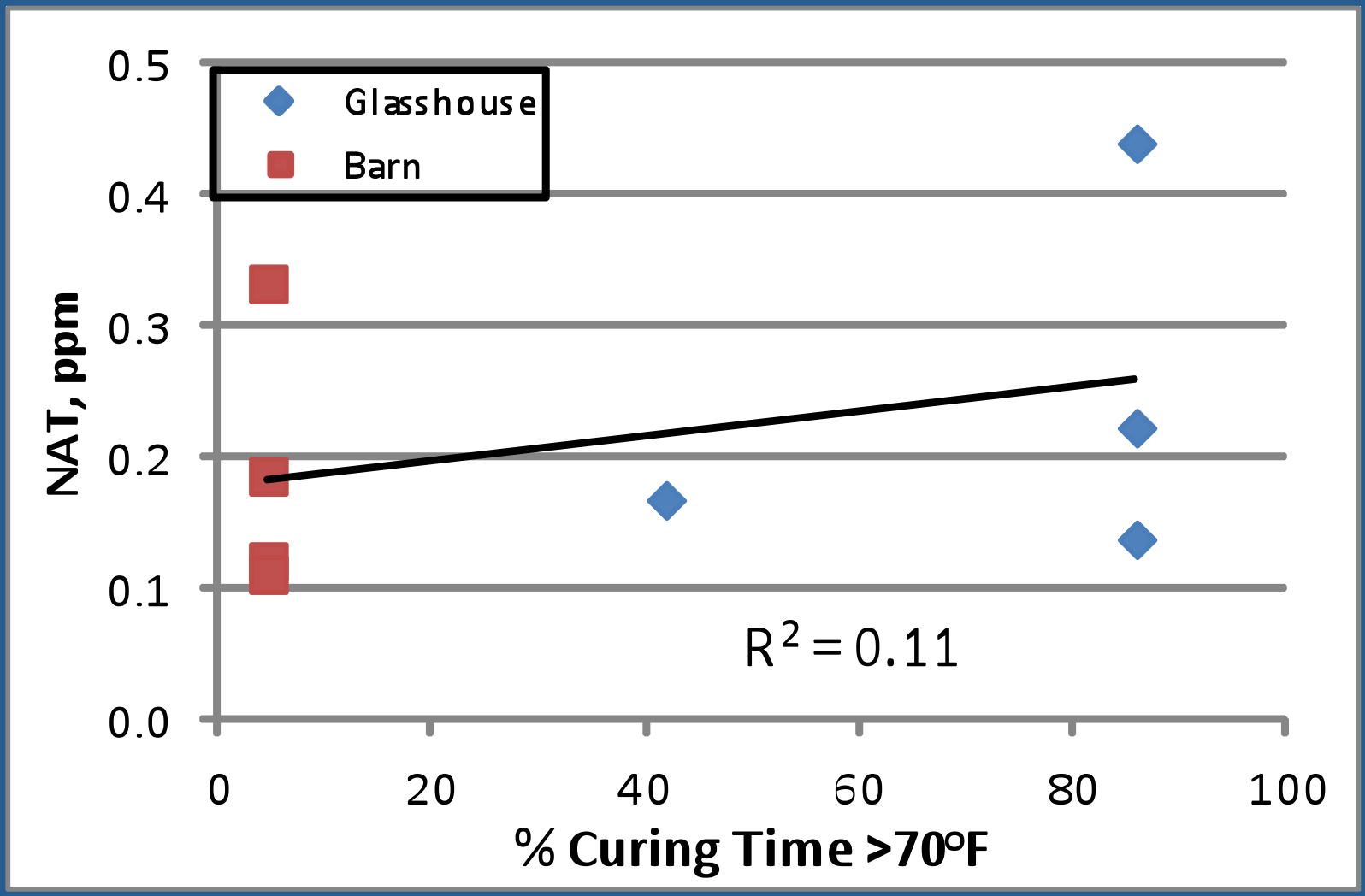
NAT, barn + glasshouse



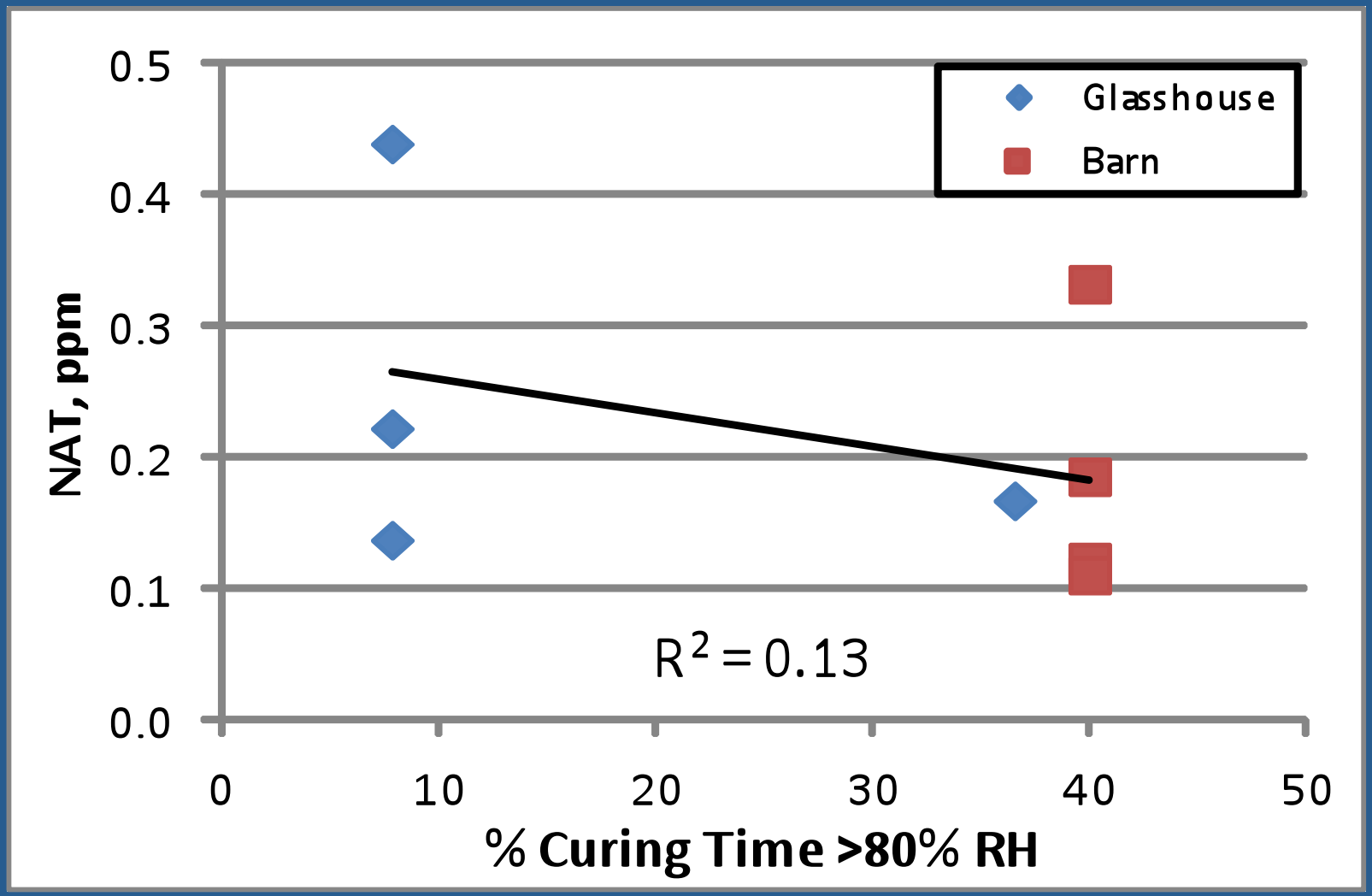
NAT, all applications



Curing temperature and NAT



Curing humidity and NAT



Discussion

- Plants not typical of field-grown plants
  - 4 months to lose moisture and brown
  - reason for TSNA in unsprayed check?
- Metagenomic analysis of bacterial populations in due course

Discussion

Effect of curing environment:

- In this test, NNN increases as temperature increases and/or as humidity decreases
  - further investigation
- NAT is not affected by temperature and/or humidity, or at most not to same extent

Does this explain why NAT concentration in some samples** is occasionally a much greater proportion of total TSNA than in most other samples?

Conclusions

Time of application of soil suspension to greenhouse-grown plants:

- Total TSNA increases with the length of time between application of soil suspension and harvest
  - increasing NAT component
  - not NNN component

Conclusions

Perhaps a differential effect of

temperature

and / or

humidity

on NNN and NAT

2012 - Curing cabinet study of temp /RH / air flow

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