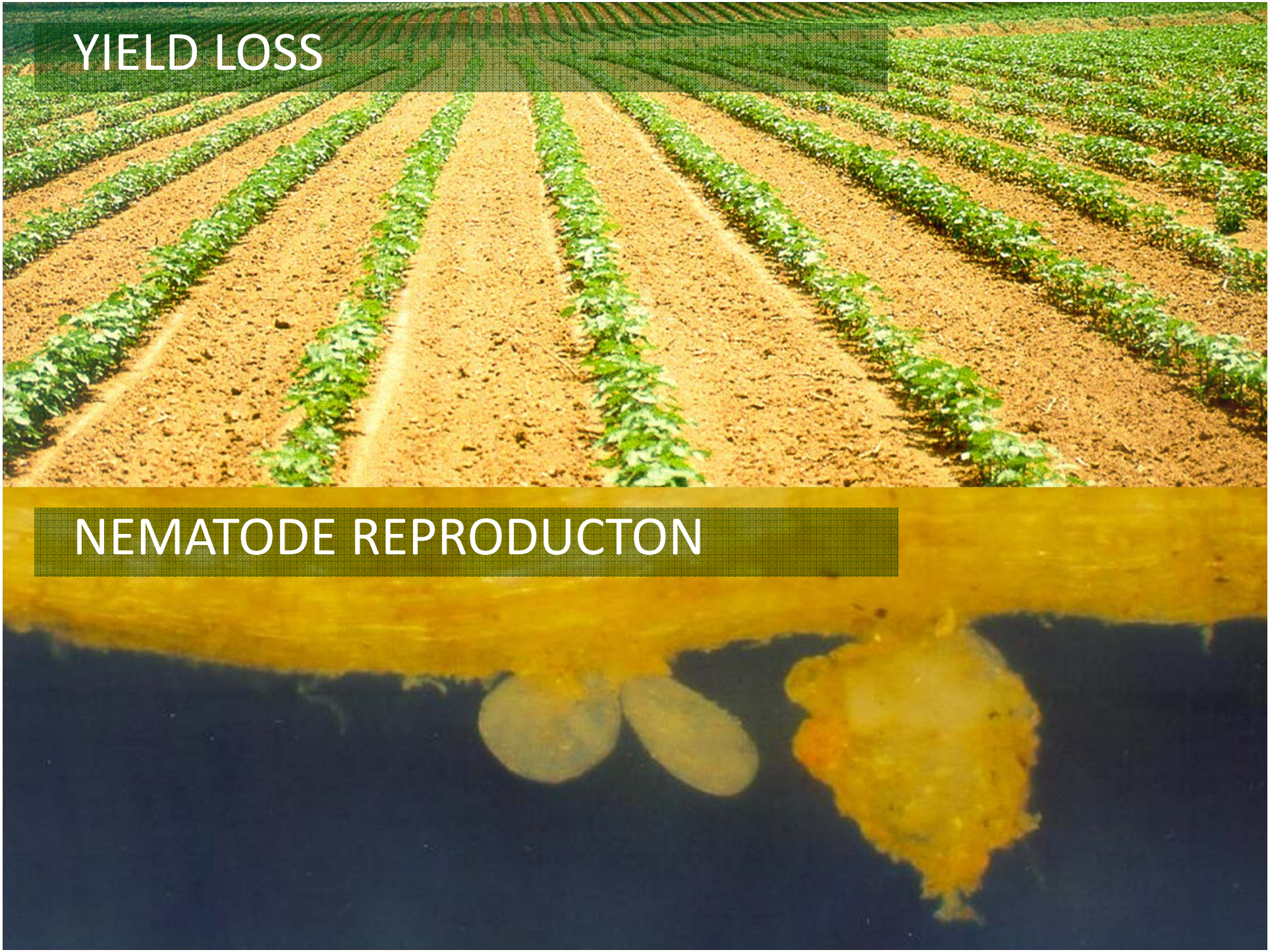
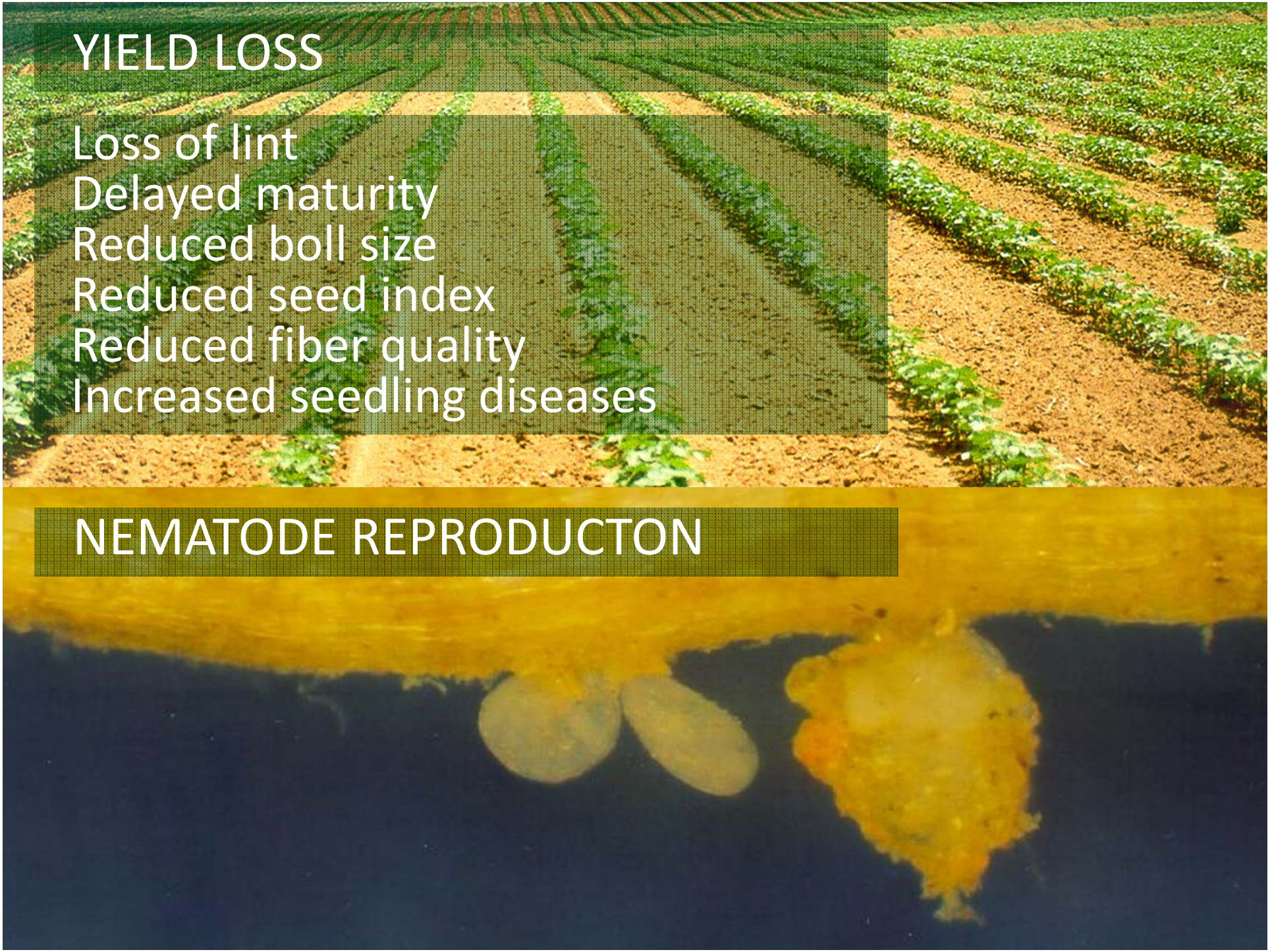
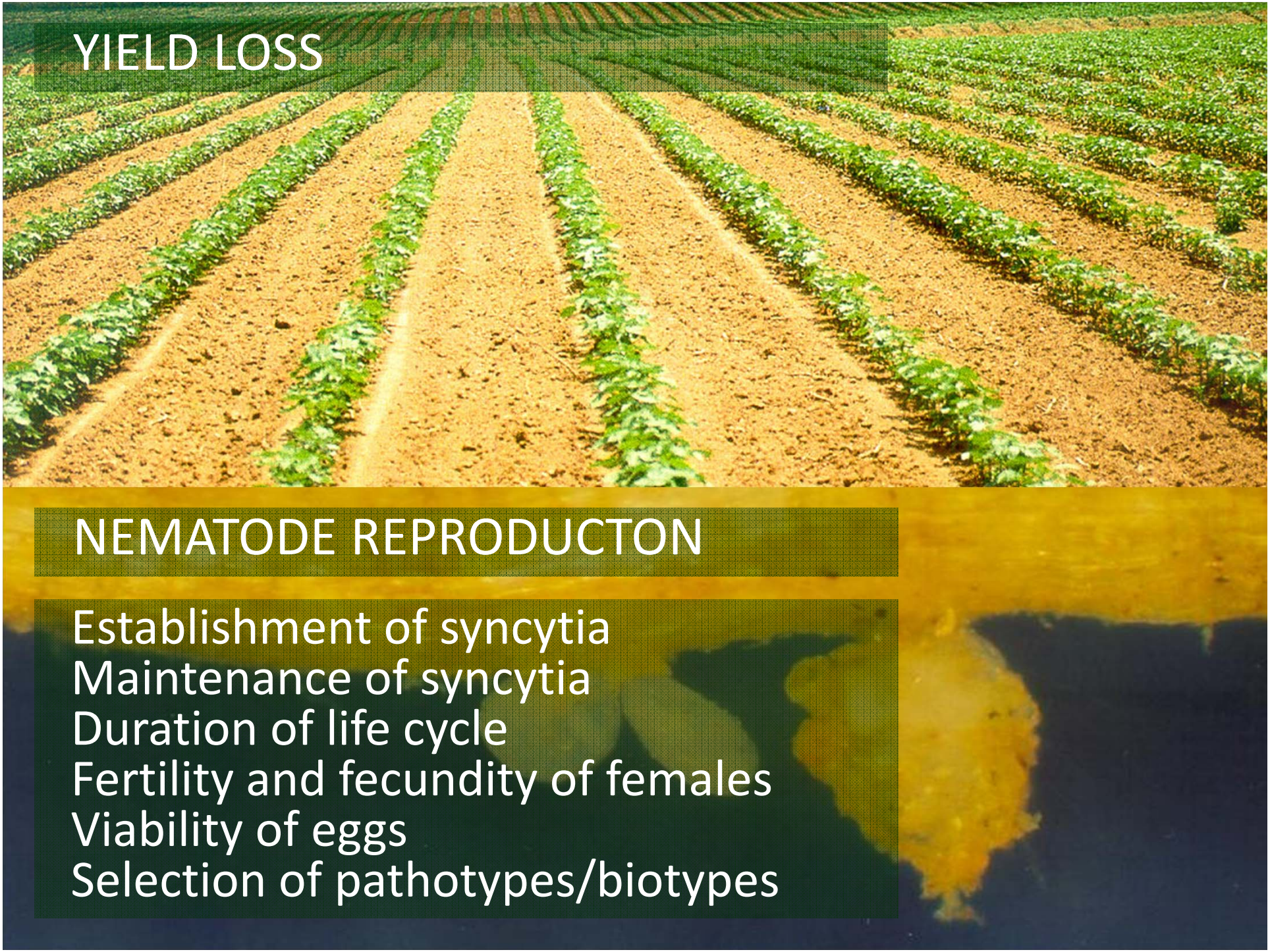
Characterization of Reniform Nematode Resistance in Cotton

Paula Agudelo
School of Agricultural, Forest, and Environmental Sciences
Clemson University


YIELD LOSS
NEMATODE REPRODUCTON

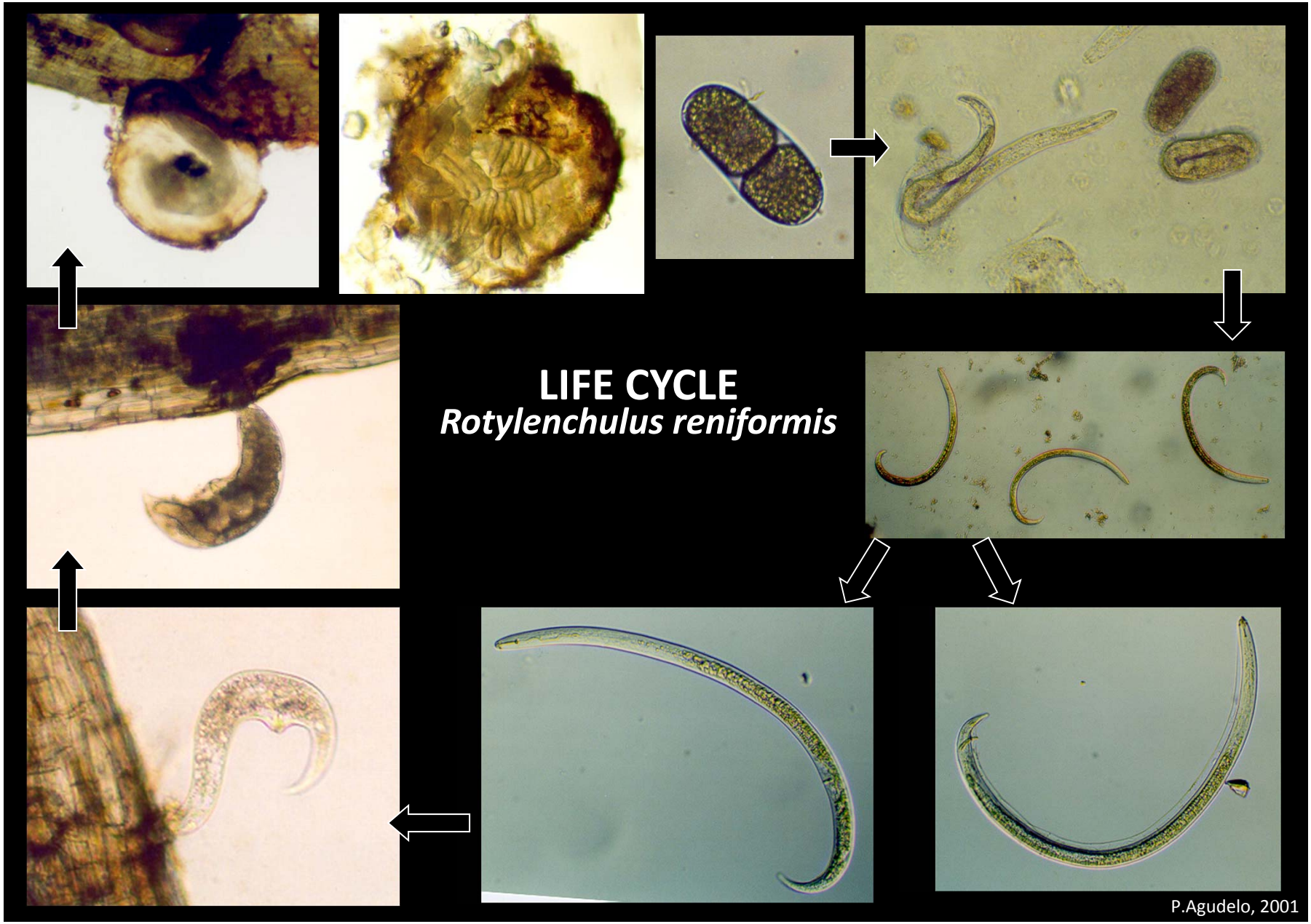

YIELD LOSS
Loss of lint
Delayed maturity
Reduced boll size
Reduced seed index
Reduced fiber quality
Increased seedling diseases
NEMATODE REPRODUCTON



YIELD LOSS

NEMATODE REPRODUCTON

Establishment of syncytia
Maintenance of syncytia
Duration of life cycle
Fertility and fecundity of females
Viability of eggs
Selection of pathotypes/biotypes


LIFE CYCLE
Rotylenchulus reniformis
P.Agudelo, 2001

REPRODUCTION

Pi: 3,000 nematodes/500 cc

Pf: 60 days after inoculation

Reproductive factor = Pf/Pi

Cotton cv. DP50

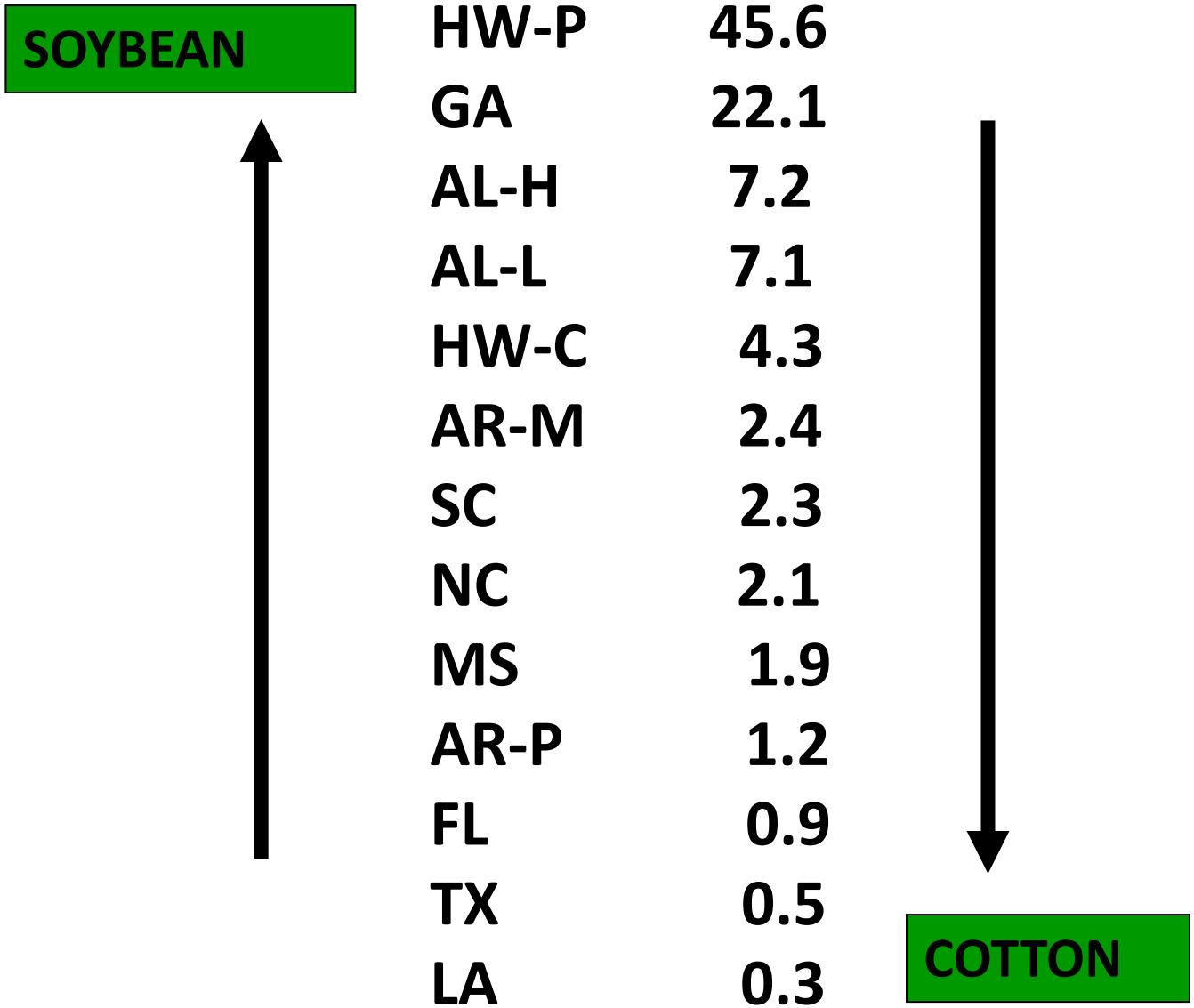
	MEAN Rf
TX	55.6
AR-P	33.5
LA	18.5
MS	14.9
FL	13.5
AR-M	9.4
AL-L	8.7
SC	5.6
NC	4.1
GA	1.2
HW-C	0.8
AL-H	0.4
HW-P	0.3

Soybean cv. Braxton

	MEAN Rf
AL-L	62.1
AR-P	38.5
TX	30.3
MS	29.3
GA	26.7
AR-M	22.0
FL	13.1
SC	12.8
HW-P	11.4
NC	8.7
LA	6.1
HW-C	3.5
AL-H	2.8

Rf Soybean cv. Braxton: Rf Cotton cv. DP50

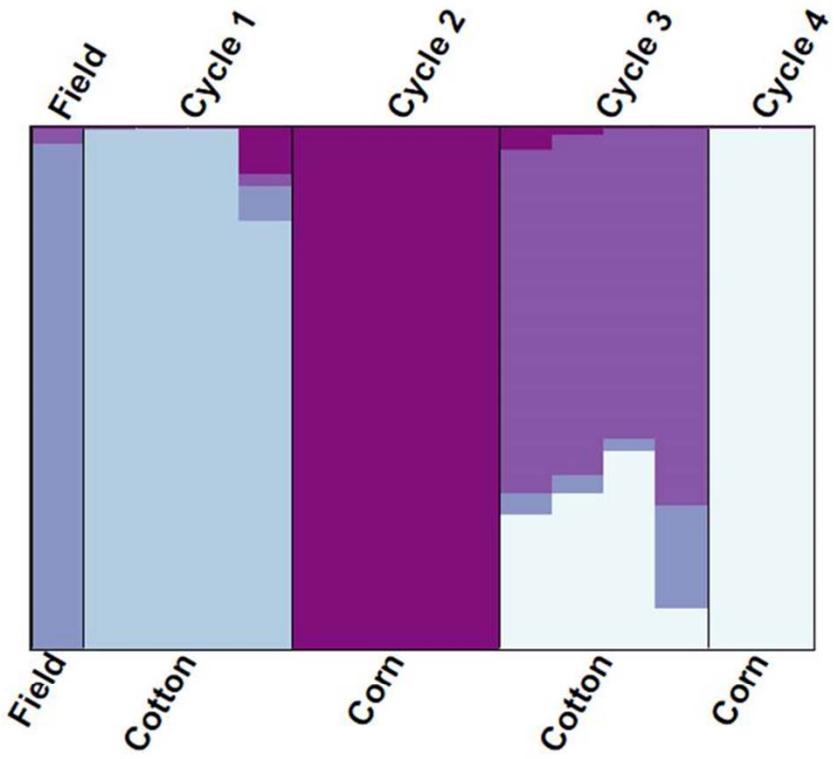
MEAN REPRODUCTIVE INDEX RATIO

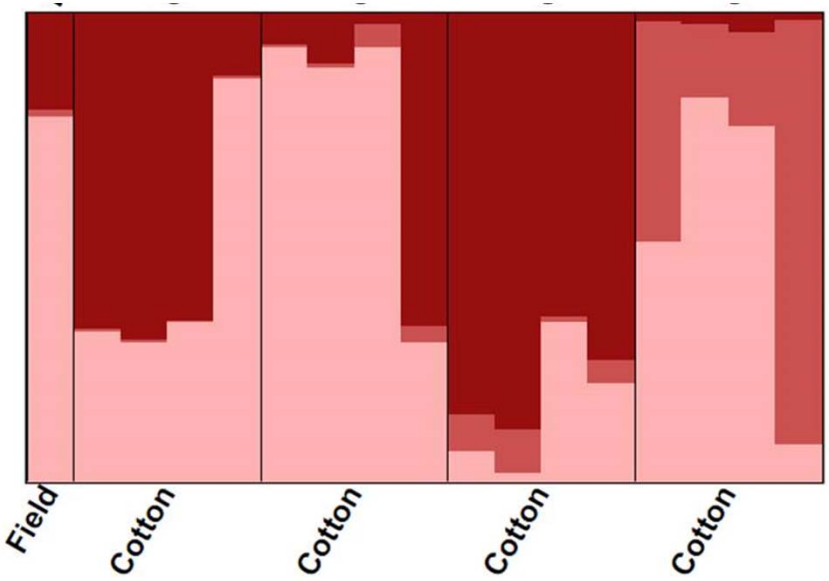


Effect of Crop Rotations on Population Structure of Reniform Nematode

Cotton, corn, cotton, corn
Corn, cotton, corn, cotton
Soybean (S), corn, soybean (S), corn
Soybean (R), cotton, soybean (R), cotton
Continuous cotton
Continuous soybean (S)

Cotton, Delta Pine 50 (PI 529566)
Corn, Funk's Waxy (PI 504055)

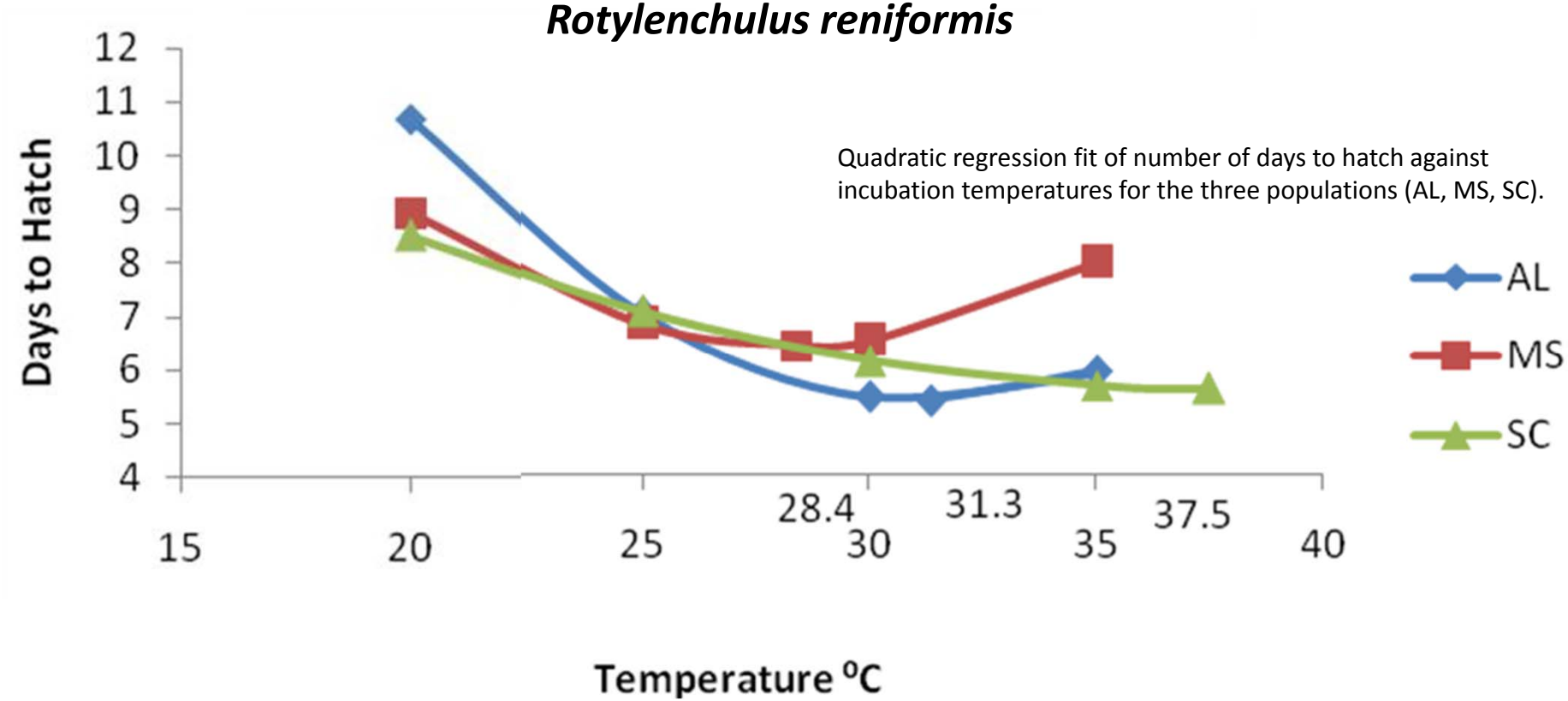




Graphical representation of *R. reniformis* genotypes following four 120-day crop rotation cycles. Population grouping assignments determined by the program STRUCTURE V 2.3. Each line on the X-axis represents the genotype of 1,000 reniform nematodes using 4 selective primer pairs with 6 selective nucleotides each. The Y-axis is percentage based indicating the homogeneity between genotypes after each crop cycle.

Leach, M., P. Agudelo, A.Lawton-Rauh. 2012. Plant Disease 96: 30-36.

Effect of Temperature on the Embryogenesis of Geographic Populations of *Rotylenchulus reniformis*



Quadratic regression fit of number of days to hatch against incubation temperatures for the three populations (AL, MS, SC).

•AL was the most sensitive to lower temperatures (slowest development at 20°C). Winter temperatures in Huxford, AL are 2-4°C warmer than in the other two locations.

•MS has the lowest normal high temperatures throughout the year, MS population had the lowest calculated optimal temperature (28.4ºC) of the three.

Leach, M.M., P. Agudelo, and P. Gerard. 2009. Effect of Temperature on the Embryogenesis of Geographic Populations of *Rotylenchulus reniformis*. Journal of Nematology 41: 23-27.

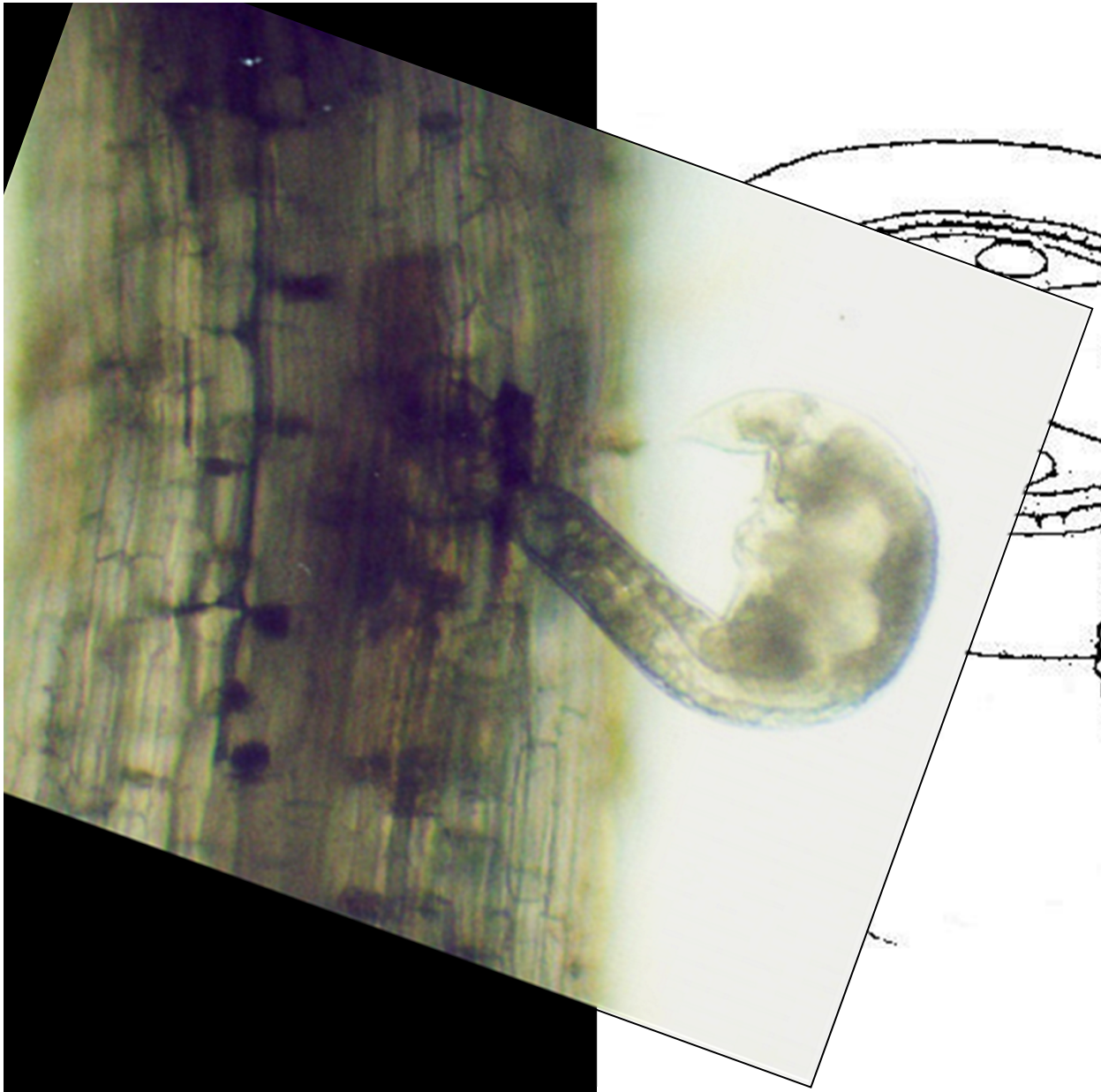
REPRODUCTION

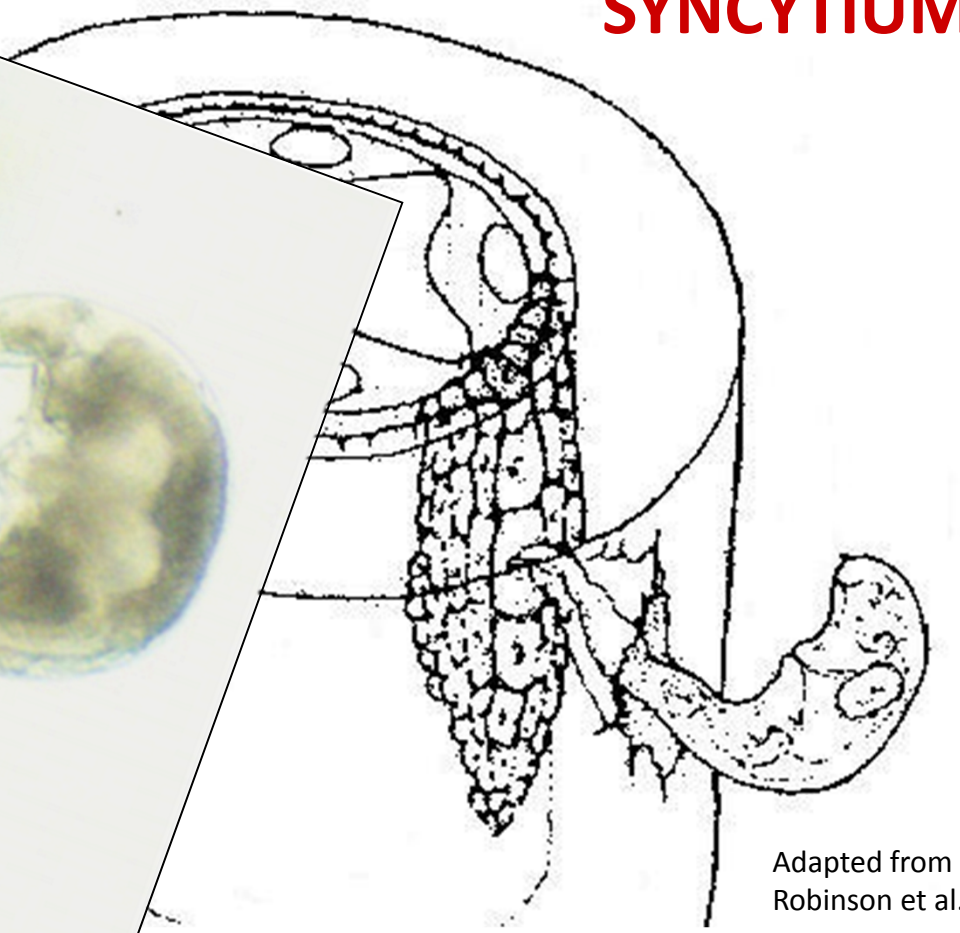
Pi: 3,000 nematodes/500 cc

Pf: 60 days after inoculation

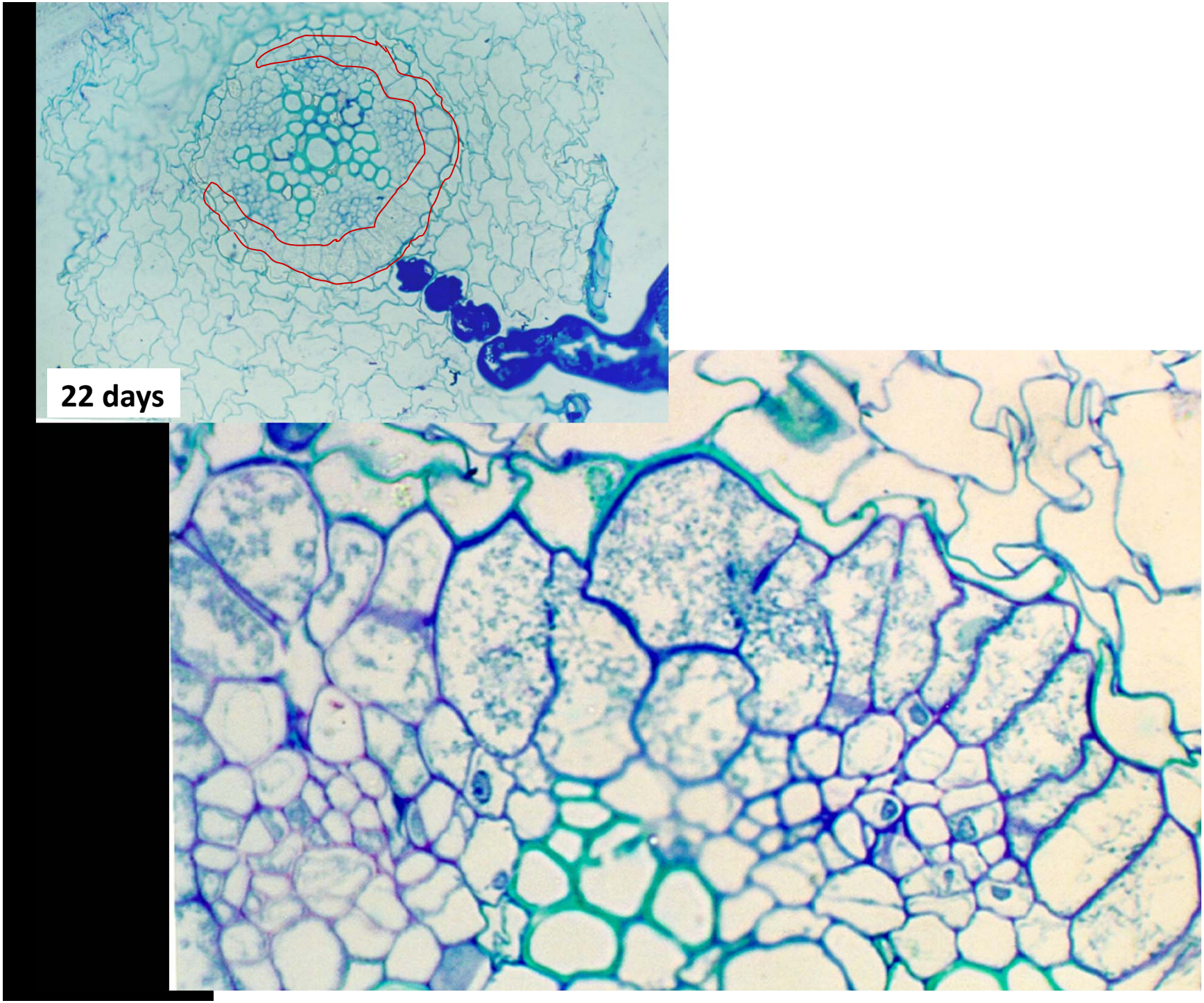
Reproductive factor = Pf/Pi

SYNCYTIUM



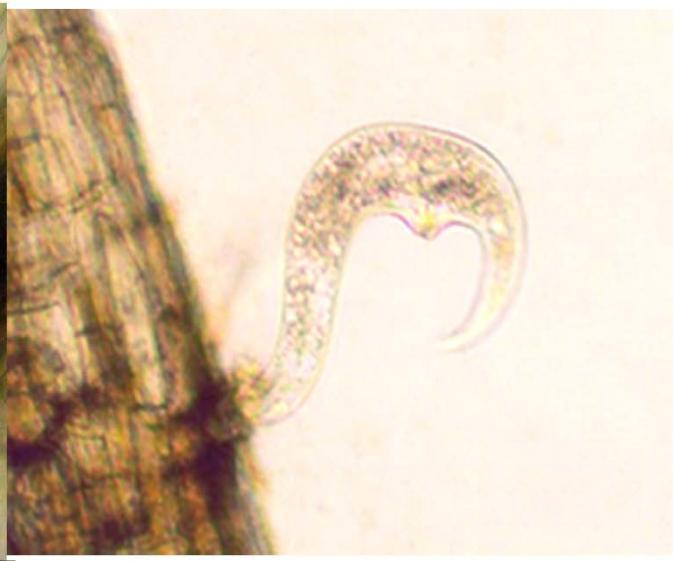


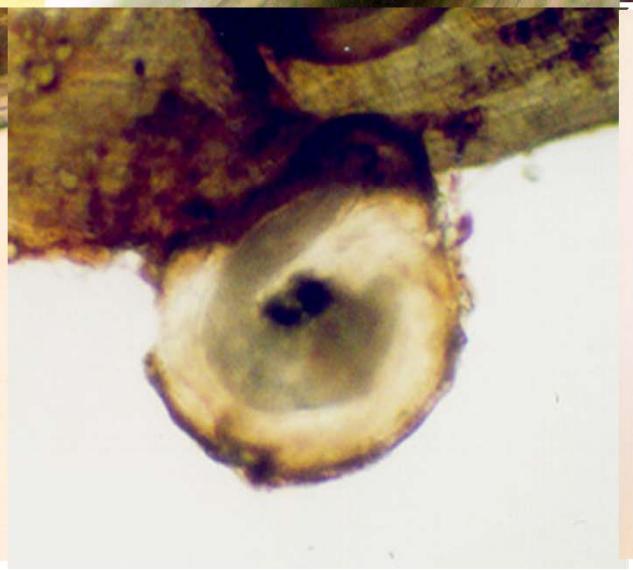
Adapted from
Robinson et al., 1997


22 days

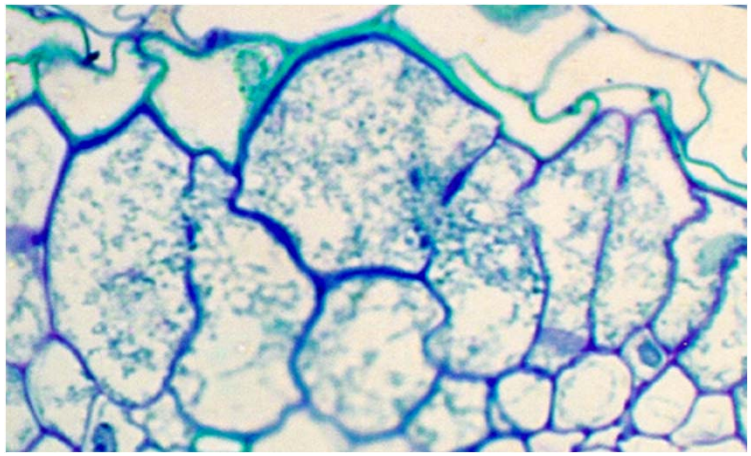
FEMALE MATURATION

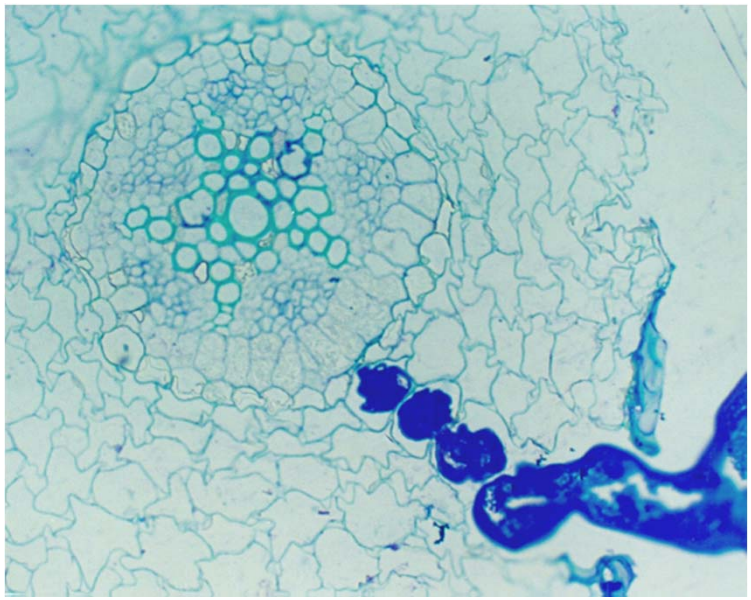




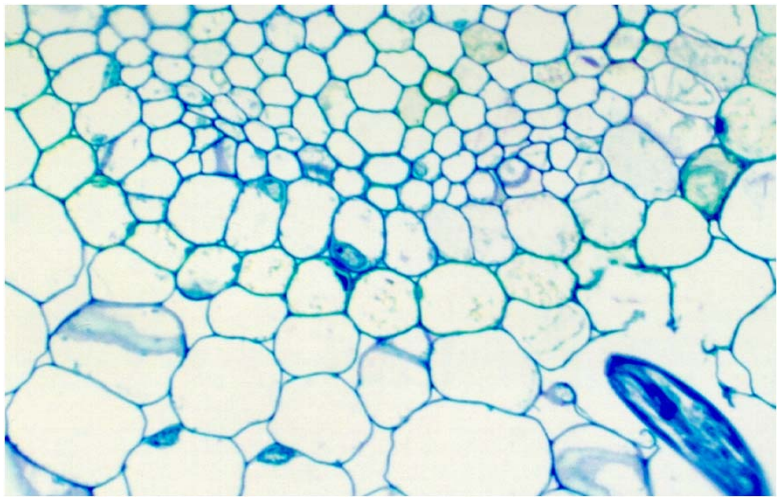


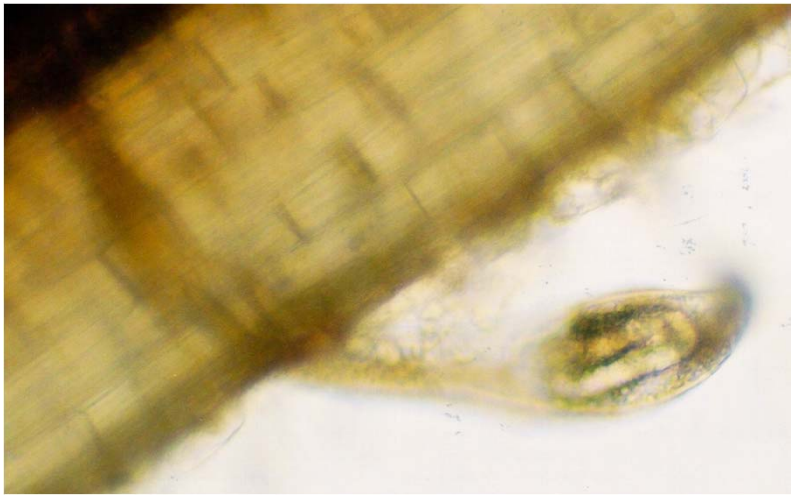

SUSCEPTIBLE





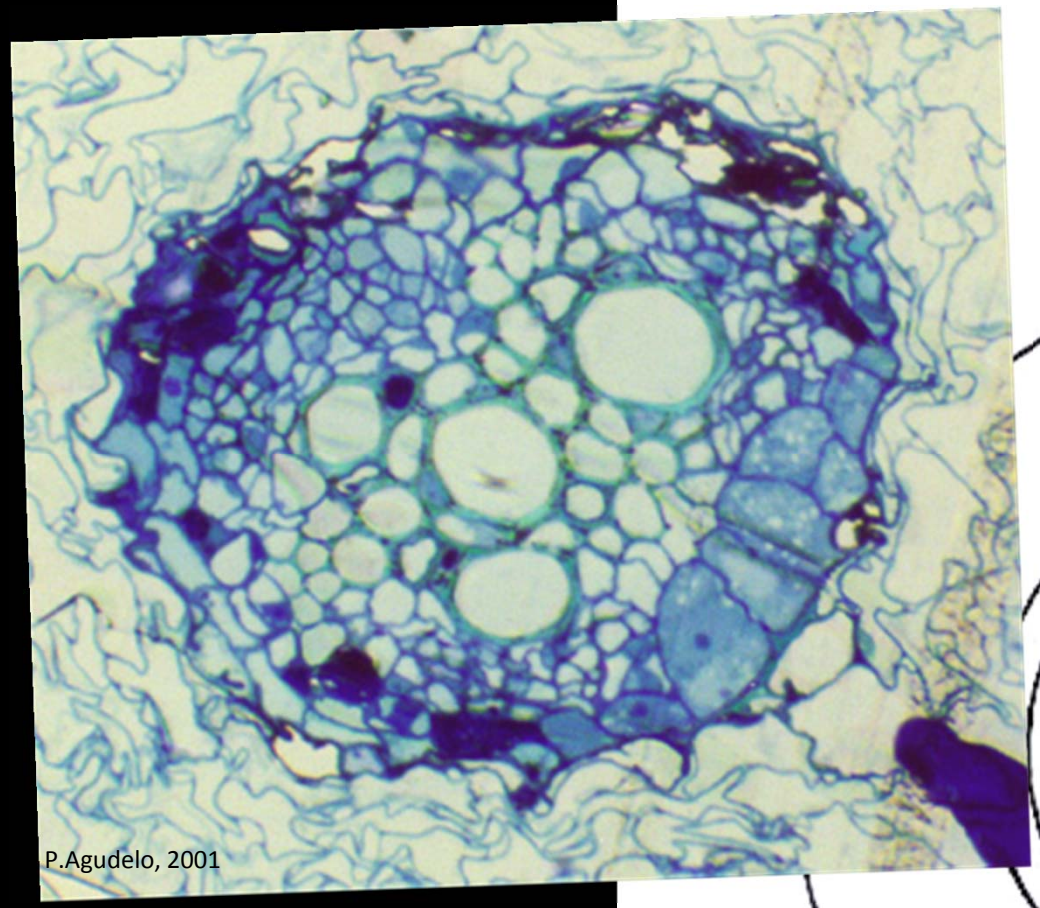
RESISTANT

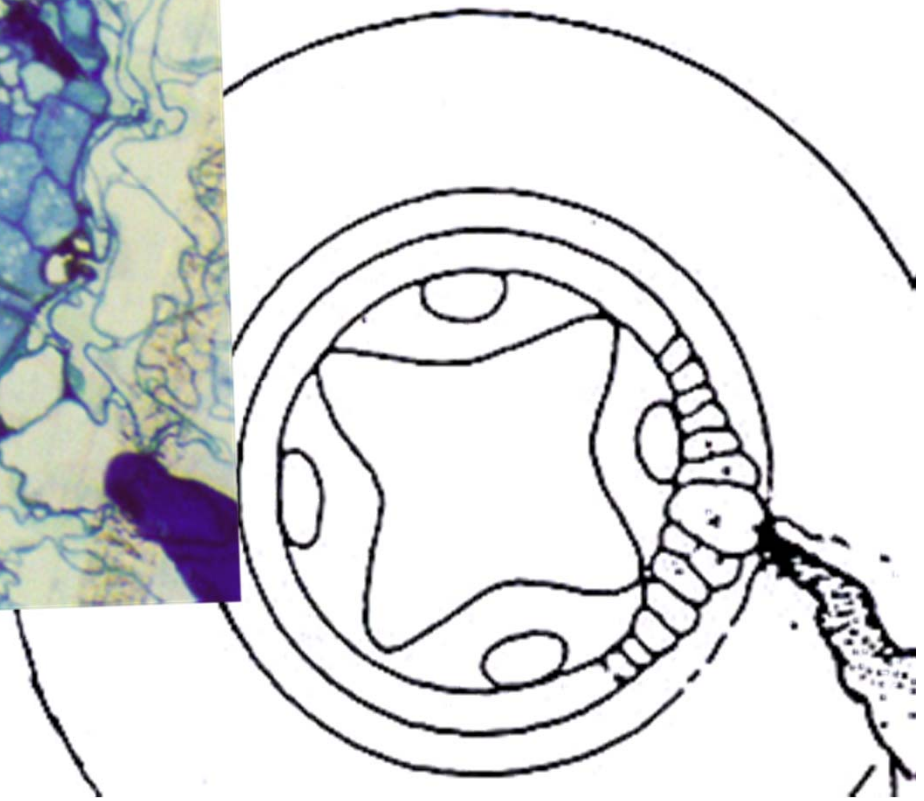




Agudelo, P., Robbins, R.T., Kim, K.S., and Stewart, J.M. 2005. Histological observations of *Rotylenchulus reniformis* on *Gossypium longicalyx* and interspecific cotton hybrids. Journal of Nematology 37:444-447.

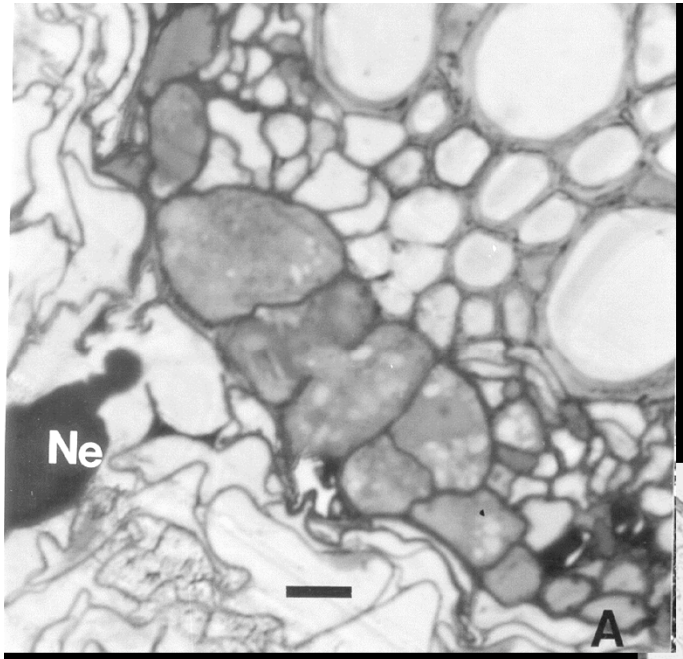
SYNCYTIUM

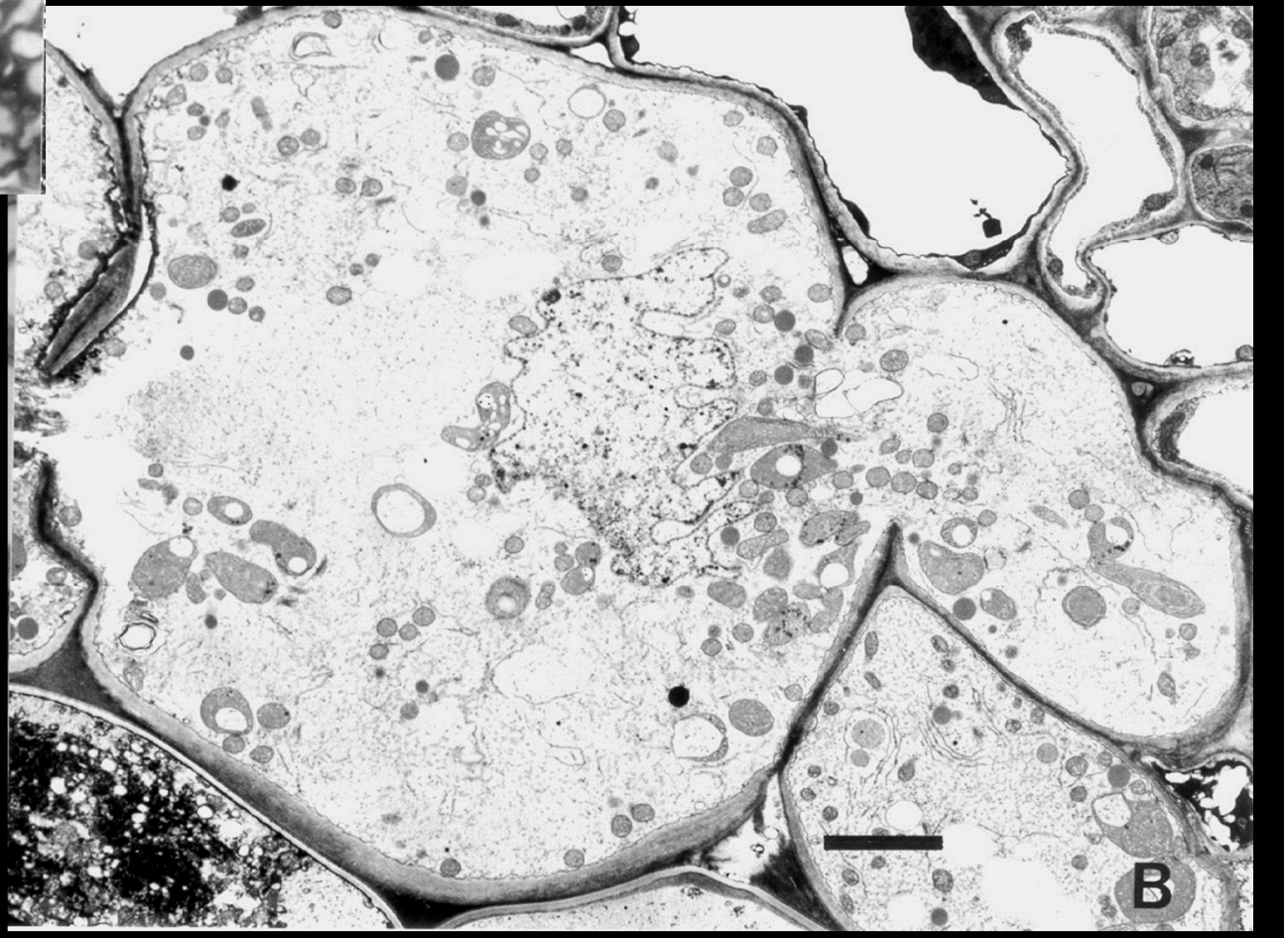




Dense granular cytoplasm
Dissolution of cell walls
Coalescence of cytoplasm
Enlarged nuclei

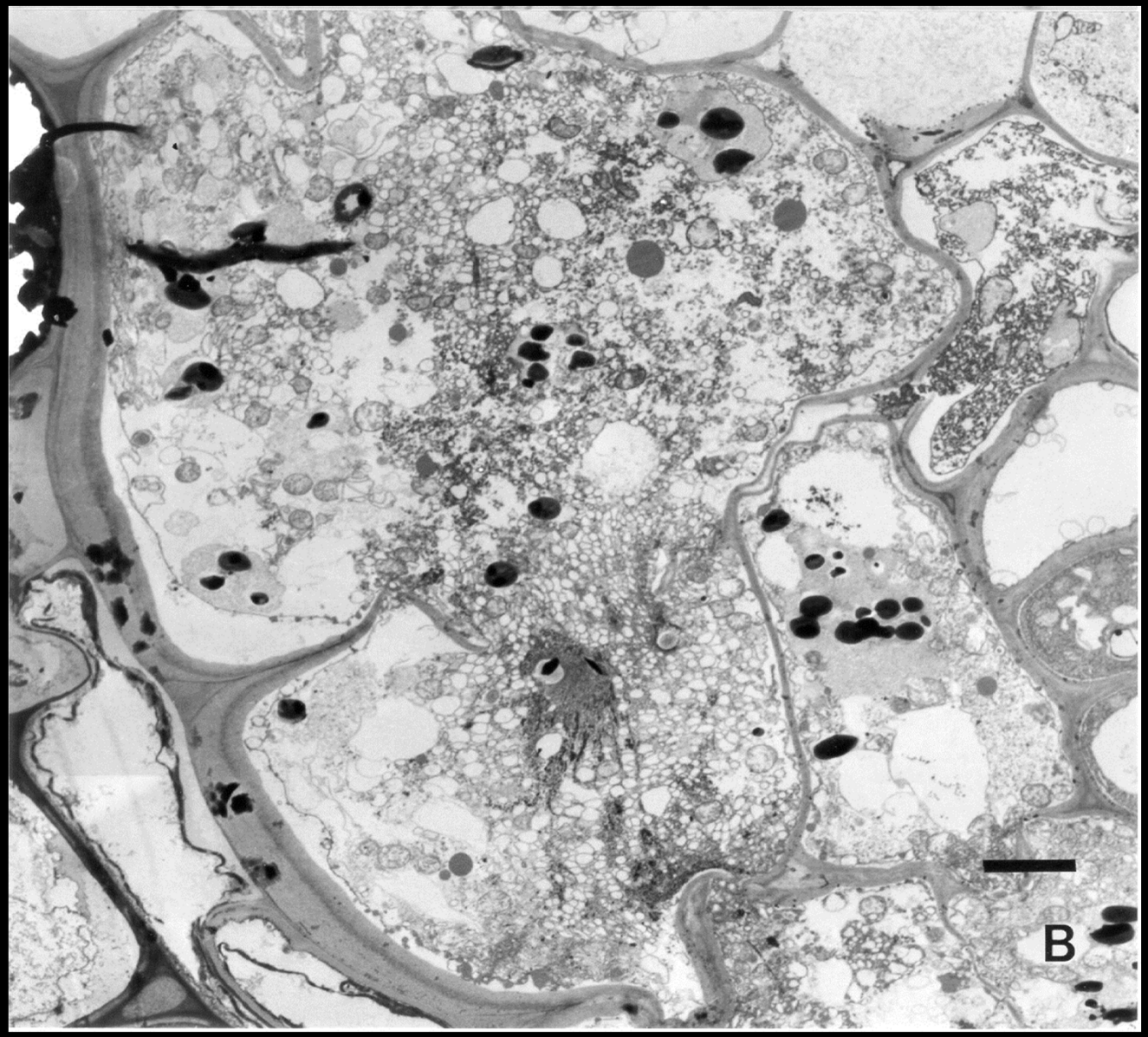
Adapted from
Robinson *et al*., 1997



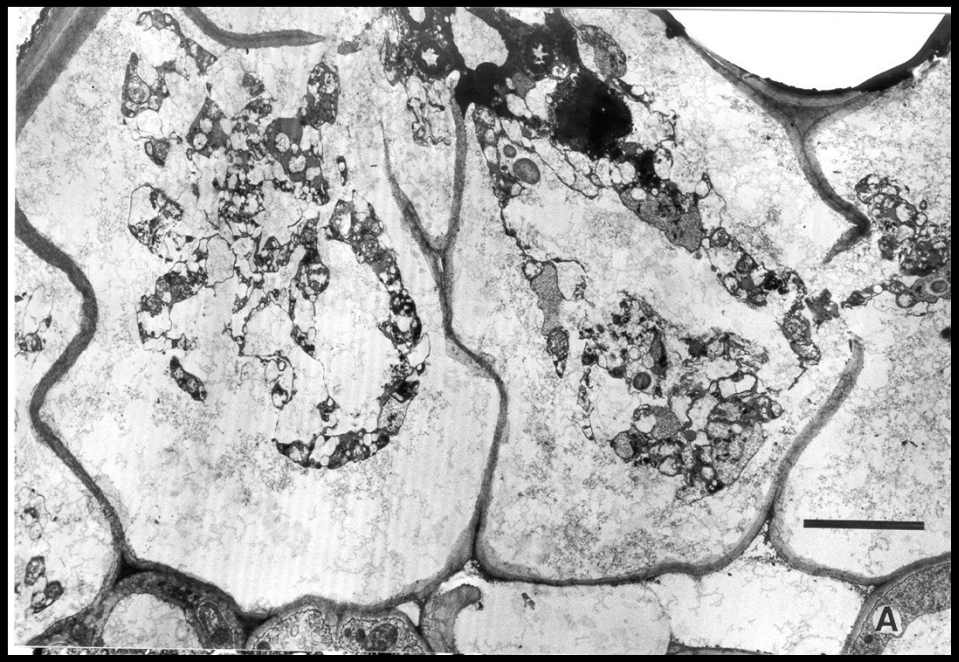


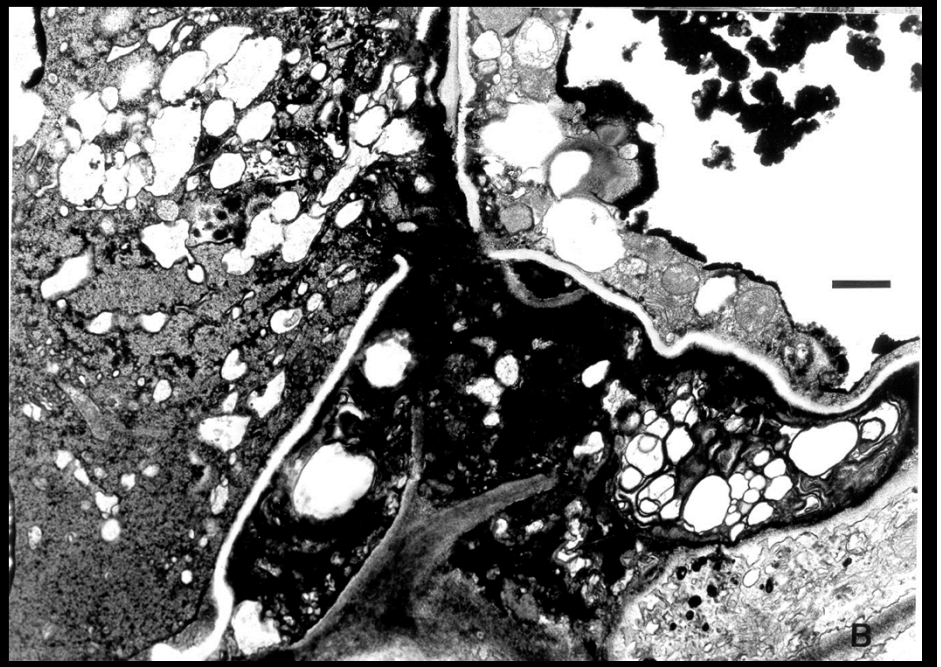
15 d.a.i.

SUSCEPTIBLE



9 days **RESISTANT**





RESISTANT

12 d.a.i.

Agudelo, P., Robbins, R.T., Kim, K.S., and Stewart, J.M. 2005. Histological changes in *Gossypium hirsutum* associated with reduced reproduction of *Rotylenchulus reniformis*. Journal of Nematology 37:185-189.

Infected and Uninfected portions of the root
3, 6, 12 days after inoculation

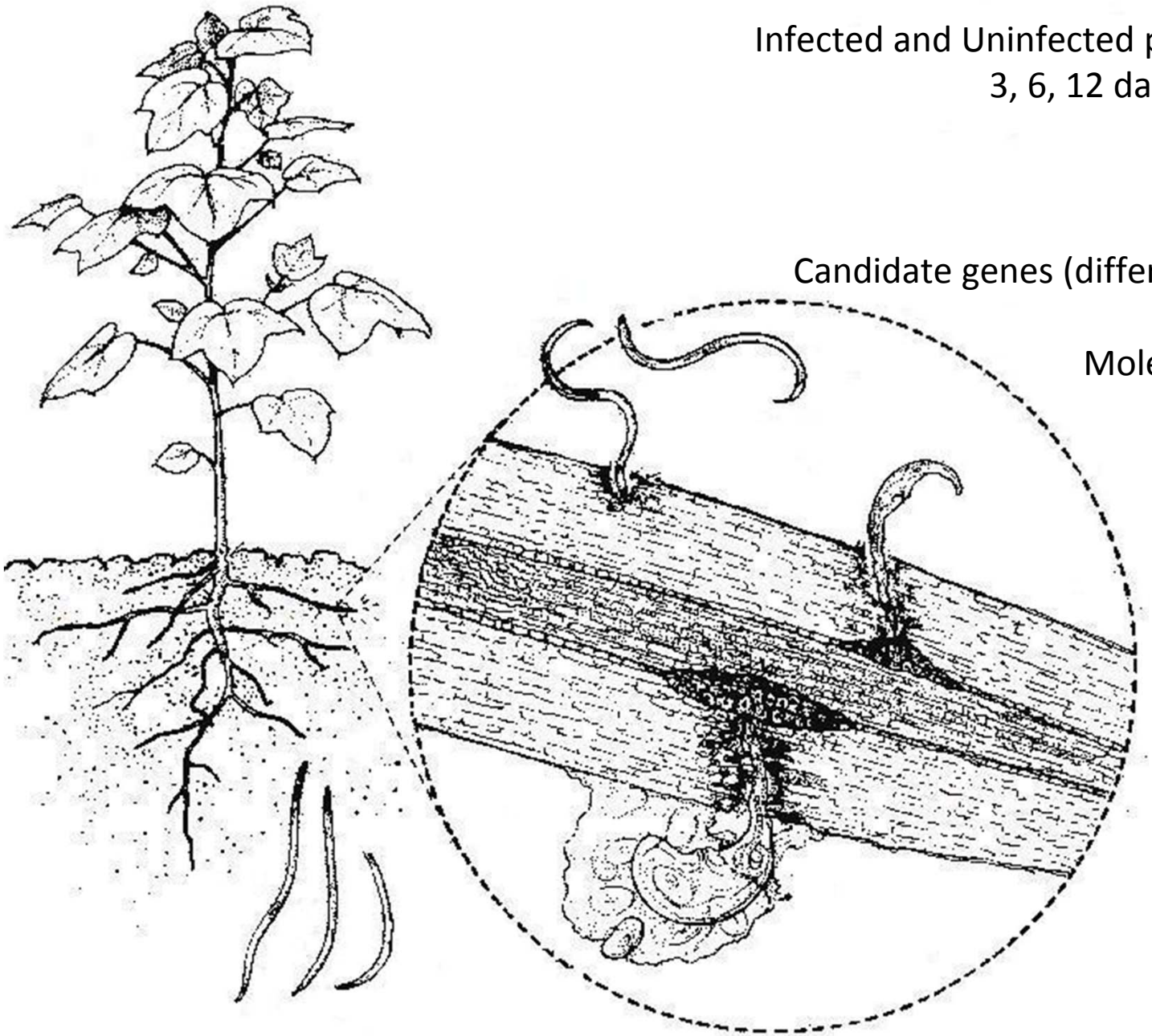
Candidate genes (differentially expressed)

↓

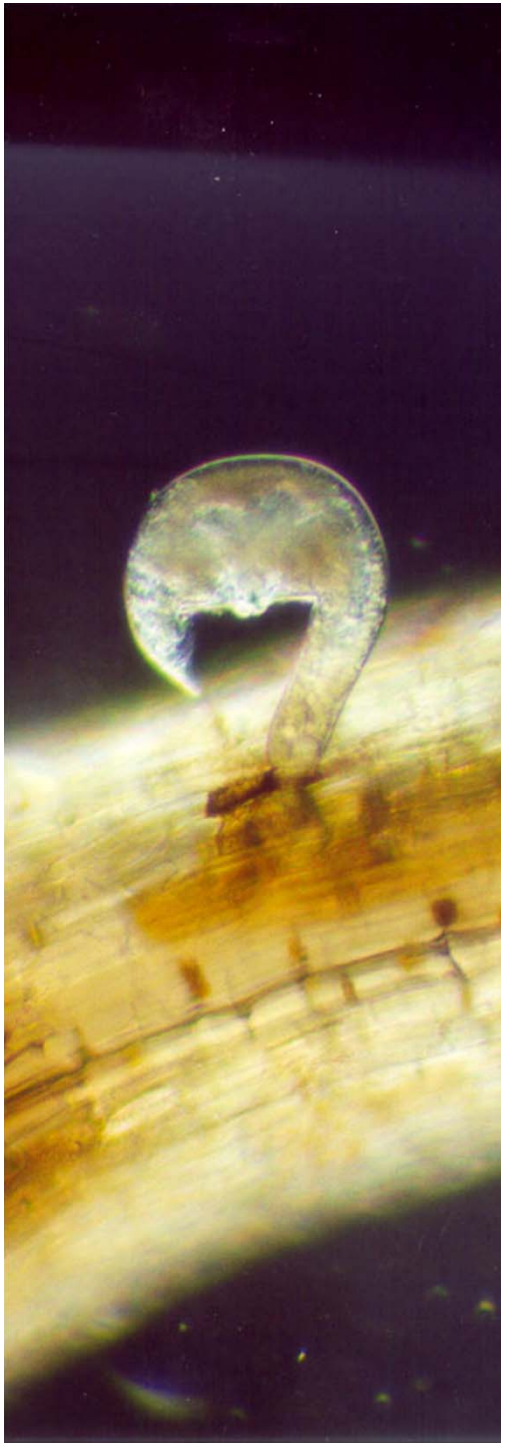
Molecular confirmation

↓

Functional studies



Drawing adapted from Robinson *et al*., 1997



cDNA library construction and sequencing.

David H. Murdock Research Institute. Six cDNA libraries were sequenced via Illumina HiSeq 2000.

Data filtering and assembly.

Trinity on a node of Palmetto Cluster (http://citi.clemson.edu/hpc) with 4 core processors and 512 GB memory. Assembled contigs were condensed by removing the alternative splicing isoforms.

Gene annotation.

All the unigenes assembled were exported and blast using Blast2GO against Non-redundant (nr) database using tblastx algorithm with cutoff E value < 10^{-6}. Reads with blast hits were annotated using Blast2GO with E value < 10^{-6}.

Differential expression analysis.

Trinity generated contigs were used as reference in the RSEM. The reads of the different conditions were compared. Transcript abundance calculated using RSEM and DESeq.

Hutcheson (Susceptible)
Perrin (RKN-Resistant)
Forrest (Reniform-Resistant)

~7 days

Inoculation
BPMV constructs
Empty vector

~7 days

Nematode inoculation

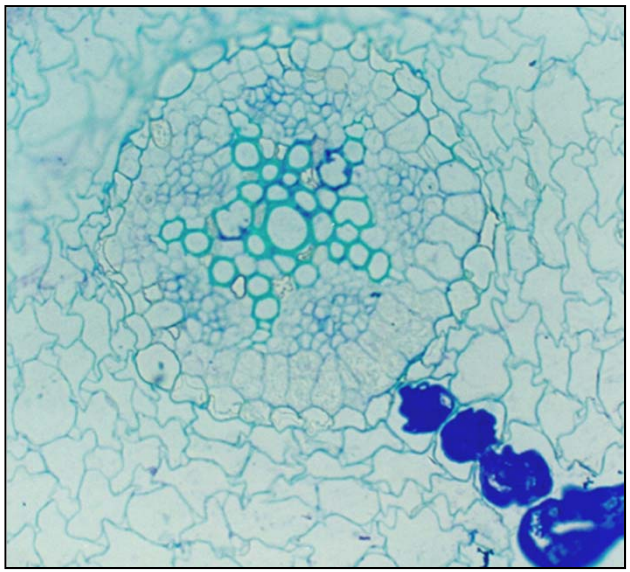
Reniform 60 days
RKN 45 days

VIGS SYSTEM

RKN
Galling and egg masses

Reniform
Reproduction factor

Histopathology:
Thick and thin sections



BPMV Constructs

ICS (isochorishmate synthase)

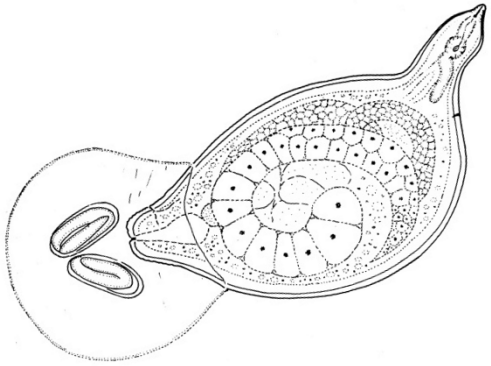
PAL (phenylalanine ammonia lyase)

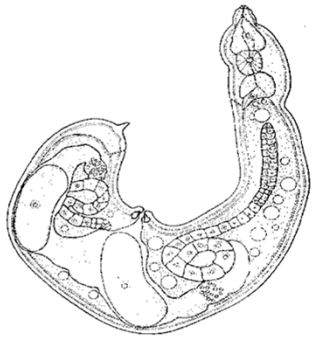
These genes are involved in salicylic acid (SA) biosynthesis.

The silenced lines make reduced SA and are susceptible to several pathogens.

D (stearoy-acyl carrier protein-desaturase)

This gene contributes to synthesis of oleic acid (18:1). A monounsaturated fatty acid which we know is involved in plant defense. D plants exhibit enhanced resistance to several pathogens.





H. glycines	***R. reniformis***	***M. incognita/M. arenaria***
J2 infective stage	immat. female infective stage	J2 infective stage
intracell. migration (endo)	intracell. migration (semi-endo)	intercell. migration (endo)
syncytia	syncytia	giant cells
no galls	no galls	galls
cyst-females	no cysts	no cysts
amphimictic	amphimictic	parthenogenetic
narrow host range	wide host range	wide host range
males survive differentially	males genetically determined	males survive differentially
vascular cylinder	pericycle	vascular cylinder
reported pathogenic variability	reported pathogenic variablity	reported pathogenic variability

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