

Reproduction of *M. arenaria* on flue-cured tobacco homozygous for Rk1 and/or Rk2

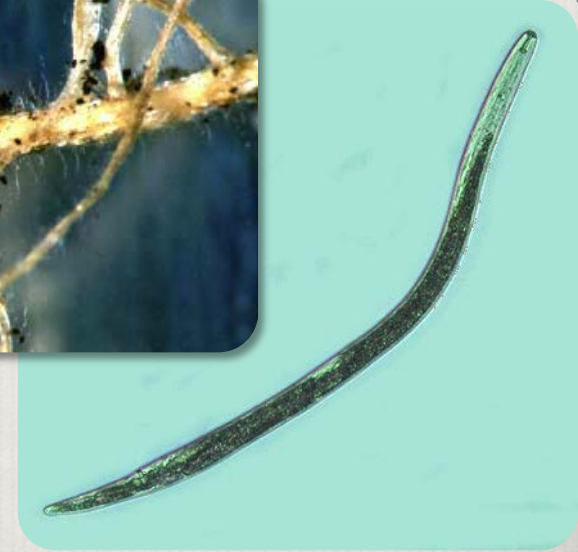
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Root Nematode Problems

⊙ 3 main parasitic nematodes in tobacco:

- ⊙ Cyst nematode
- ⊙ Root-knot nematode
- ⊙ Lesion nematode



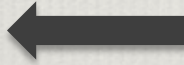
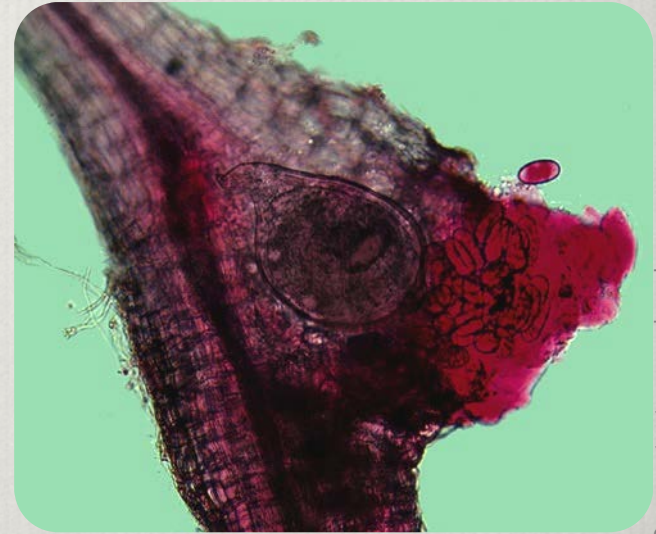
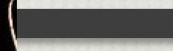
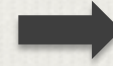
⊙ Root-knot symptoms

- ⊙ Root galls
- ⊙ Stunting
- ⊙ Wilting
- ⊙ Chlorosis
- ⊙ Root decay
- ⊙ **Yield loss**





Images: C. Johnson, Virginia Tech

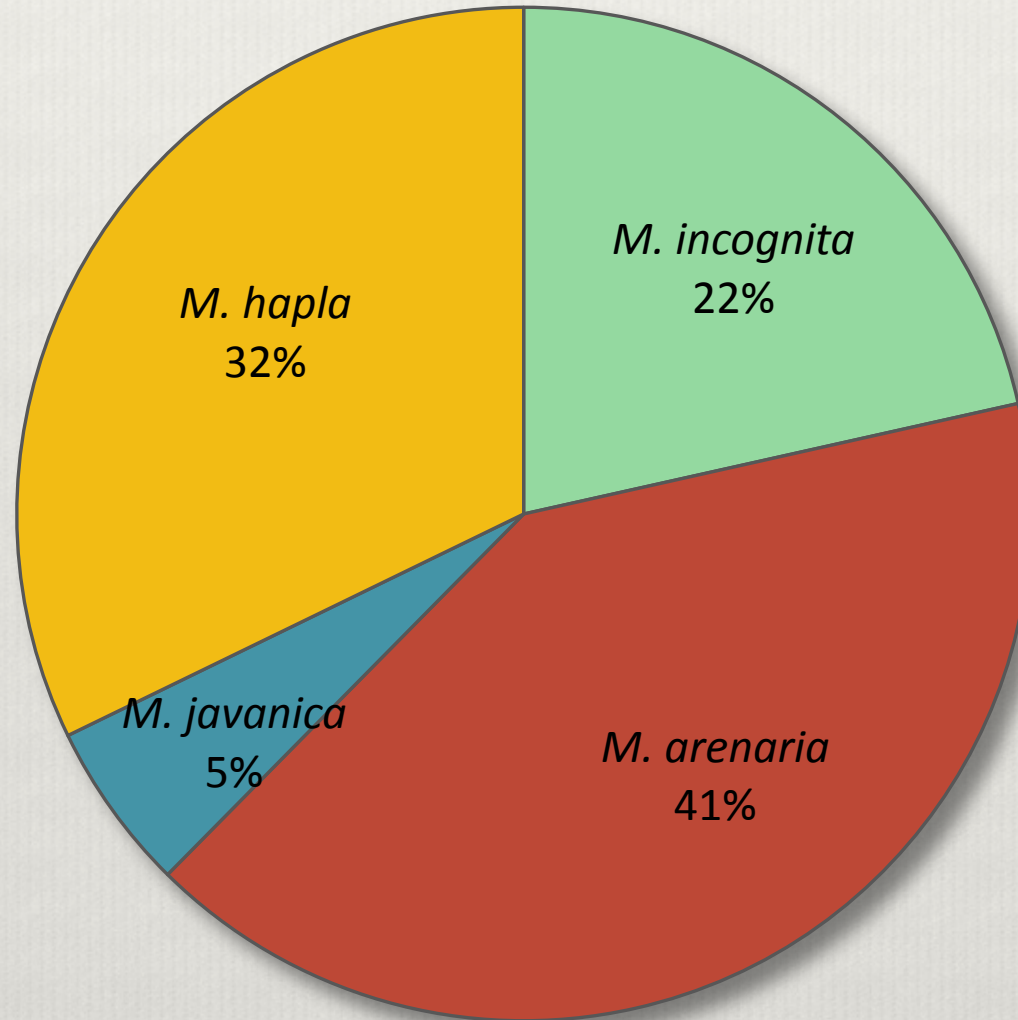


McGawley, via Nemapix

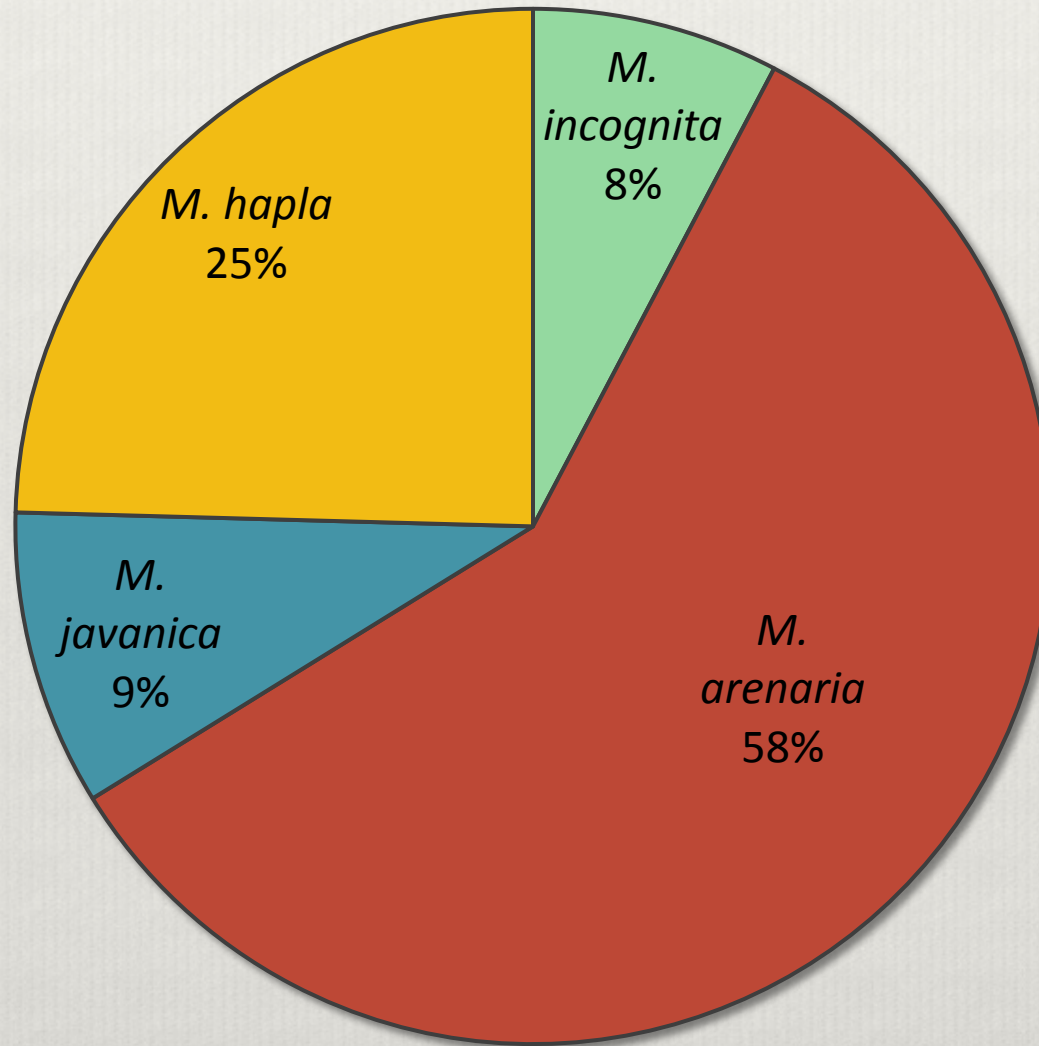
Current Control Options

- ⦿ Crop rotation
- ⦿ **Resistant varieties**
- ⦿ Nematicides

2004 VA Nematode Survey, Flue-Cured Tobacco



2010 VA Nematode Survey, Flue-Cured Tobacco



Resistance Genes

- ⊙ Genes Rk1 and Rk2 are responsible for root knot resistance in tobacco
- ⊙ Rk1: resistance to races 1 and 3 of *M. incognita*
 - ⊙ Bred into tobacco from *N. tomentosa*
 - ⊙ Most commercial tobacco cultivars have this gene
- ⊙ Rk2: resistance (?) to *M. javanica*
 - ⊙ Breeding line developed into cultivated tobacco from a land race of Zimbabwe tobacco

Tobacco entries

- ◎ C371G: standard susceptible
- ◎ SC72 and NC95: Rk1 gene
- ◎ T1511: Rk2 gene
- ◎ STNCB and NOD8: Rk1 and Rk2; available in the US within the last 5 years



Objective of Experiment

- To determine if having both resistance genes (Rk1Rk1 and Rk2Rk2) gives more resistance in tobacco to *M. arenaria* than just one or the other

Materials & Methods



- ◎ 6 tobacco entries, 6 replications

Entry	Resistance Genes
C371G	Susceptible
T1511	Rk2
SC 72	Rk1
NC 95	Rk1
STNCB	Rk1 & Rk2
NOD 8	Rk1 & Rk2



- ◎ Inoculate each with 5,000 RK eggs when plants have 4 true leaves (5-10 cm height)
- ◎ 60 days after inoculation, cut tops off, rinse roots

Materials & Methods Cont'd.



- Sub-divided root system in half by weight
- Stained ½ the roots red with Phloxine B
- Collected three 1-gram samples from stained roots and counted the number of egg masses to get an estimate of egg masses per gram roots for each plant



Materials & Methods Cont'd.

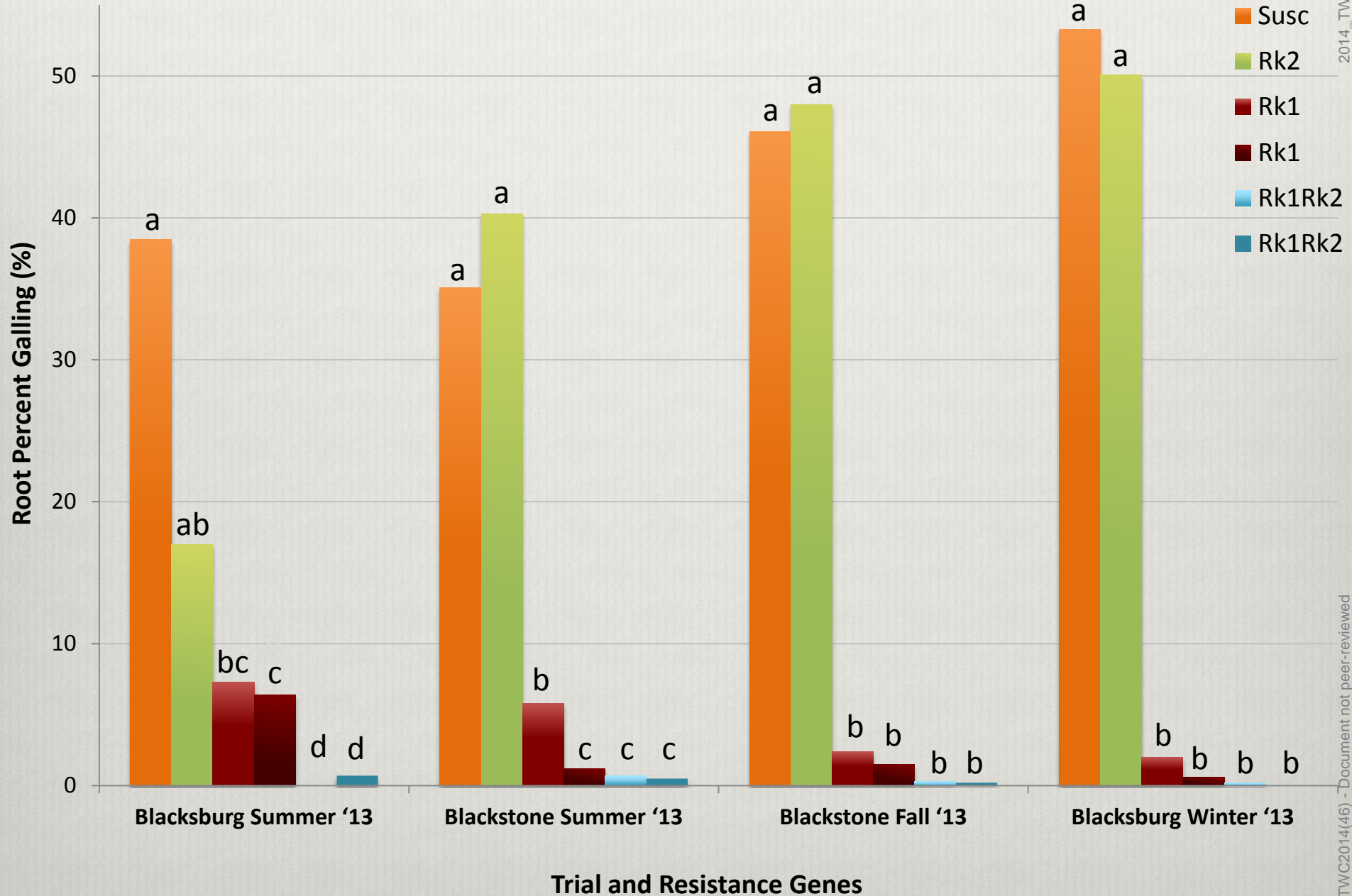
- Other half of roots: bleach-extracted eggs from root surface, suspended in 500 mL water and counted two 10-mL aliquots
- Averaged the counts and calculated eggs for 500 mL/0.5 root weight to get an estimate of eggs/gram roots



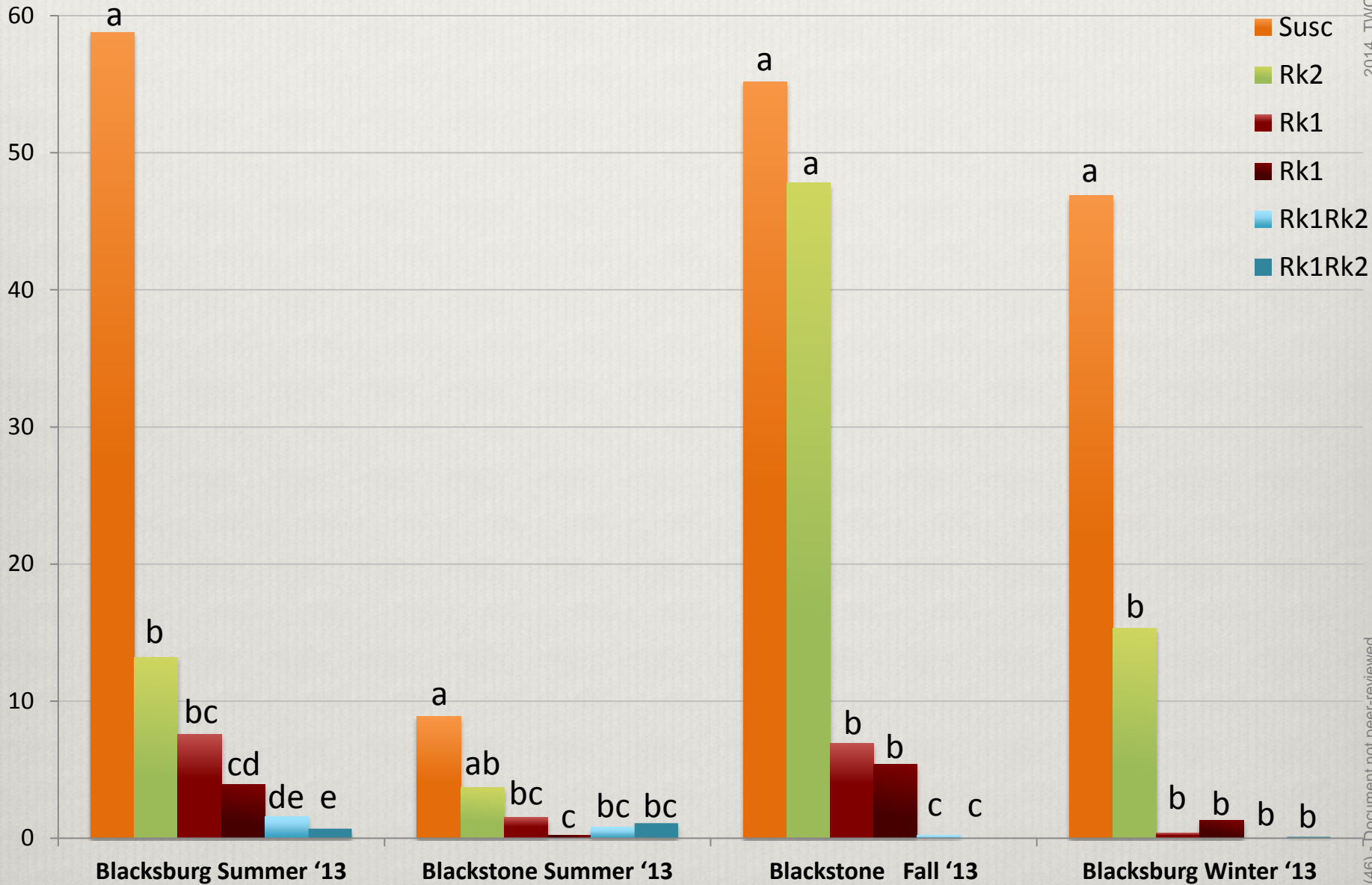
Analysis

- Data was analyzed in JMP
 - Data presented are non-transformed means. Means followed by the same letter(s) are not significantly different according to statistical analysis of transformed $[\log_{10}(x+1)]$ data and the Tukey-Kramer honest significant difference (HSD) test.

Root Percent Galling by Trial

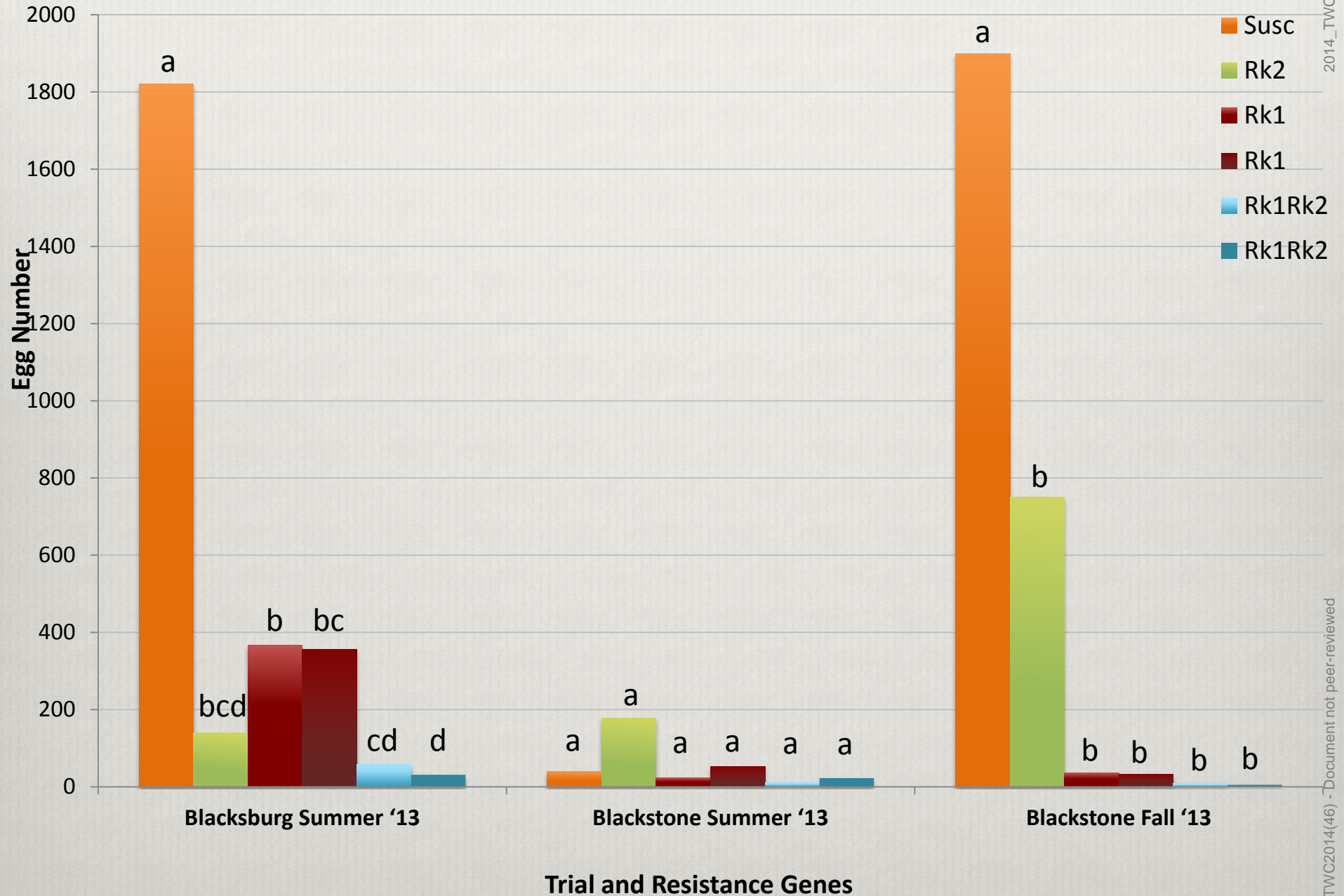


Egg Mass Number per Gram Roots by Trial



Trial and Resistance Genes

Egg Number per Gram Roots by Trial



Conclusions

- ⊙ Plants with Rk1 alone, and Rk1 and Rk2 together significantly reduced root galling, egg masses, and eggs than the control
- ⊙ Combining Rk1 and Rk2 genes further reduced galling (significantly in 1 of 4 trials), egg masses (significantly in 2 of 4 trials), and eggs (significantly in 1 of 3 trials)

Implications

- ⊙ Better understanding of the specific effects of Rk1 and Rk2 on root-knot nematode parasitism should help plant breeders improve tobacco resistance to *M. arenaria*

Acknowledgements



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