



My
opponents
are
loathsome
miscreants!

Is it Time for a Transgenic Tomato Variety?!

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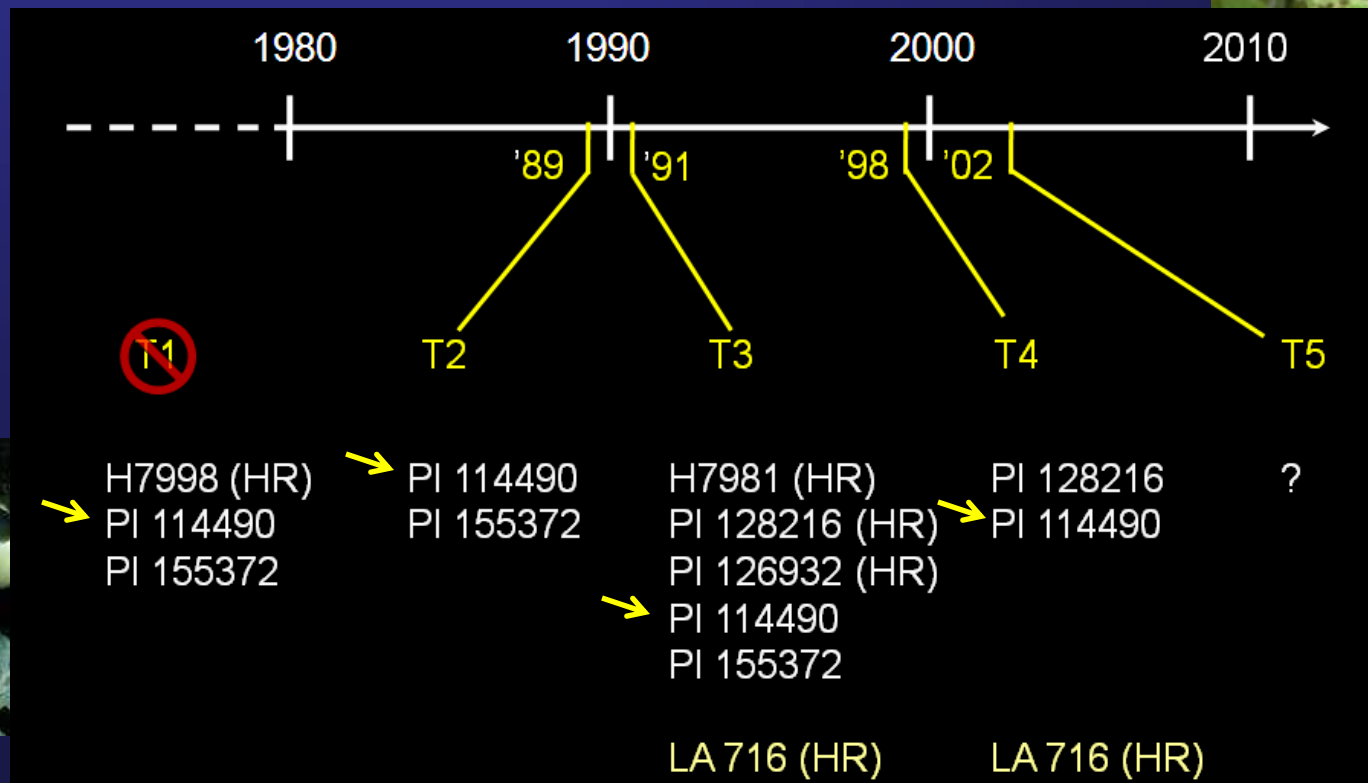
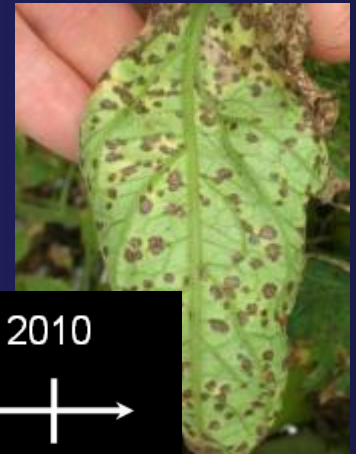




Bacterial Spot

- Causal agent:

- *Xanthomonas euvesicatoria* (Race T1)
- *Xanthomonas vesicatoria* (Race T2)
- *Xanthomonas perforans* (Races T3, T4, T5)



Two Blades Foundation

- Mission to support the development and deployment of disease resistance in crops
- Driven by advances in molecular plant sciences and sequencing permitting access to a larger repertoire of disease resistance genes
- Goal to reduce crop losses due to disease, enhance food security
- Commercial and humanitarian applications

Effective genetic resistance to BLS

Bs2 Project

- Project first undertaken by 2Blades in 1992
- At that time:
 - BLS was a widespread problem on tomato and pepper
 - Known resistances in pepper (BS1-3)
 - Xanthomonas effectors were characterized

Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*

Brian Kearney & Brian J. Staskawicz

Department of Plant Pathology, University of California, Berkeley, California 94720, USA

1990 Nature 346: 385-6

TABLE 1 Distribution of *avrBs2* activity and DNA homology within *Xanthomonas*

Strain	Hypersensitivity on ECW20R	<i>avrBs2</i> Homology
<i>X. c. vesicatoria</i>	yes	yes
<i>X. c. alfalfae</i>	yes	yes
<i>X. c. phaseoli</i>	yes	yes
<i>X. c. malvacearum</i>	yes	yes
<i>X. c. campestris</i>	yes	yes
<i>X. c. vitians</i>	yes	yes
<i>X. c. vignicola</i>	yes	yes
<i>X. c. glycines</i>	no	yes
<i>X. c. holcicola</i>	no	yes
<i>X. c. oryzae</i>	—	yes
<i>X. c. citri</i>	—	yes
<i>X. fragariae</i>	no	no


- AvrBs2 widely distributed
- Function conserved

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Crop Development Process



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A photograph showing three individuals in full-body yellow hazmat suits with blue hoods and gloves, working in a field of tall green grass and weeds. One person is standing on the left, another is kneeling in the center, and a third is lying on the ground on the right, holding a large white cylindrical object. A man in a pink polo shirt and white shorts stands on the right, looking towards the camera. A red speech bubble is overlaid on the image, pointing towards the man in the pink shirt.

I told
them it
was just
a pepper
gene in a
tomato!

Total and extra-large marketable yield, fruit size and cull weights for tomato inbreds and hybrids with and without the pepper *Bs2* gene, Fall 2011, GCREC.

Entry ^z	Marketable yield (25 lb box/A)		Fruit Size (oz.)	Culls (% by wt.)	Bacterial spot disease severity ^y
	Total	Extra-large			
Fla. 8000 <i>Bs2</i> homo	2362 a ^x	906 bc	5.1 cd	27	5.4
Fla. 8314 <i>Bs2</i> homo	2237 ab	1232 a	5.6 a-c	21	4.0
Fla. 8000 <i>Bs2</i> hemi	1918 b	1060 ab	5.8 ab	26	4.3
Florida 47	1099 c	682 c	6.2 a	23	5.5
Fla. 8314	1093 c	588 c	5.5 bc	23	5.6
Fla. 8000	1028 c	253 d	4.8 d	28	5.1

^z Genotypes with *Bs2* gene indicated by *Bs2*, hemi = 1 copy, homo = 2 copies.

^y Rated on the Horsfall-Barrett Scale, 4 = 6-12% defoliation; 5 = 12-25% defoliation; 6 = 25-50% defoliation.

^x Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$.

Bacterial spot disease severity for tomato inbreds and hybrids with and without the Bs2 pepper gene, GCREC Spring 2012.

Genotype	Disease Severity ^z
VF 36	9.25 a ^y
Fla. 8111 B	8.75 a
Florida 47	7.38 b
Sebring	7.13 bc
Xv4 F ₁	6.5 b-d
Fla. 8000	6.5 b-d
Florida 91	6.25 b-e
Fla. 8314	6.25 b-e
Xv4 line	6.13 c-e
Sanibel	5.67 de
104009-29 (susceptible)	5.25 e
104009-8 Bs2	2.75 f
VF36 Bs2 hemi	2.5 f
104009-13 Bs2	2.5 f
VF 36 Bs2 homo	2.5 f
104009-5 Bs2	2.45 f
104009-26 Bs2	2.25 f
Fla. 8111B Bs2 homo	2.25 f
Xv4 Bs2 F ₁	2.25 f
Fla. 8314 Bs2 homo	2.0 f
104009-12 Bs2	2.0 f
Fla. 8000 Bs2 homo	2.0 f

^z Horsfall- Barratt scale, higher number means more disease.

^y Mean separation by Duncan's Multiple Range Test at $P \leq 0.05$.



VF 36

Fla. 8314 *Bs2*

Total and extra-large marketable yield, fruit size, and cull weights for tomato inbreds and hybrids with and without the pepper *Bs2* gene, Spring 2012, GCREC.

Entry ^z	Marketable Yield (25 lb box/A)		Fruit size (oz)	Culls (% by wt.)
	Total	Extra-large		
Fla. 8000 <i>Bs2</i>	2122 a ^y	566 bc	5.1 f	28.4 e
Fla. 8314 <i>Bs2</i>	1725 ab	849 a	6.2 cd	31.8 de
Fla. 8000	1648 b	403 c-e	5.3 ef	42.0 cd
Xv4 <i>Bs2</i> F ₁	1615 b	507 b-d	5.3 ef	43.0 cd
9-3-20 SUSC	1579 b	228 de	4.9 f	44.4 b-d
9-3-5 <i>Bs2</i>	1524 b	201 e	4.8 f	46.5 bc
Florida 91	1357 b-c	855 a	6.9 b	47.9 bc
Sanibel	1003 cd	467 c-e	6.2 cd	56.9 a-c
Fla. 8314	967 cd	395 c-e	5.8 de	56.3 a-c
Fla. 8111 <i>Bs2</i>	892 cd	750 ab	7.9 a	57.6 a-c
Fla. 8111	764 d	371 c-e	6.2 cd	67.9 a
Sebring	707 d	367 c-e	6.5 bc	64.7 a
Florida 47	665 d	272 de	5.9 de	68.0 a

^z *Bs2* indicates genotype is homozygous for the *Bs2* gene.

^y Mean separation in columns by Duncan's Multiple Range Test at $P \leq 0.05$.







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Conqueror III

Summer Squash Varieties:



Conqueror III is a green-stem straightneck with transgenic resistance to CMV, WMV and ZYMV and intermediate resistance to papaya ring spot. The fruit is a uniform, creamy yellow color and the plant is vigorous and semi-open.

Fruit Color	Glossy lemon yellow, Green peduncle
Fruit shape	Straight, Slightly crooked neck, Tapered
Fruit size	6-7 in.; 16-18 cm
Plant type	Vigorous medium
Relative Days to Maturity	41

Independence II



Judgement III



Justice III



Liberator III



Patriot II



Prelude II



XPT 1832 III



Chemistry Cost

Per season:

Compound	Ave price \$/ lb AI	Appln Rate	# Applns (with BLS)	Total cost FL	Total cost FL/ EC/ Mex
Mancozeb (Dithane DF)	\$1.65	1.5-2	44.8	\$3,592,512	\$14,414.400
Copper hydroxide (Kocide 101)	\$3.25	1.5	44.8	\$7,076,160	\$28,392,000

2009 prices from one representative provider

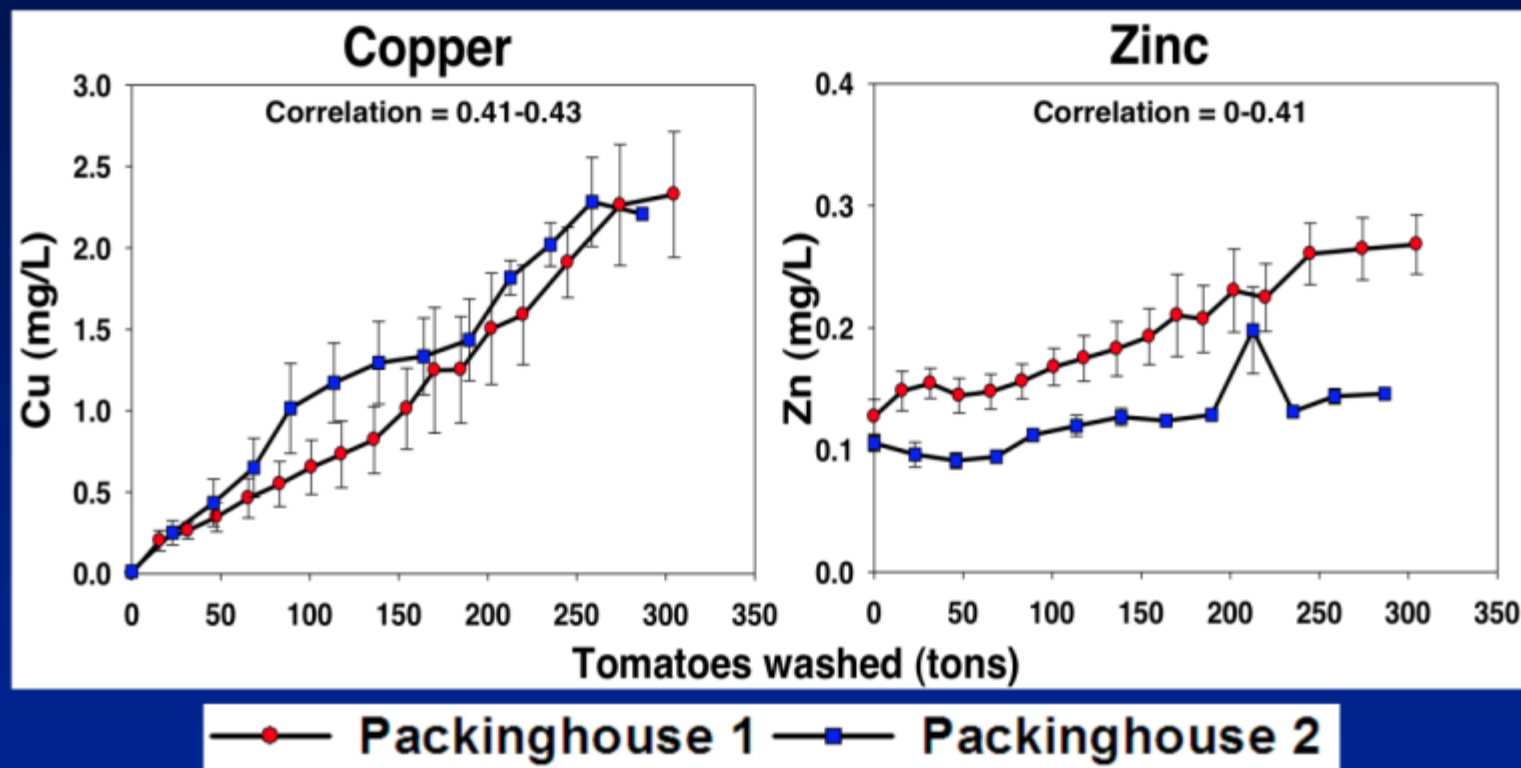
Applications rates and number of applications from discussions with Glades Crop Care (2009)

FL acres average 32,400; Acres for FL, East Coast and Mexico are 130,000.

Costs do not include labor

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EVOLUTION OF COPPER AND ZINC IN WASTEWATER



- ❖ Copper increased linearly with the washing of tomatoes.
- ❖ Zinc increased more in PKG 1 than PKG 2.

Transgenic BLS Resistant Fresh Market Tomato: From proof of principle to product

- Improved gene cassette
 - Only pepper and tomato DNA
 - No antibiotic selection gene
- Florida adapted varieties-reliable yields
- Green technology-less pesticides needed
- This fall we are testing hybrids that have *Bs2* and resistance to TYLCV, fusarium crown rot, fusarium wilt race 3, TSWV
- In the future we hope to improve resistance durability by adding *Xv4* and *EFR* genes.



Can this
really
be the
end?





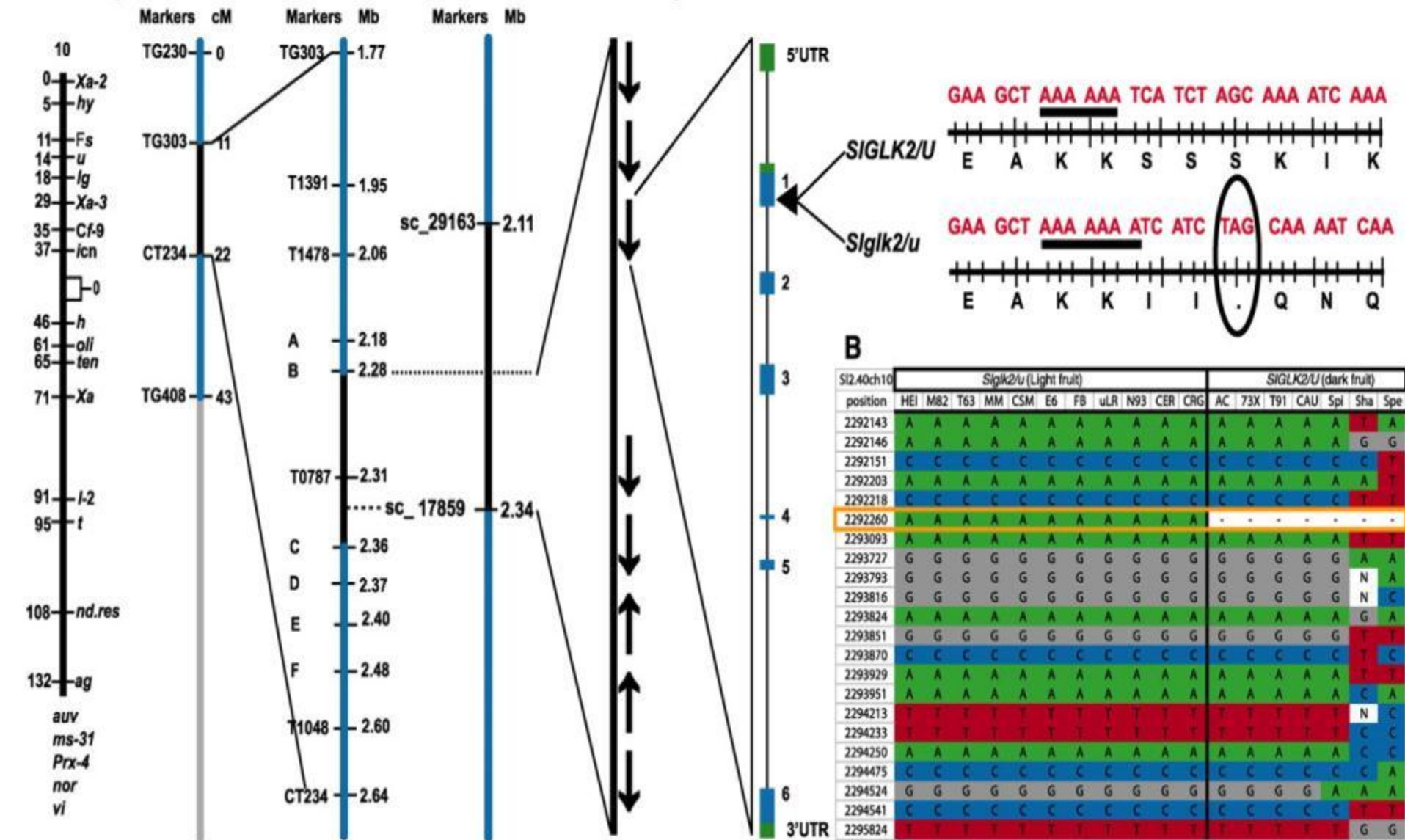


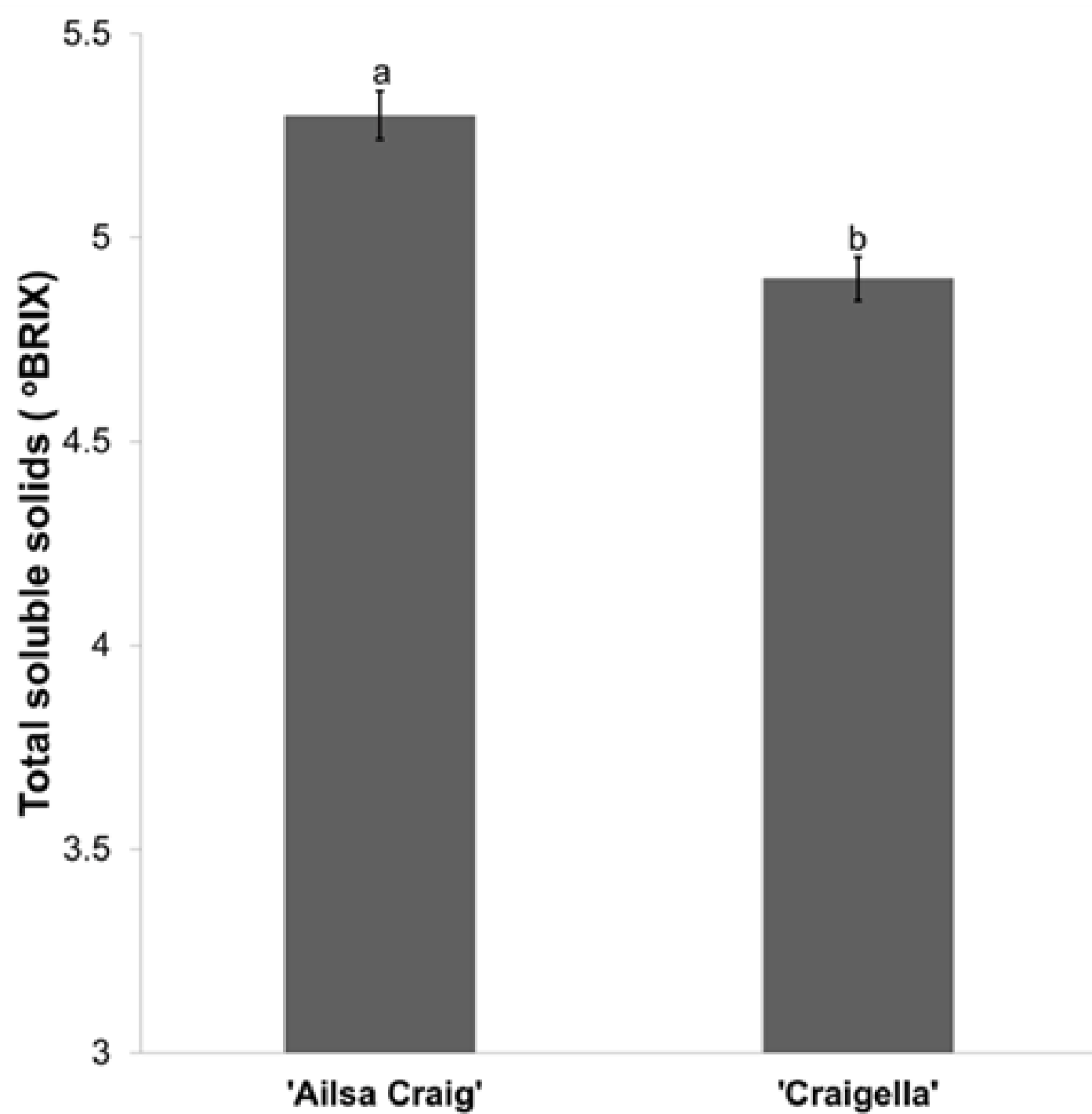
Regulating Tomato Fruit Chloroplast Development
Uniform ripening Encodes a Golden 2-like Transcription Factor

Ann L. T. Powell *et al.*
***Science* 336, 1711 (2012);**
DOI: 10.1126/science.1222218

“For ~70 years, breeders have selected tomato varieties with uniformly light green fruit before ripening, a characteristic that facilitates maturity determinations and promotes even ripening at the stem end. However, light green fruit ripen with reduced sugars, compromising traits that are valuable for processed products and the flavor of fresh fruit (fig. S1).”

Classical Map IL 10-1 F2 IL10-1xM82 RIL MMxT0937 **Candidate genes** *SIGLK2/U*







Ailsa Craig



Ailsa Craig *og^c*



Ailsa Craig *hp*



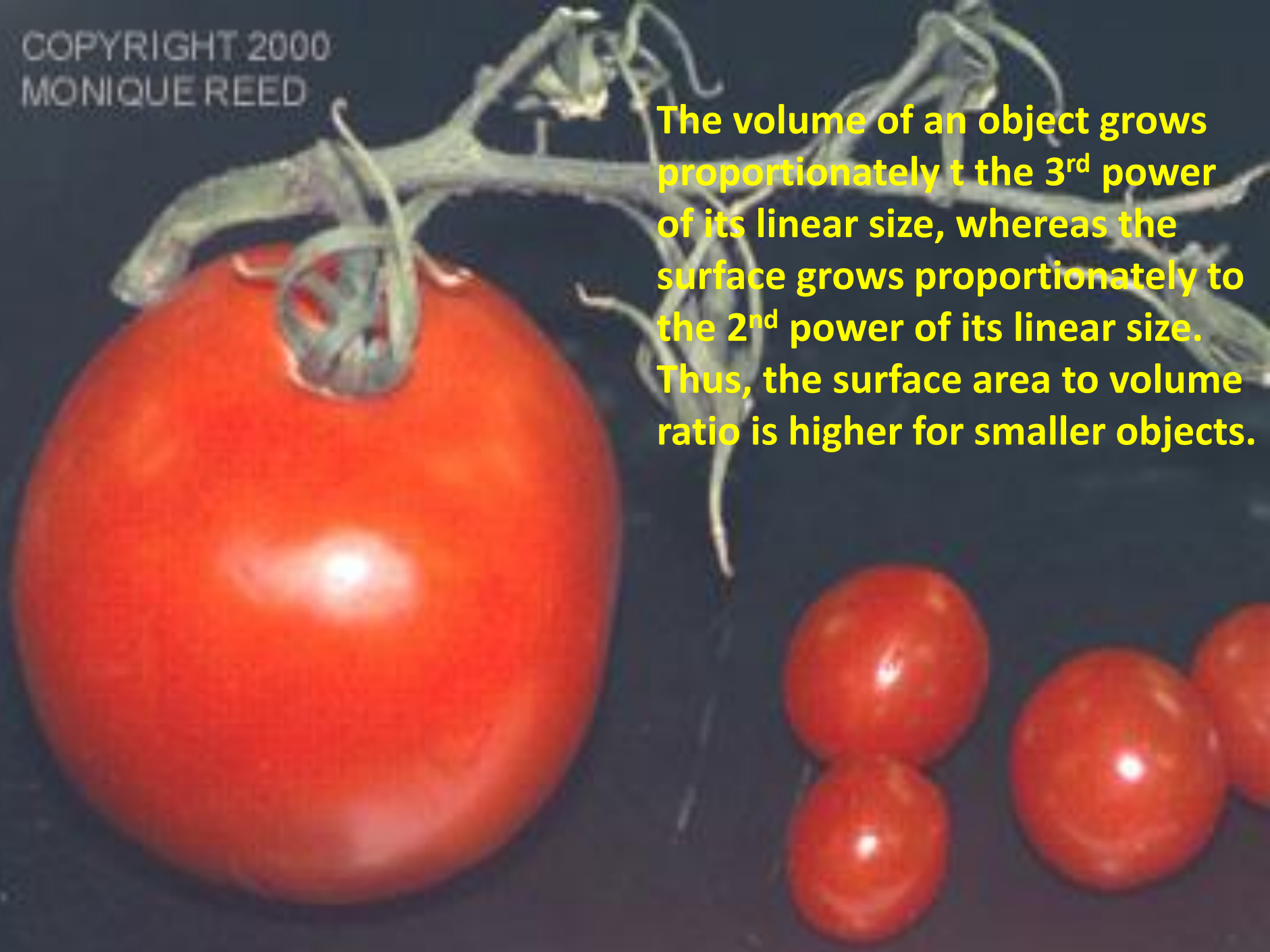
Ailsa Craig *hp og^c*

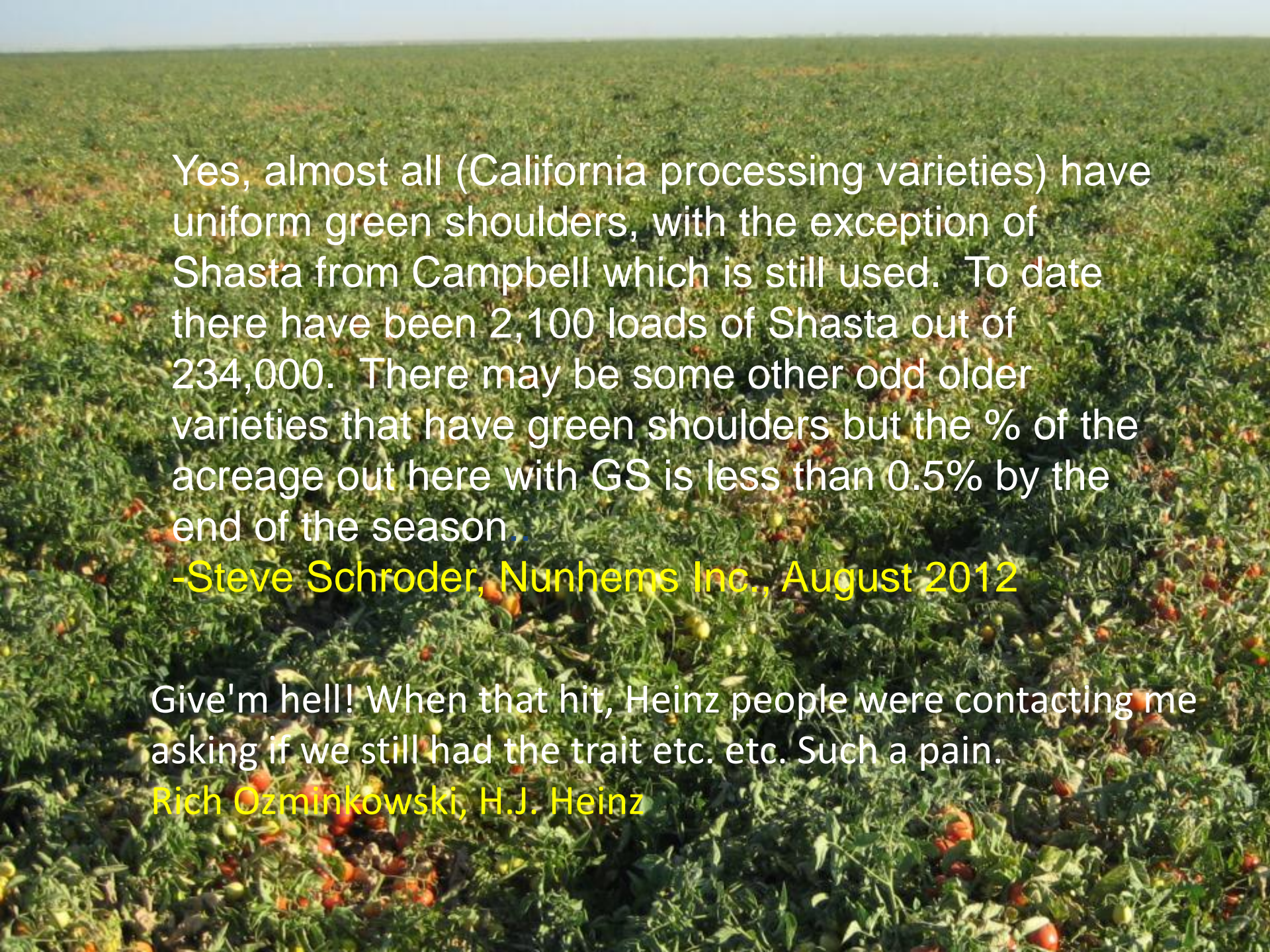
A Piece of the Puzzle Indeed; Why (some of) the Science Article May Be Odiferous

- ☐ Only 1 isogenic comparison was made
- ☐ The varieties compared had small (cherry) fruit
- ☐ Fruit load (harvest index) was not considered
- ☐ What about “green shouldered” fruit that are inside a tomato canopy?
- ☐ Can it be assumed that the effect is the same for *ug* as well as *uu* varieties?
- ☐ Genetic control of tomato flavor is complex, it's not just sugar levels

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The volume of an object grows proportionately to the 3rd power of its linear size, whereas the surface grows proportionately to the 2nd power of its linear size. Thus, the surface area to volume ratio is higher for smaller objects.





Yes, almost all (California processing varieties) have uniform green shoulders, with the exception of Shasta from Campbell which is still used. To date there have been 2,100 loads of Shasta out of 234,000. There may be some other odd older varieties that have green shoulders but the % of the acreage out here with GS is less than 0.5% by the end of the season..

-Steve Schroder, Nunhems Inc., August 2012

Give'm hell! When that hit, Heinz people were contacting me asking if we still had the trait etc. etc. Such a pain.

Rich Ozminkowski, H.J. Heinz

