Understanding evolution of resistance to pyramided Bt crops in *Helicoverpa zea*

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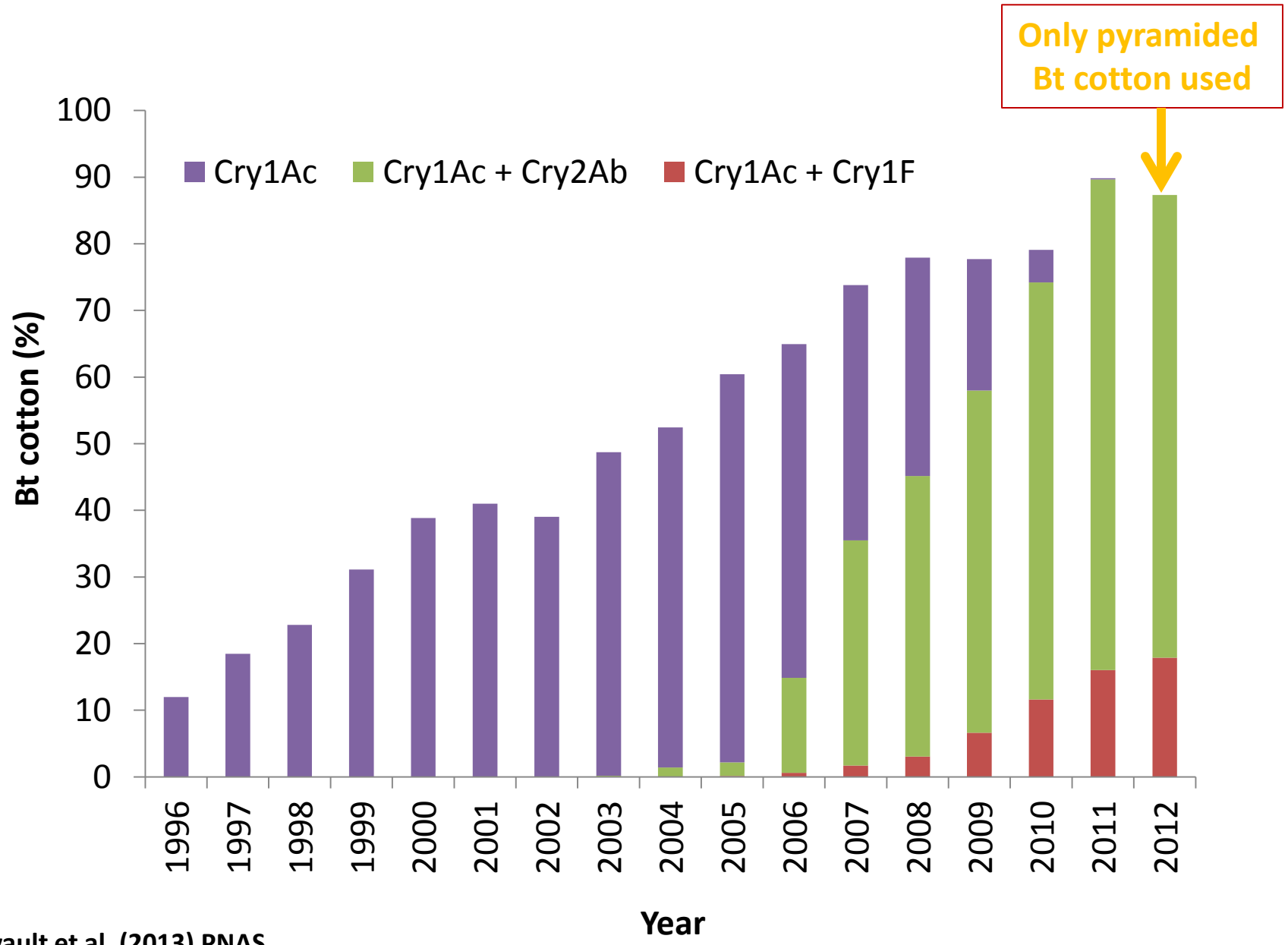
Pyramided Bt crops

- **Crops that produce two or more distinct Bt toxins that kill the same pest**

- **Pyramids are designed to delay evolution of resistance in pests and improve pest control**

Pyramided Bt cotton and Bt corn

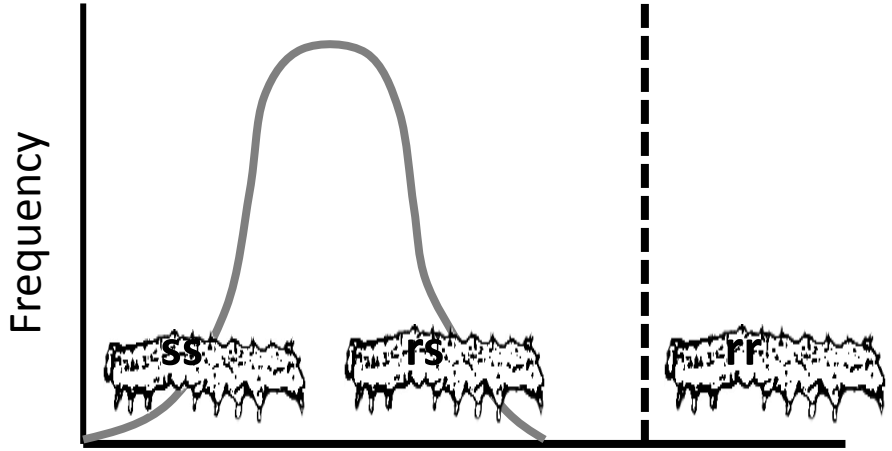
Percentage of total upland cotton planted to Bt cotton from 1996 to 2012 in the US



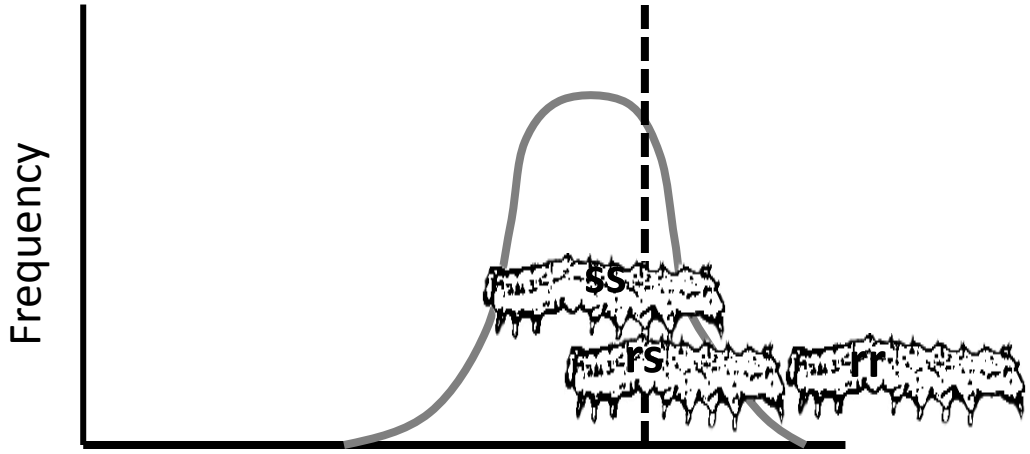
Brévault et al. (2013) PNAS

Success of refuge strategy for delaying resistance to pyramids depends on pest susceptibility to Bt toxins

Pest susceptible to Bt toxins → **Optimal conditions for durability of pyramids met**



Pest less susceptible to Bt toxins → **Optimal conditions for durability of pyramids less likely to be met**



Carrière et al. (2010) Evol. Appl.

Pectinophora gossypiella (US)

Helicoverpa zea

Susceptibility to Bt toxins

High ←

Low →

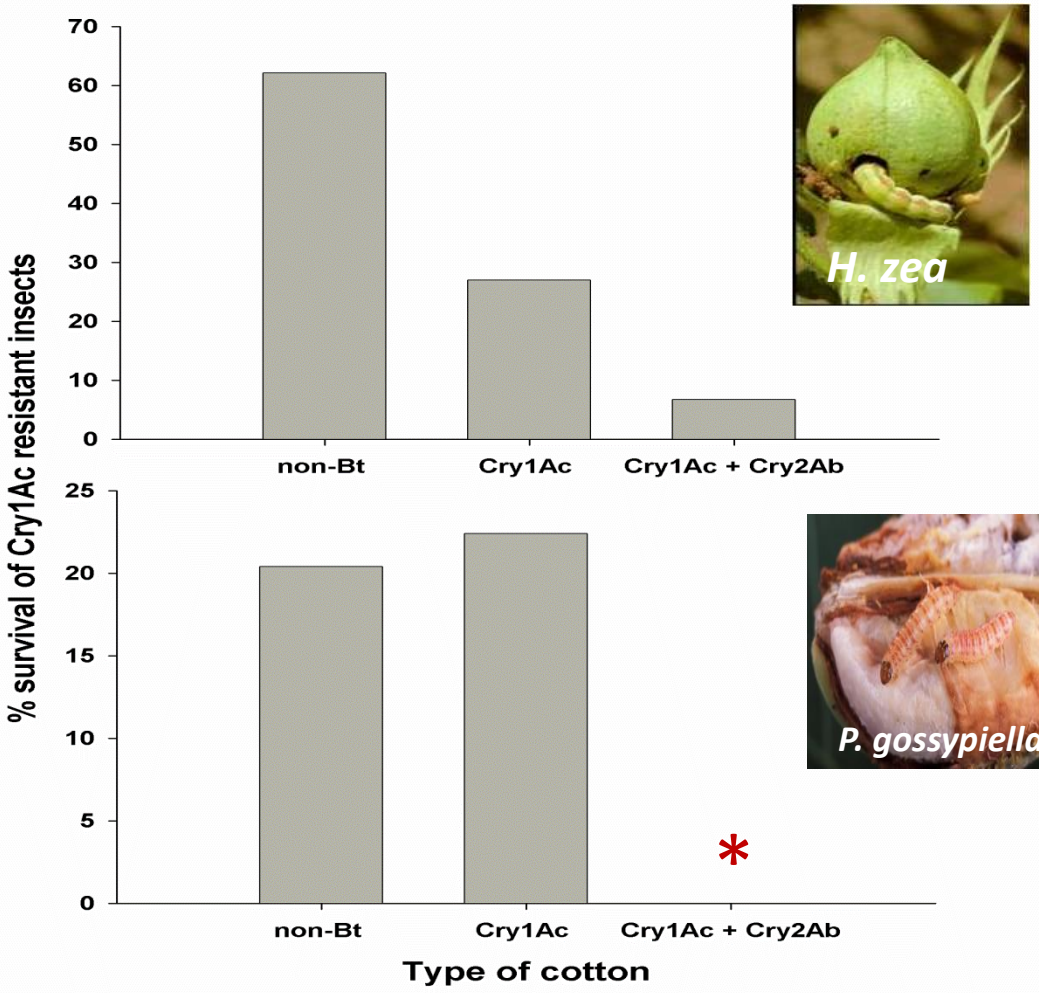
Likelihood of resistance

Susceptibility to Cry1Ac and Cry2Ab in pink bollworm (*Pectinophora gossypiella*) and cotton bollworm (*Helicoverpa zea*)

Pest	Toxin	LC_{50}	Ratio
H. zea	Cry1Ac	0.870	72.5
P. gossypiella	Cry1Ac	0.012	
H. zea	Cry2Ab	17.476	485.4
P. gossypiella	Cry2Ab	0.036	

Sivasupramaniam et al. (2008) JEE

Redundant killing: each toxin alone kills most susceptible insects, which means that *individuals resistant to one toxin are killed by other toxin in the pyramid*



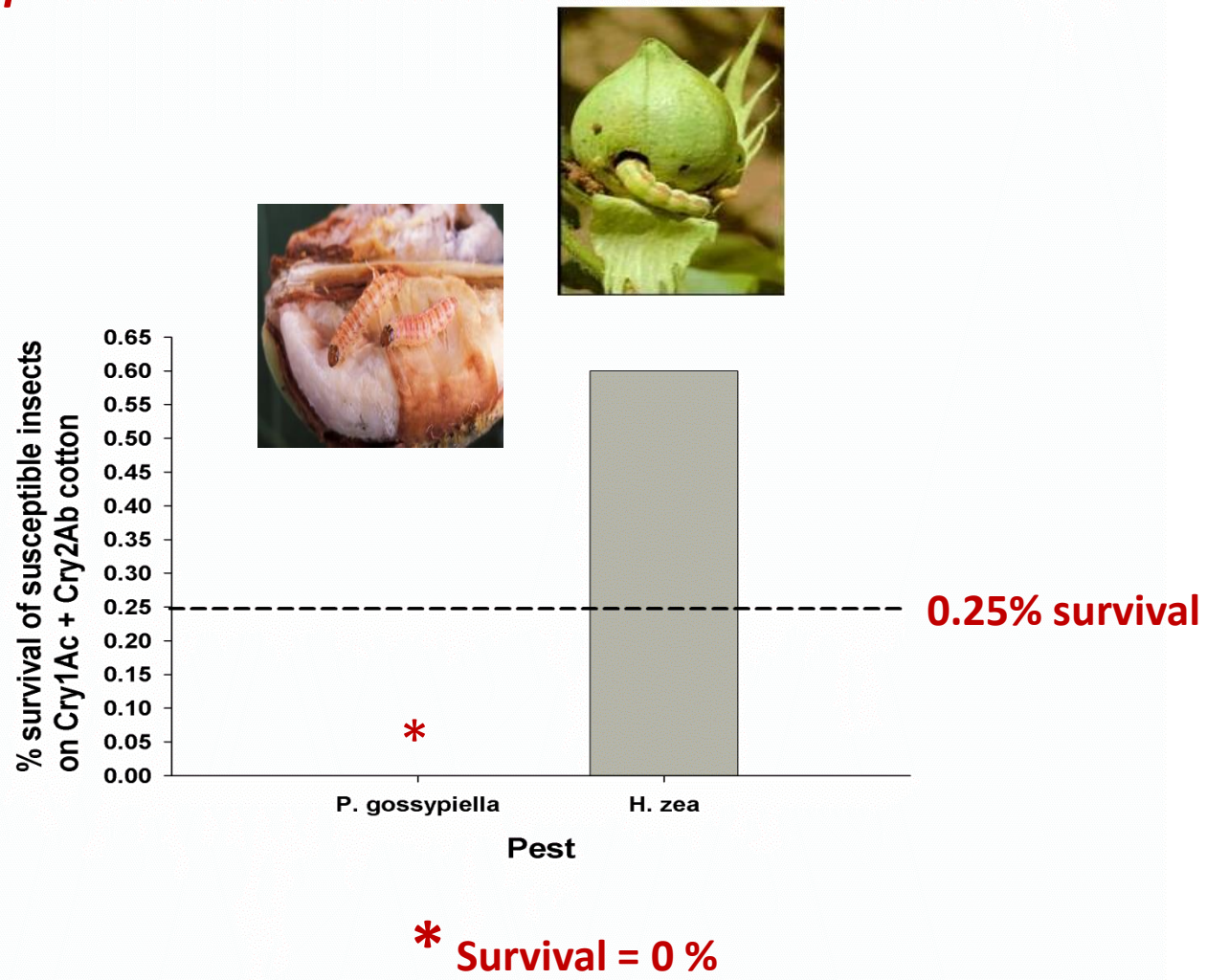
Redundant killing incomplete

Redundant killing complete

* Survival = 0 %

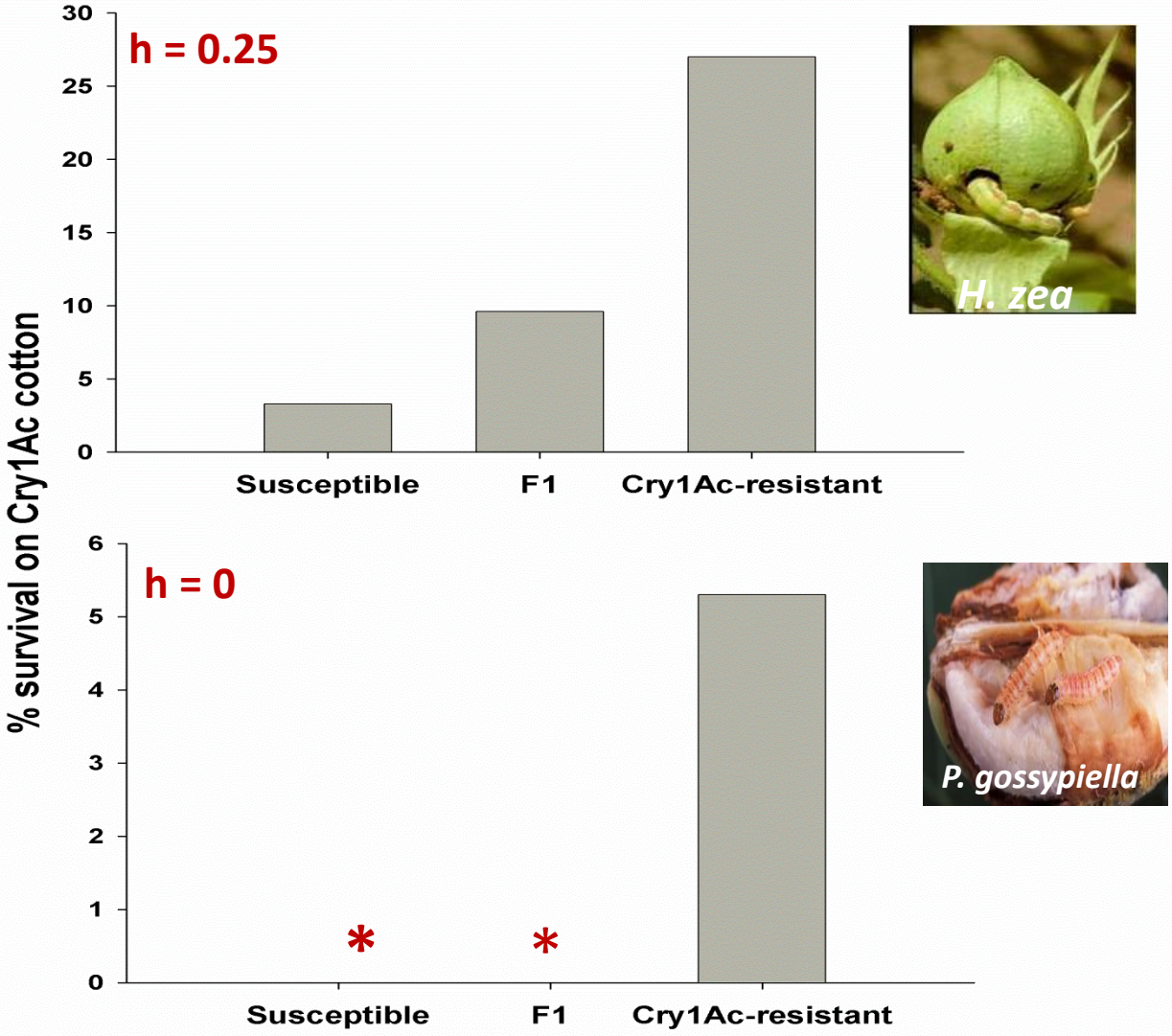
Brévault et al. (2013) PNAS; Tabashnik et al. (2002) AEM

Survival of susceptible insects on pyramids: each toxin of a two-toxin pyramid should kill at least 95% of susceptible individuals for redundant killing to be effective, which means that ***two-toxin pyramids are expected to kill at least 99.75% of susceptible insects***



Brévault et al. (2013) PNAS; Tabashnik et al. (2002) AEM

Dominance of resistance: resistance to each toxin of a pyramid is recessive, which occurs when ***all insects heterozygous for resistance are killed by single toxins in pyramids***



Brévault et al. (2013) PNAS; Liu et al. (2001) JEE

* Survival = 0 %

Cross-resistance: should be ***absent*** between toxins produced by a pyramid if susceptible insects can survive on pyramid; should be ***low*** if susceptible insects do not survive on pyramids

Survival to Cry2Ab in diet overlay bioassays

Strain	LC_{50} (µg/cm^2)	95% Fiducial limits
Susceptible	0.8	0.6 – 1.2
Cry1Ac-resistant	2.6	2.1 – 3.1

P < 0.05

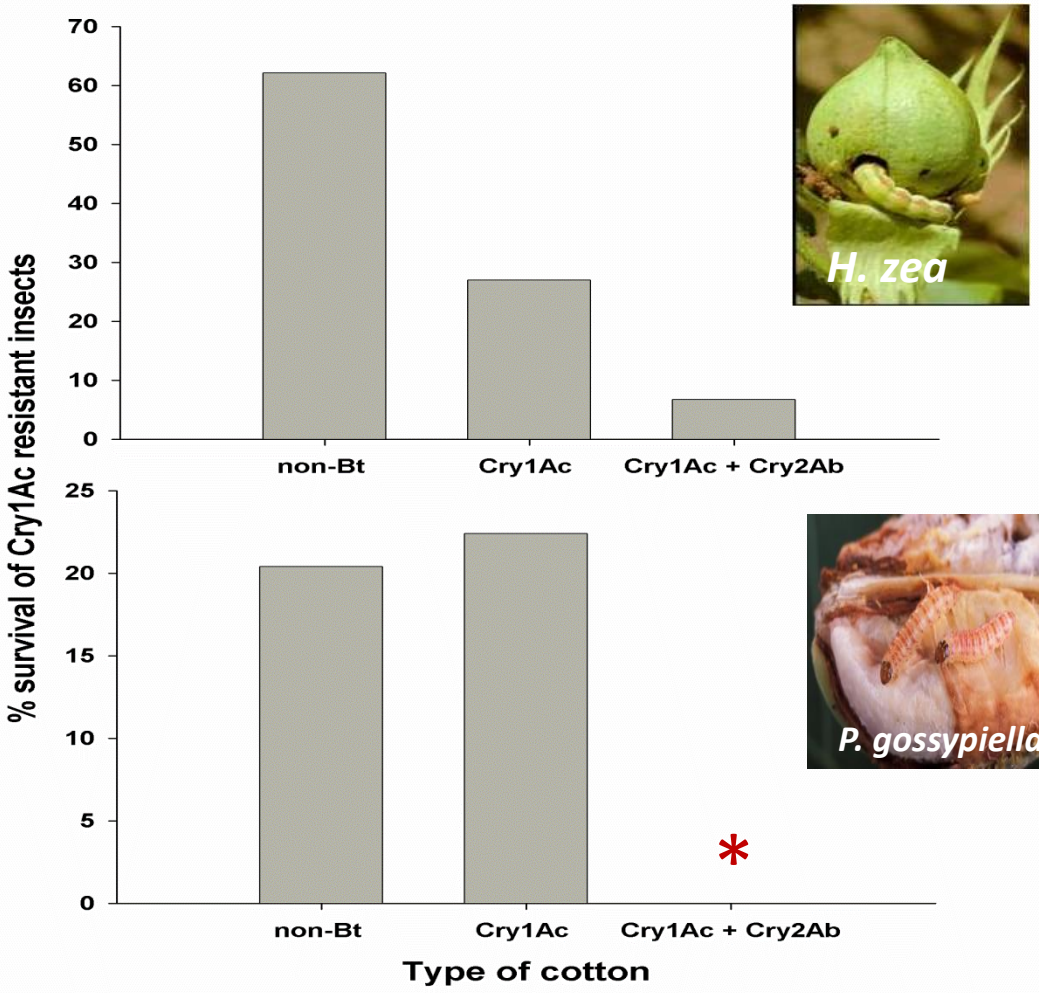
Survival to single concentration of Cry2Ab in diet incorporation bioassays

Strain	% survival at 1 µg/ml
Susceptible 1	0
Susceptible 2	0
Cry1Ac-resistant 1	7.1
Cry1Ac-resistant 2	10.0
Cry1Ac-resistant 3	5.1

P = 0.027

Tabashnik et al (2002) JEE; Welch et al. (*in prep*)

Redundant killing: each toxin alone kills most susceptible insects, which means that *individuals resistant to one toxin are killed by other toxin in the pyramid*



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Summary

Susceptibility to toxins Cry1Ac and Cry2Ab is much higher in *P. gossypiella* than *H. zea,* which affects how assumptions underlying success of pyramid strategy are met

Assumption	*P. gossypiella*	*H. zea*
Redundant killing	yes	no
Mortality of susceptible insects > 99.75%	yes	no
Recessive resistance to Cry1Ac	yes	no
“Meaningful” cross-resistance	no	yes

Implications

1) Conditions that favor durability of pyramided Bt crops are less likely to be met in pests with low than high susceptibility to Bt toxins

2) Pests with low susceptibility to Bt toxins have the greatest likelihood of evolving resistance: resistance management strategies for pyramided Bt crops will be improved if deviations from ideal conditions in such pests are taken into account

Acknowledgements

This research was funded by U.S. Department of Agriculture (USDA) Biotechnology Risk Assessment Grant Award 2011-33522-30729.