

**NATURAL REFUGE FOR COTTON
BOLLWORM AND TOBACCO
BUDWORM**

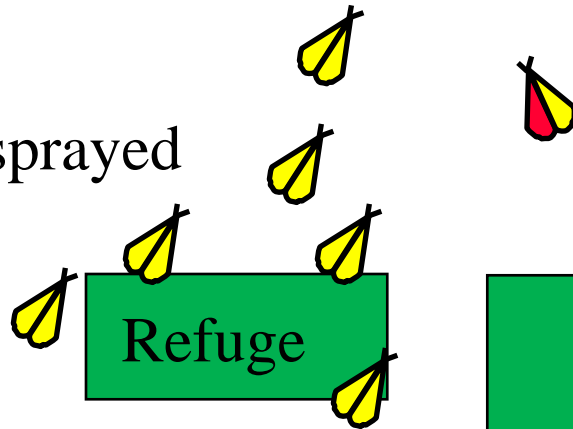
***PRACTICAL CONTRIBUTION OF HOST PLANT
DIVERSITY TO COTTON IRM***

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JOHN GREENPLATE
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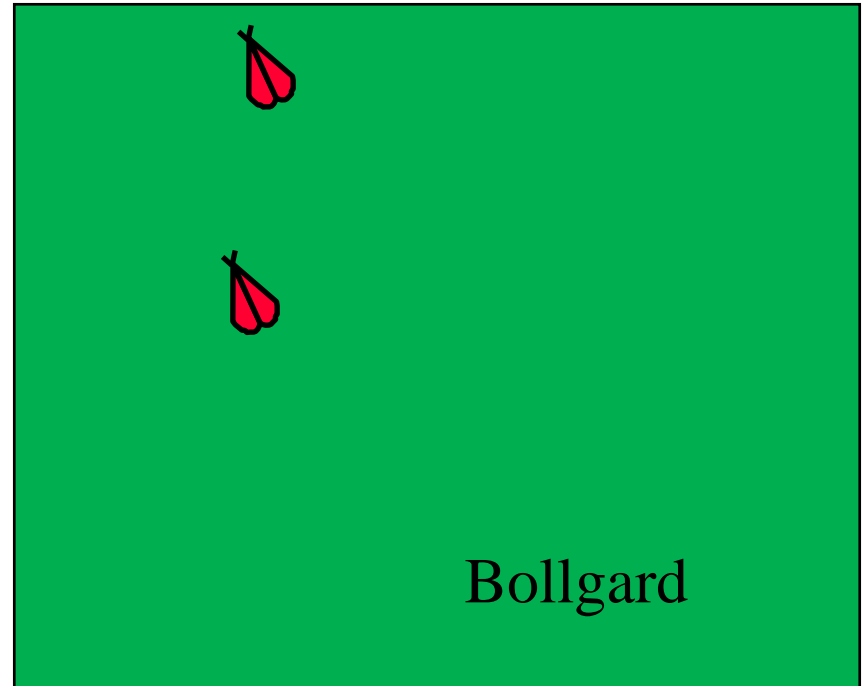
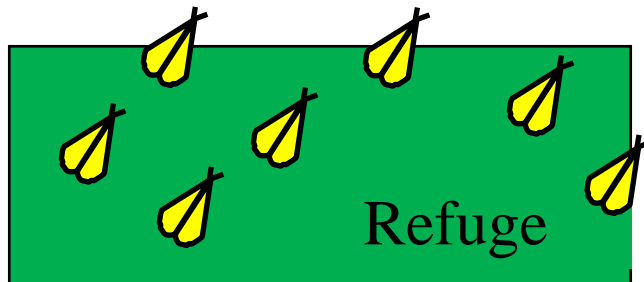
Original refuge requirement for Bollgard[®] cotton in US

5% unsprayed



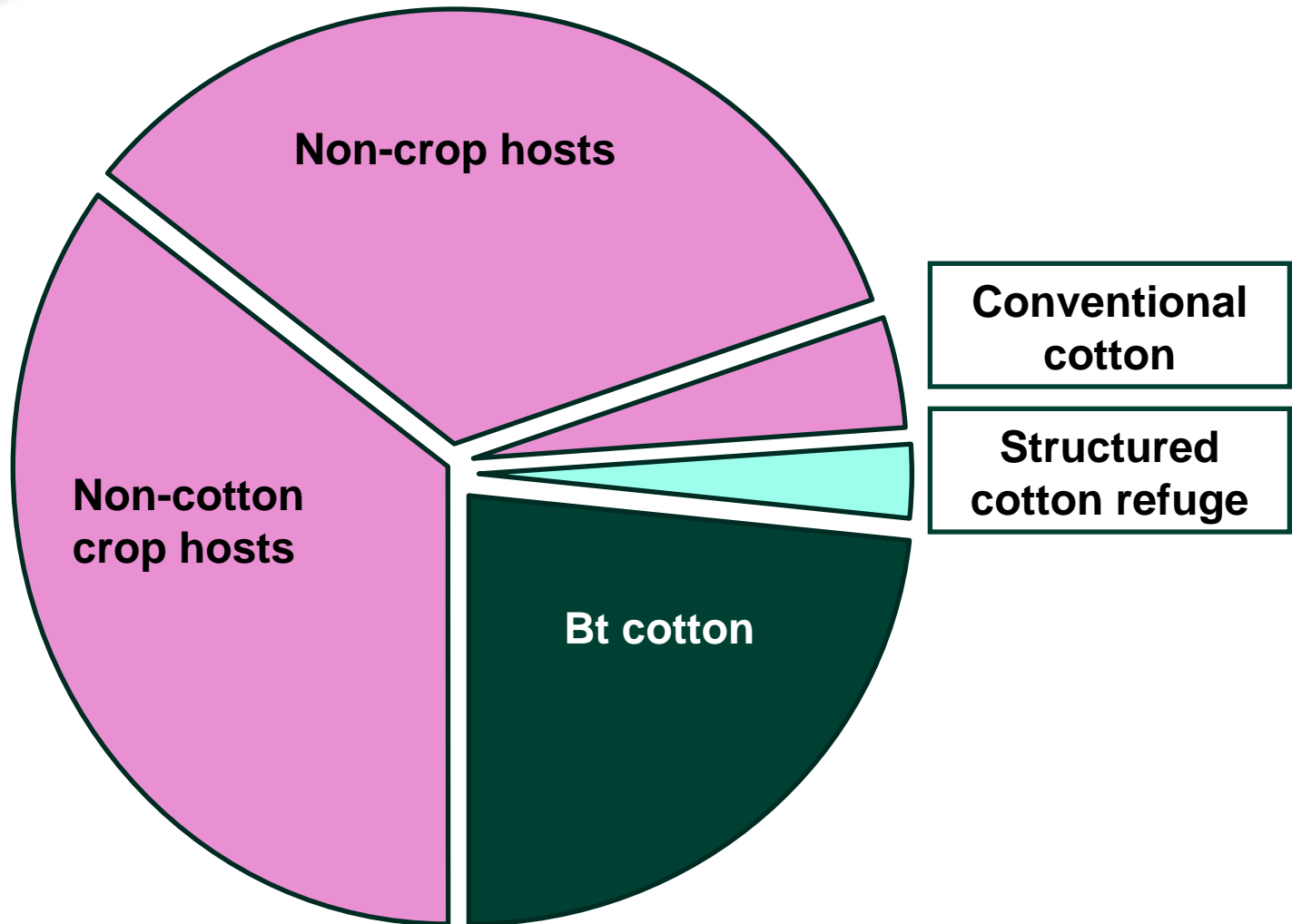
OR


20% sprayed



Refuge for *Bt* Cotton

Effective refuge = structured refuge + natural (unstructured) refuge





Idea of natural or unstructured refuge considered 2000-2001

- Costs of structured refuge
 - Growers
 - Monsanto
- Bollgard[®] II approaching marketplace
 - 2 MOA amenable to reduced refuge
- EPA Conditions of Bollgard[®] reregistration



Condition of the Bollgard Reregistration in 2001

5% external unsprayed refuge option was to expire on September 30, 2004.

One of EPA's conditions:

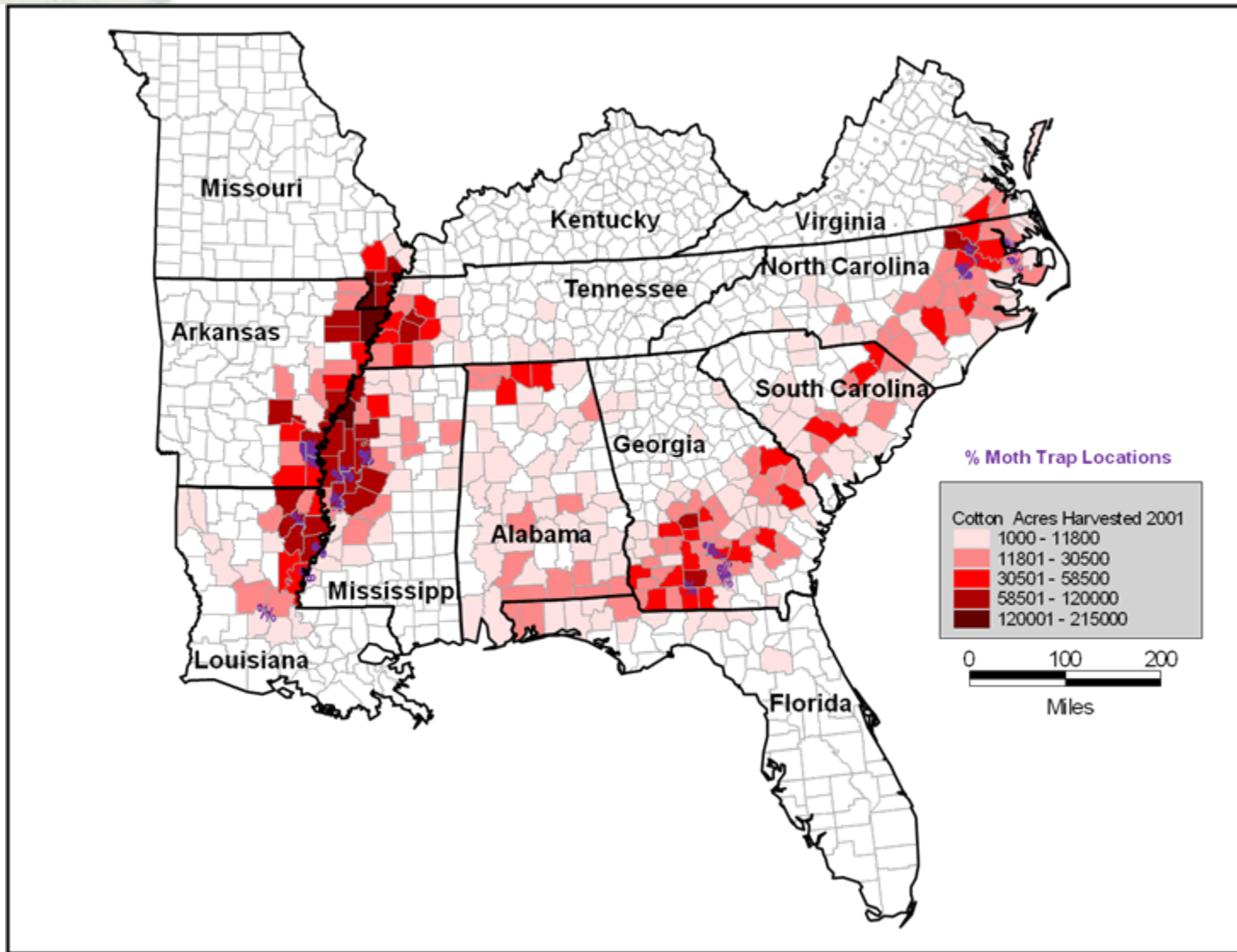
“The registrant must conduct research on whether alternate hosts of *Helicoverpa zea* (bollworm) provide an effective refuge for *Bt* cotton.”




Origins of TBW and CBW

- 2002-2006
- Landscape cropping pattern data collected
 - Satellite imagery
 - Published crop acreage data
- CBW crop production estimates
- Extensive adult moth trapping
- Analytical methods utilized to establish larval origins
 - $^{13}\text{C}/^{12}\text{C}$ ratios – differentiate C3 and C4 plant sources
 - Gossypol – differentiate cotton and non-cotton sources

CBW origins 2002-2003 study



Jackson et al. 2008. *Entomologia Experimentalis et Applicata*. 126:89-106.



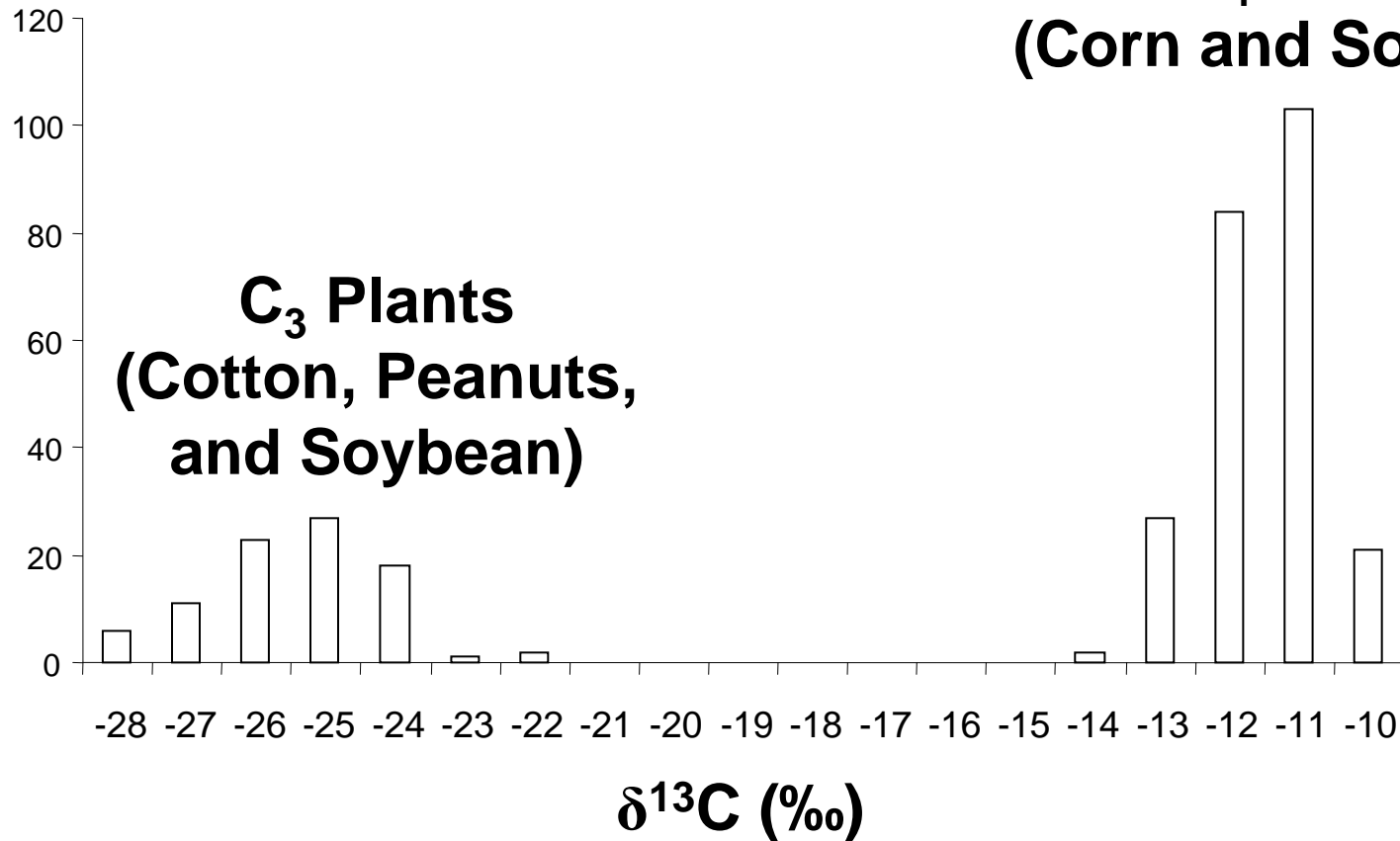
Stable Carbon Isotope Analysis: C₃/C₄ Host Plant Determination

- Larval host plant physiology is reflected in the wings of adult CBW
- **C₃ plants (e.g., cotton, peanut, and soybean) are more depleted in ¹³C relative to ¹²C than C₄ plants (e.g., corn and grain sorghum)**

$\delta^{13}\text{C}$ Values from Field-collected CBW

**C₄ Plants
(Corn and Sorghum)**

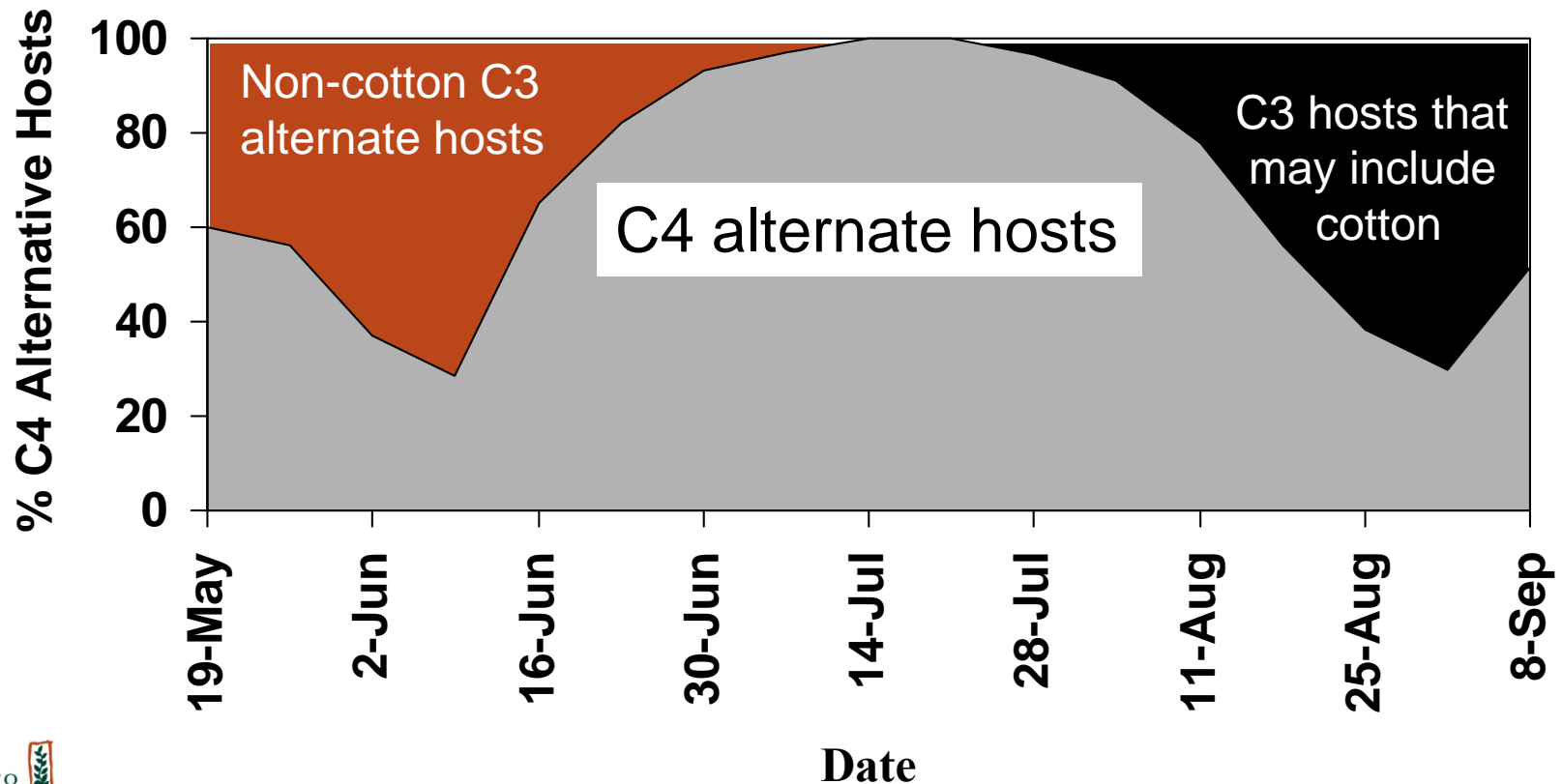
Frequency of moths



**C₃ Plants
(Cotton, Peanuts,
and Soybean)**

Results of C₃/C₄ CBW Studies

- The majority of cotton bollworm are produced by non-cotton hosts





Complementary CBW survey 2005-2006

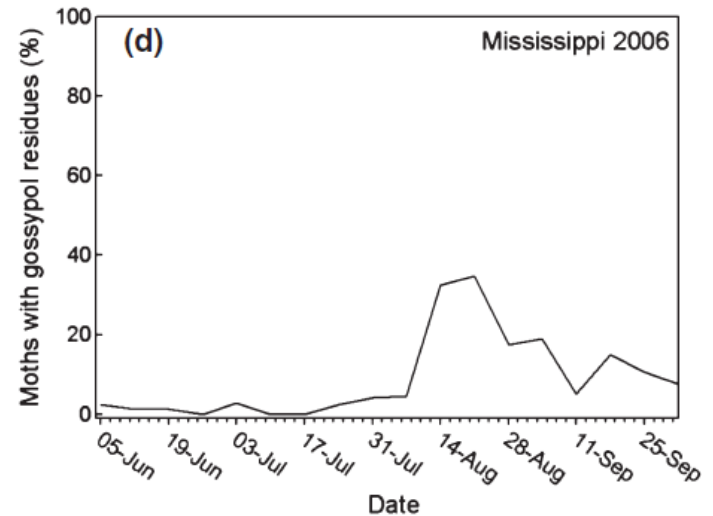
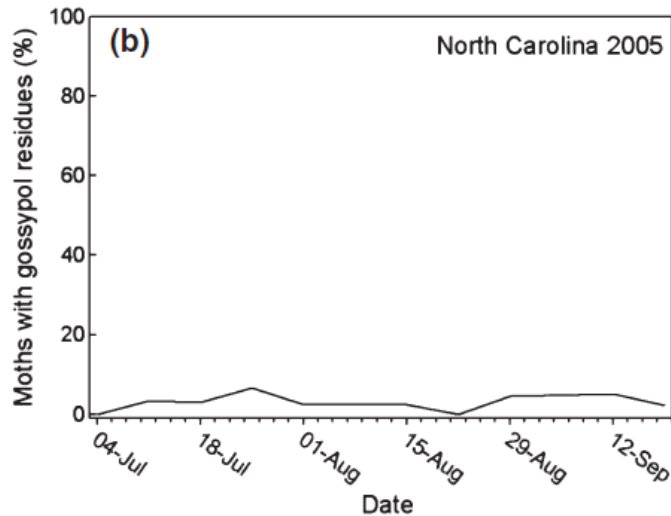
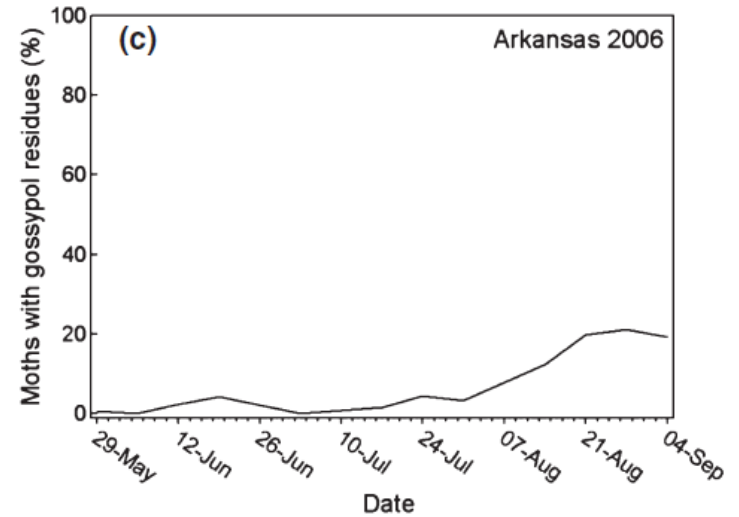
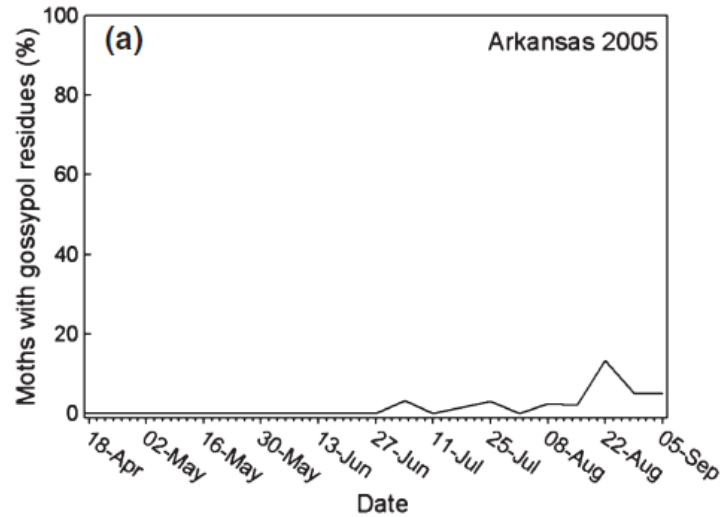
- Gossypol: terpene aldehyde exclusive to cotton and close relatives
- Found in tissues ingested by larvae
- 1-3% of total gossypol ingested by larvae retained as a derivative in the adult moth
- HPLC/Mass Spec analysis of gossypol & derivatives in adult enables determination of larval diet source as cotton or non-cotton



CBW 2005-2006 gossypol survey

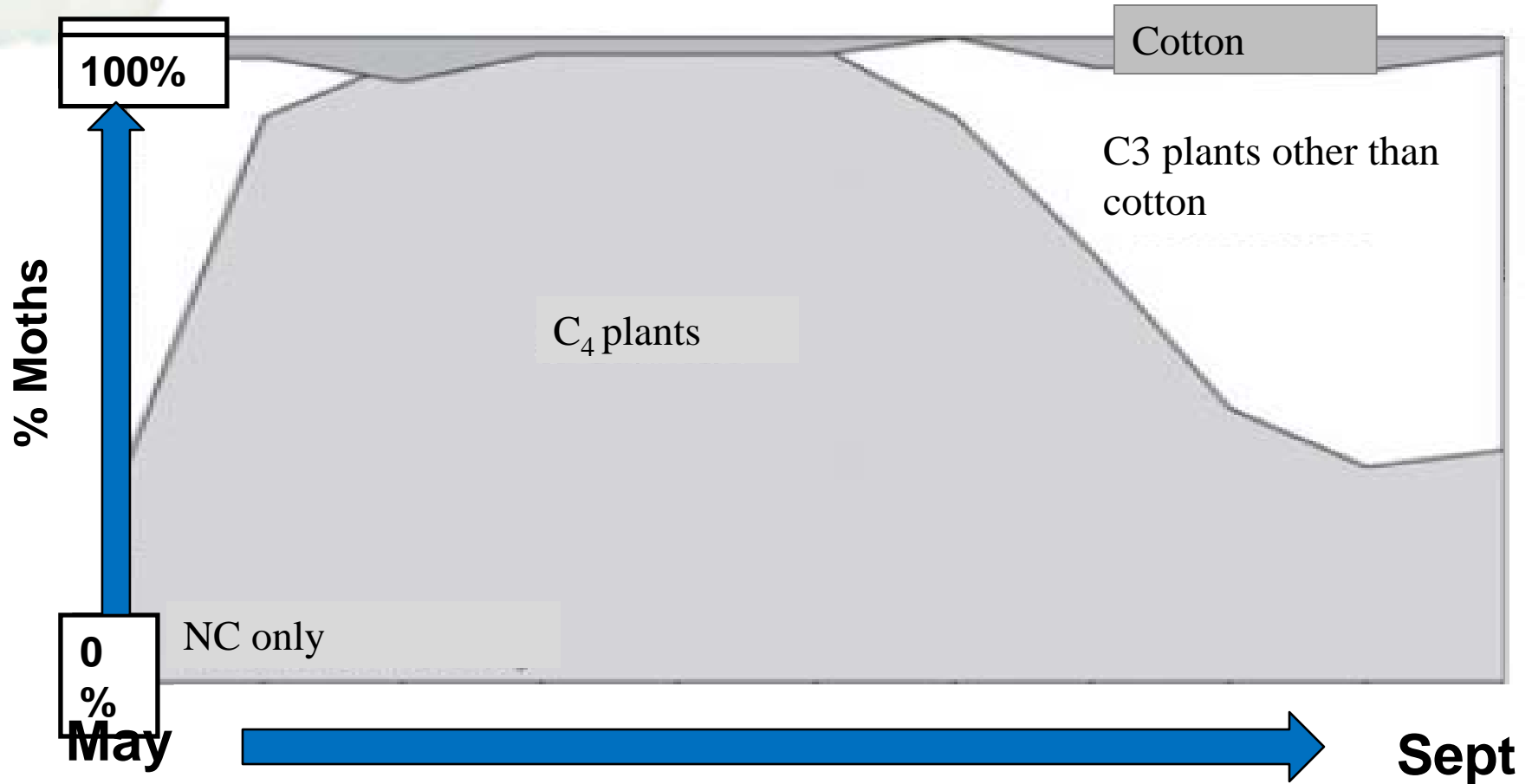
- AR (2005; 2006); MS (2006); NC (2005)
- Pheromone traps located in regions with historic high bollworm densities and high adoption of Bollgard/Bollgard II
 - AR 14 sites in '05 and '06
 - NC 10 sites in '05
 - MS 18 sites in '06
- 2005 – 1773 moths
- 2006 – 2749 moths

CBW moths from cotton 2005-2006



CBW origins

Integrated summary of C₃/C₄ and gossypol data



Head et al. 2010. *J. Appl. Ecol.* 47:583-592.



CBW Conclusions

- C_3/C_4 study shows majority of CBW are produced by C_4 hosts
- Published host utilization data agree (Jackson et al 2008) and suggest strong contribution of maize and sorghum
- Gossypol study suggests that cotton is a relatively small contributor to total populations of CBW adults found in and around Bt cotton fields



Non-Bt cotton



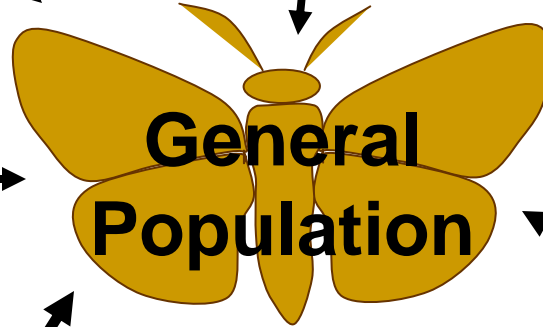
Weed Hosts



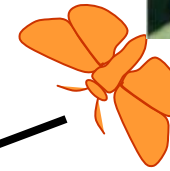
Soybeans



Peanuts



Bollworm



Grain sorghum



Field corn



Bollgard cotton



***Heliothis virescens* has many non-cotton hosts**

What do they contribute as refuge?

	1st generation (March - early June)	2nd/3rd generation (June - August)	4th + generation (August - October)
Carolinas	Wild hosts: deergrass, beggarweed, morning glory, prickly sida, bicolor lespedeza, toadflax, Desmodium spp., Ipomoea spp.	Crops: tobacco, cotton, peanut, soybean Wild hosts: deergrass	Crops: regrowth cotton, peanut, soybean, tobacco Wild hosts: deergrass, beggarweed, morning glory, prickly sida, others
Mississippi Delta	Wild hosts: clover, velvetleaf, vetch, geranium	Crops: cotton, soybean, some vegetables	Crops: cotton, soybean Wild hosts: beggarweed, burcucumber, empress tree, spider flower, prickly sida, Royal Paulownia, others
Texas	Wild hosts: paintbrush, bluebonnet, vetch, wild tobacco Crops: alfalfa	Crops: cotton, tomato	Crops: alfalfa, tomato Wild hosts: Amantillo, hillside verbena, wild tobacco



TBW origins 2004-2005

- Attempted to identify the sources of tobacco budworms found in and around cotton fields
- Trapped adults adjacent to cotton fields in intensive cotton-growing regions with high *Bt* cotton adoption
- Analyzed for presence of gossypol
 - Cotton vs. non-cotton sources
- Linked composition of moth populations to cropping patterns at various scales

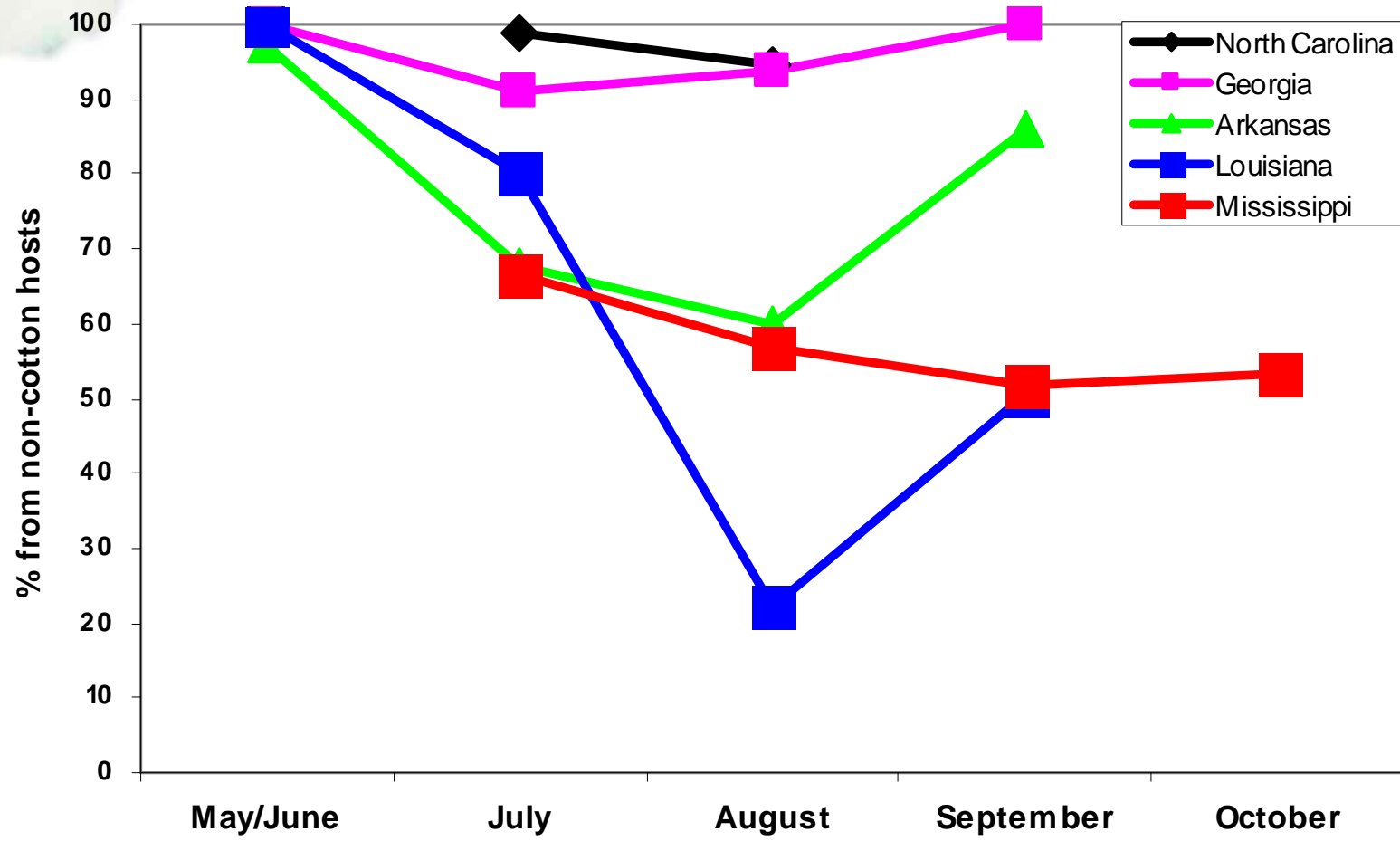
States and Counties where TBW were Collected for Analysis

States	Counties with traps 2004	Counties with traps 2005
Arkansas	Drew, Little River, Mississippi	Craighead, Drew, Mississippi
Georgia	Decatur, Dooly, Mitchell, Seminole, Terrell	Appling, Decatur, Dooly, Mitchell, Terrell, Tift
Louisiana	Bossier, Catahoula, Franklin, Rapides, Tensas	Bossier, Catahoula, Franklin, Rapides, Tensas
Mississippi	Bolivar, Carroll, Chickasaw, Clay, Coahoma, Grenada, Humphreys, Itawamba, Lee, Leflore, Lowndes, Madison, Monroe, Noxubee, Prentiss, Tunica, Washington, Yazoo	Bolivar, Carroll, Chickasaw, Clay, Coahoma, Grenada, Humphreys, Itawamba, Lee, Leflore, Lowndes, Madison, Monroe, Noxubee, Prentiss, Tunica, Washington, Yazoo
North Carolina	Lenoir, Pitt, Wilson	Edgecombe, Halifax, Lenoir, Pitt, Wilson
Tennessee	Carroll, Crockett, Dyer, Fayette, Gibson, Haywood, Lake, Lauderdale, Madison, Tipton	Carroll, Crockett, Dyer, Fayette, Gibson, Haywood, Lake, Lauderdale, Madison, Tipton
Texas	-	Austin, Burleson, Fort Bend, San Patricio

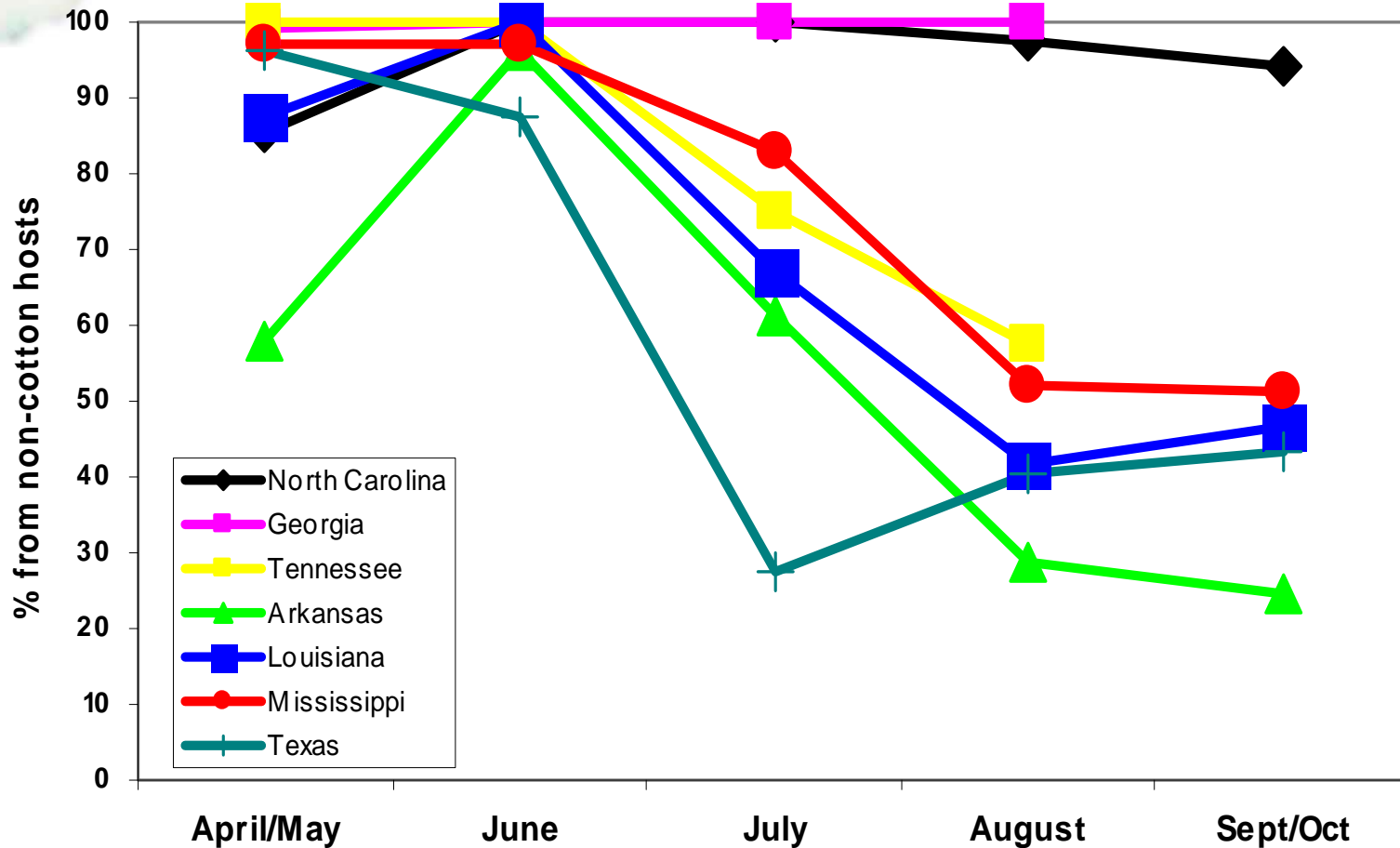
TBW Collected and Assayed for Gossypol in 2004 and 2005

State	2004				2005			
	# collected	# assayed	Counties	Locations	# collected	# assayed	Counties	Locations
AR	399	399	3	9	4327	1216	3	41
GA	8252	896	5	9	5396	544	6	8
LA	487	417	3	4	936	245	5	7
MS	1049	989	18	42	1892	1076	16	40
NC	6794	684	3	9	2865	1392	5	38
TN	<20	0	-	-	126	82	10	15
TX	-	-	-	-	1662	688	4	8

Proportion of Tobacco Budworm from Alternative (non-cotton) Hosts: 2004



Proportion of Tobacco Budworm from Alternative (non-cotton) Hosts: 2005





TBW Conclusions

- Gossypol analyses suggest significant contribution of non-cotton host plants to total population of adult TBW moths found in and around Bt cotton fields
- Sustained high abundance of natural hosts demonstrated
 - Eastern Cotton Belt (e.g., Abney et al. '04)
 - Mid-South (e.g., Schneider and Cross '99)
- In all states, effective refuge calculated to be at least 20% throughout season



Non-Bt cotton



**Weed
Hosts**



Soybeans



Peanuts



Budworm

Alfalfa



Tobacco



Bollgard cotton







Final Disposition


- 2nd generation cotton traits (Bollgard II:2003) provide additional IRM value (2 MOA) for both TBW and CBW
- Conservative models predict that Natural Refuge for TBW and CBW is sufficient to ensure the durability of Bollgard II cotton
- EPA – Based upon these data and subsequent modeling results:
 - 5% refuge retained for Bollgard
 - No structured cotton refuge required for Bollgard II and products like it in US (excluding PBW region)




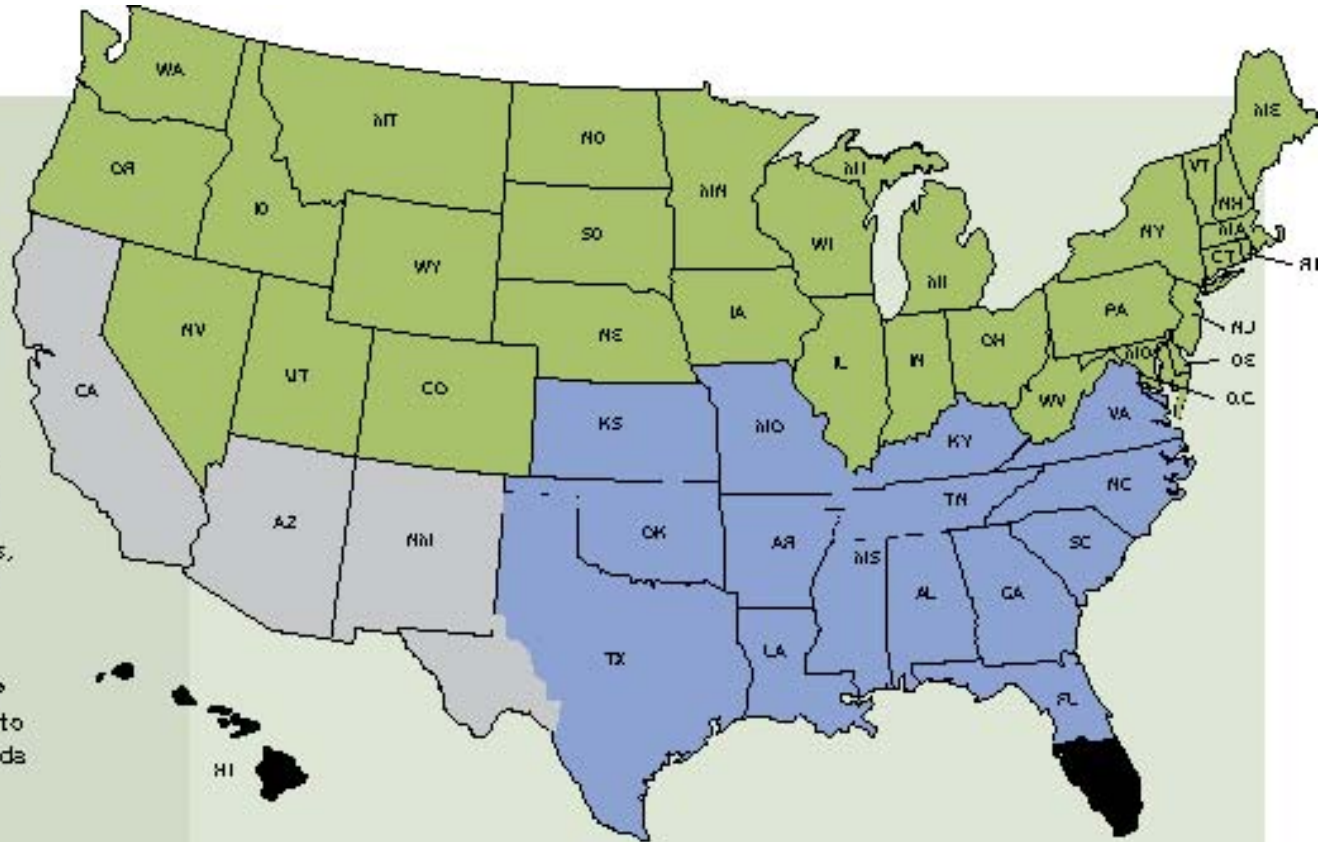
US Natural Refuge Area

 Natural Refuge

 Refuge Required

 EPA-Designated Pink Bollworm Area
A refuge **MUST** be planted in California, Arizona and New Mexico, and the Texas counties of Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward and Winkler.

 No Safe
Sale or commercial planting of Genuity® Bollgard II® is prohibited in Hawaii, Puerto Rico, the U.S. Virgin Islands, and in Florida south of Route 60 (near Tampa).





Implications for other systems

- Heterogeneous cropping systems in the developing world may provide environments where natural refuge can contribute significantly to cotton IRM
 - In areas where major pests (and resistance threats) are polyphagous (*H. armigera*)
 - When 2nd generation Bt traits (2+ MOA) are deployed



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