Environmental effects on an HPPD inhibiting herbicide’s persistence in different soils over a four-year period

Cheryl L. Dunne
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Abstract

Multiple year applications of bicyclopyrone increased the risk of carryover to soybeans compared to single year applications at some trial sites, but the results were not consistent from year to year. The environmental conditions promote the best degradation of bicyclopyrone were those with ample soil moisture and milder climates similar to those found in the southern United States. These conditions are also the most favorable for soil microbial activity. For northern states, from application through corn harvest (June through September) and springtime the following year before soybean planting (April – May) will be the only times for degradation prior soybean planting. Sufficient soil moisture and warm/mild temperatures, and longer growing periods can help to accomplish degradation. High CEC or coarse texture soils in combination with drier, cooler climates and shorter growing periods increase the likelihood of bicyclopyrone carryover into soybeans.

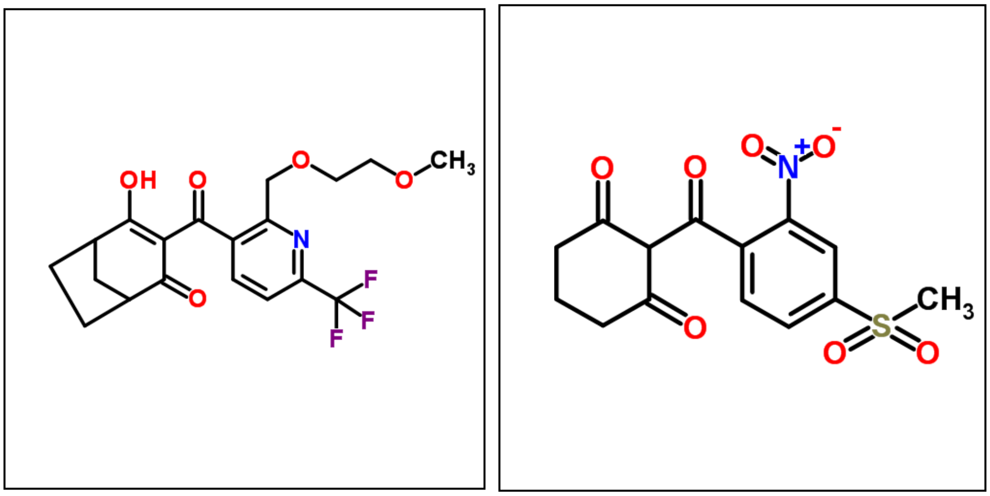
Introduction

HPPD inhibiting herbicides block the enzyme, p-Hydroxyphenylpyruvate dioxygenase (HPPD). This enzyme is involved with the formation of carotenoids, which protect chlorophyll in plants from being destroyed by sunlight. If chlorophyll is destroyed, the plants turn white and die. HPPD inhibiting herbicides tend to have a greater effect on broad leafed plants or dicots versus grasses or monocots, and generally result in a faster, more efficient system of metabolizing the herbicide, thus making it ineffective once inside the plant. Triketone HPPD inhibitors were discovered by a biologist interested in allelopathic control of weeds in the back yard. This biologist observed that very few weeds emerged underneath bottlebrush plants (*Callistemon citrinus*). Soil from beneath these plants was sampled and extracted in the lab and the triketones were discovered. Syngenta registered the triketone, mesotrione, as Callisto® herbicide in 2001. Mesotrione is taken up by both the roots and shoots of plants and has residual activity in the soil (Cornes, 2001). Residual herbicides provide the benefit of extended weed control in a crop; however, they may persist longer than expected under certain environmental conditions and is dependent on the herbicide applied (Colquhoun, 2006). The half-life of mesotrione in soil ranges from 5 to as many as 62 days (Dyson, 2002), depending on soil type, organic carbon, and soil pH. Complaints from customers regarding carryover of mesotrione from maize to rotational crops have been documented (personal conversation with Dr. G. Vail, Syngenta). The carryover from HPPD herbicides (mesotrione) has occurred in fields whose soil texture was sand or sandy loam; or, following a fertilizer application of anhydrous ammonia that was “knifed in”. Long strips of bleached plants could be seen across the field where the anhydrous ammonia caused a localized change in soil pH, thus causing the release of mesotrione residues from the soil colloids.

Due to the success of Callisto® herbicide, Syngenta has developed another HPPD inhibiting herbicide, bicyclopyrone, for use in maize. This herbicide has not yet received registration and is currently in the experimental phase. The half-life of bicyclopyrone has not been fully evaluated though it may be similar or slightly longer than that of mesotrione because of the methoxy group tail (Vail, internal communication). Molecular structures and some physical chemical properties are listed below (Figure 1.)

Figure 1.

Bicyclopyrone Mesotrione



CSID:11488158, http://www.chemspider.com/
Chemical-Structure.11488158.html
(Accessed 16:54, Nov 17, 2012)

Log Kow (KOWWIN v1.67 estimate) = 1.58
Water Solubility at 25 deg C (mg/L): 138.7
Soil Adsorption Coef. (PCKOCWIN v1.66):
Koc : 13.11, Log Koc: 1.118
pKa: 3.06

CSID:153301, http://www.chemspider.com/
Chemical-Structure.153301.html
(Accessed 16:49, Nov 17, 2012)

Log Kow (KOWWIN v1.67 estimate) = 1.49
Water Solubility at 25 deg C (mg/L): 157.5
Soil Adsorption Coefficient (PCKOCWIN v1.66):
Koc : 141.7, Log Koc: 2.151
pKa 3.1

The Kow (oil/water partitioning coefficient) data of the two molecules above are similar with bicyclopyrone have a slightly higher value. Bicyclopyrone is slightly less water soluble than mesotrione and the Koc of bicyclopyrone is significantly less (about 10X) than the Koc of mesotrione which alludes to the fact that bicyclopyrone is likely more mobile in the soil profile and thus is more likely to leach. Both compounds are slightly acidic in nature with a pKa about 3.1 and are mainly degraded by microbial activity (2012, Vail, internal communication), however the chemical structure of bicyclopyrone may render it less favorable for microbial breakdown than mesotrione.

Early field trials and soil bioassays evaluating the soil activity of bicyclopyrone (BIR) had shown that residues in the soil were at levels great enough to cause injury to sensitive rotational crops like soybeans the following year. This was not consistent among the field trial locations since trial sites did exhibit any injury symptoms to rotational crops sown. With the fact that the degradation of HPPD herbicides is mainly biologically driven, drought, drainage, air temperatures below 50 F / 10 C can negatively affect degradation by slowing it down (Dharmasri, 2004, internal Syngenta document). Low pH, which increases Koc and high clay and/or soil organic matter, leads to higher binding, slowing down degradation (Dyson, 2002). Longer growing seasons similar to those in southern United States provide a more favorable environment for degradation. For north-western states, the season of application will be the only time for degradation until soybean planting. Good winter/spring moisture and mild winters can help accomplish degradation. Though many factors can be involved with degradation of herbicide in the soil, adsorption onto soil colloids can have a key role in the rate of degradation (Dyson, 2002), especially since the molecule has to be free in the soil solution before the microorganisms can use them (Sylvia, 2005). The degradation of the HPPD herbicides, for example mesotrione, were reduced to less than 1 ng/g soil on a rare occasion (Dharmasri, 2004, internal Syngenta document). Therefore, these compounds almost never completely degrade by the time soybeans are replanted in the north. HPPD-sensitive soybean varieties could be an issue compared to tolerant varieties that show no injury symptoms when exposed to eight times the amount of bicyclopyrone residues in soil that would ordinarily injure the sensitive varieties.

Objectives

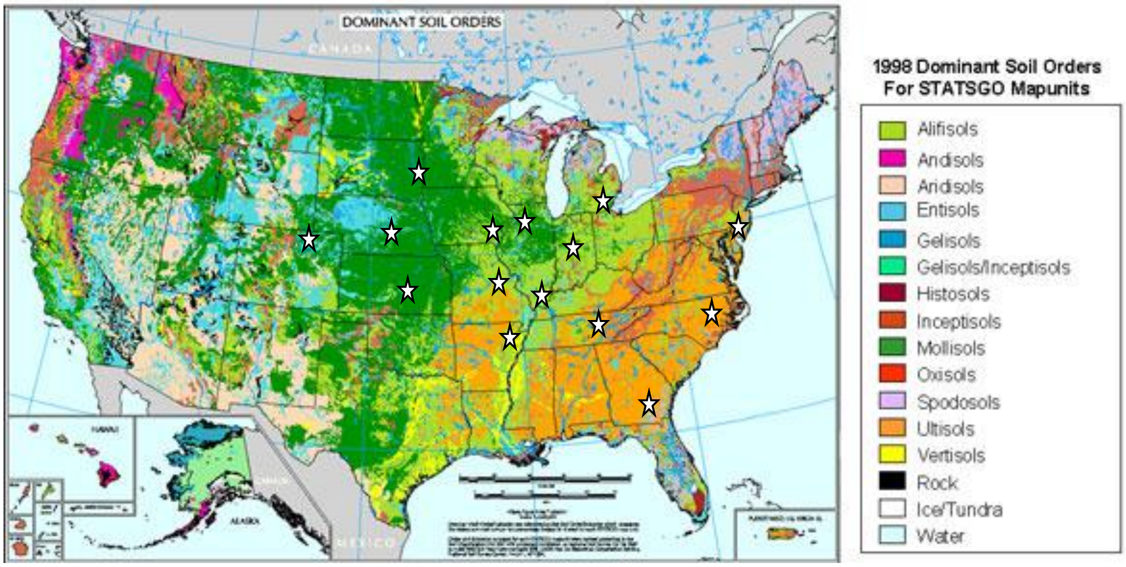
Determine if multiple year applications of this herbicide increase the risk of carryover to soybeans compared to single year applications by accumulating residues in the soil? Which environmental conditions promote the best or the least degradation of this compound? This information will provide guidance for writing the product label once the EPA registers bicyclopyrone. The proposed study followed a quantitative research approach.

Research Design and Procedures

FIELD TREATMENT APPLICATION

The project involves fifteen field trial locations in the United States (Figure 2). Trial locations (state and county) can be found in Table 2 along with the soil characterizations. Most of the trials were placed on Ultisols, Mollisols, or Alfisols. The Nebraska site was placed on an Entisol.

Figure 2. Trial locations and Dominant soil orders of the United States



ftp://ftp-fc.sc.egov.usda.gov/NSSC/Soil_Taxonomy/maps.pdf

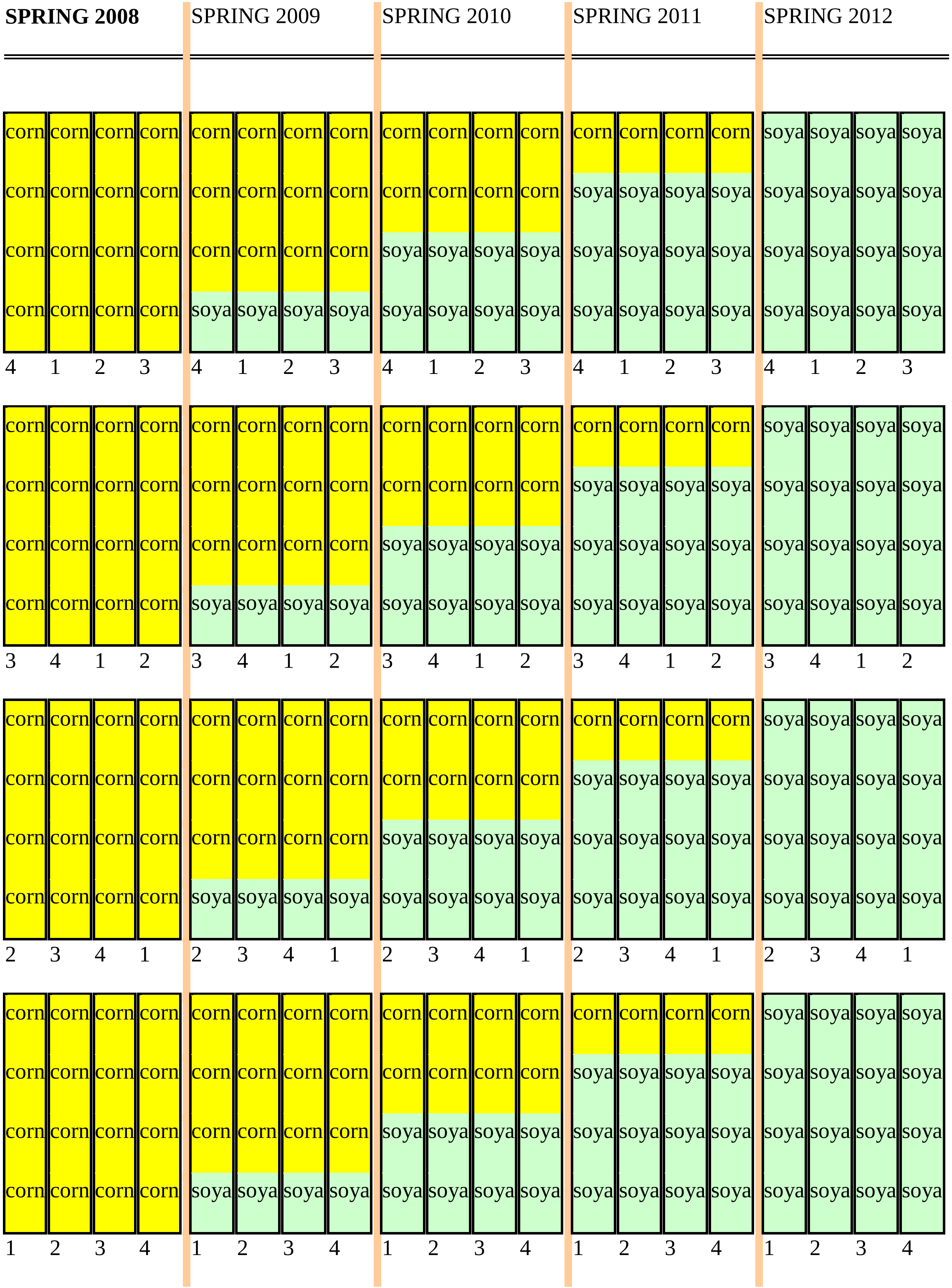
The experimental herbicide, bicyclopyrone (formulated as A13765C at 250 g active ingredient per kg) was applied preemergence to field planted corn at 200 and 400 g ai/ha over a four year period using the treatment list and application timings in Table 1. First applications were made in 2008 with last applications made in 2011.

Table 1. Treatment list used for field trials

Trt	Treatment/Product	g ai/ha	Applic. Code	year applied
1	UNTREATED CHECK			2008
2	A13765	200	A	2008
3	A13765	400	A	2008
4	UNTREATED CHECK			2009
5	A13765	200	A	2008
	A13765	200	B	2009
6	A13765	400	A	2008
	A13765	400	B	2009
7	UNTREATED CHECK			2009
8	A13765	200	B	2009
9	A13765	400	B	2009
10	UNTREATED CHECK			2010
11	A13765	200	A	2008
	A13765	200	B	2009
	A13765	200	C	2010
12	A13765	400	A	2008
	A13765	400	B	2009
	A13765	400	C	2010
13	UNTREATED CHECK			2010
14	A13765	200	C	2010
15	A13765	400	C	2010
16	UNTREATED CHECK			2011
17	A13765	200	A	2008
	A13765	200	B	2009
	A13765	200	C	2010
	A13765	200	D	2011
18	A13765	400	A	2008
	A13765	400	B	2009
	A13765	400	C	2010
	A13765	400	D	2011
19	UNTREATED CHECK			2011
20	A13765	200	D	2011
21	A13765	400	D	2011

The Application code corresponds to the timing of the preemergence application: A= 2008, B= 2009, C= 2010, and D= 2011. Thus, treatments with application codes A and B received applications in 2008 and 2009; A, B, and C in 2008, 2009, and 2010, and so forth. Glyphosate Tolerant (GT) corn was sown into research plots in order to maintain a weed free trial with glyphosate. Plot size was six rows of corn planted 25 feet in length with the entire trial equaling 18 rows of corn by 630 feet in length the first year (Figure 3). Once corn was planted, treatments were applied to bare ground (preemergence application timing). No applications were made to research plots planted in soybeans other than glyphosate, which was used to maintain weed-free plots. All treatments were applied with normal application timings for the region at 20 gallons per acre (187 L/ha) for a more uniform distribution of the herbicide on the soil surface. In order to minimize the impact of corn residues on germination and growth of the rotational crop, soybeans, corn was harvested with a full-size, commercial combine. Harvested grain was destroyed and any remaining stalks and foliage were rotary mowed. Each year beginning in 2009, soybeans were planted in place of corn in order represent a crop rotation as described in the treatment list and the plot map (Figure 3). In 2012, the whole trial site was planted in soybeans. Three soybean varieties ranging from high, moderate, and low sensitivity to HPPD herbicide residues in soil were planted in the field at crop rotation time each year according to the plot map (Figure 3). Soil samples were taken at soybean planting time in 2009, 2010, 2011, and 2012. Each soil core was 30 cm deep by 5 cm diameter. Four cores were taken per research plot (three plots per treatment) equaling 12 cores per treatment. See appendix A for detailed sampling information.

Figure 3. Field plot diagram over five years.



SPRING 2008				SPRING 2009				SPRING 2010				SPRING 2011				SPRING 2012			
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
corn	corn	corn	corn	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya	soya
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4

Soil samples from selected treatments were taken using the following schedule:

- 2009: In spring, sample prior PRE application (Treatments 1, 2, and 3).
- 2010: In spring (Treatments 4, 5, 6, 8, and 9).
- 2011: In spring, (Treatments 10, 11, 12, 14, and 15).
- 2012: In spring, (Treatments 17, 18, 19, 20, 21)

SOIL BIOASSAY PROCEDURE

Frozen soil cores were sawed into three sections, 0 – 10, 11 – 20, and 21 – 30 cm depths. Once cores were sectioned, the soil was removed, thawed, composited, sieved through a ¼ inch screen, mixed thoroughly, and divided evenly (approximately 270 g) among nine plastic pots. This was done for each depth within each treatment. Three HPPD herbicide indicator plant species, red clover, and two soybean varieties (an HPPD sensitive and an HPPD tolerant), were sown into the pots containing the soil. Plants grew for three weeks in the greenhouse. Visual injury (chlorosis/stunting) ratings were performed three weeks after planting by comparing plants grown in soil taken from the untreated check to plants grown in the soil taken from the selected treatments from the same site using a 0 to 100 percent scale where “0” was no injury observed and “100” was complete plant death. A composite sample of soil (0 – 30 cm depth) for each site was collected and sent to a local soil-testing laboratory for characterization (Table 2.)

Table 2. Soil Characteristics of research field sites.

State	County	pH	OM %	ENR* Lbs/A	P-Olsen ppm	CEC meq/100g	Sand %	Silt %	Clay %	Texture	Irrigated?
New Jersey	Salem	5.2	1.4	38.0	46	10.6	56	29	15	SANDY LOAM	No
South Dakota	Minnehaha	6.2	3.9	55.0	16	29.1	26	43	31	CLAY LOAM	No
Georgia	Telfair	5.0	0.3	6.5	15	3.9	84	11	5	LOAMY SAND	Yes
Colorado	Weld	7.9	1.0	39.0	34	18.2	80	11	9	LOAMY SAND	Yes
Tennessee	Knox	5.6	1.5	38.0	27	8.9	42	37	21	LOAM	No
Nebraska	Hall	8.1	0.7	22.0	4	19.4	76	19	5	LOAMY SAND	Yes
Iowa	Keokuk	5.7	3.1	21.0	11	21.2	16	53	31	SILTY CLAY LOAM	No
Michigan	Lenawee	7.1	1.8	38.0	19	13.3	66	19	15	SANDY LOAM	No
Kansas	Pawnee	6.8	1.9	8.5	46	20	36	37	27	LOAM	Yes
Illinois	Stark	6.4	2.7	27.5	16	13.9	23	52	25	SILT LOAM	No
Missouri	Boone	6.7	2.6	57.5	13	20.1	35	38	27	L OAM	No
North Carolina	Wayne	5.6	0.5	12.5	32	2.4	75	20	5	LOAMY SAND	No
Southern Illinois	Jefferson	6.1	1.6	14	17	8	19	66	15	SILT LOAM	No
Indiana	Hamilton	5.6	2.7	56	15	13.8	31	44	25	LOAM	No
Arkansas	Jackson	6	1.1	20.5	9	8.1	39	44	17	LOAM	No

*Estimated nitrogen release

Research Analysis

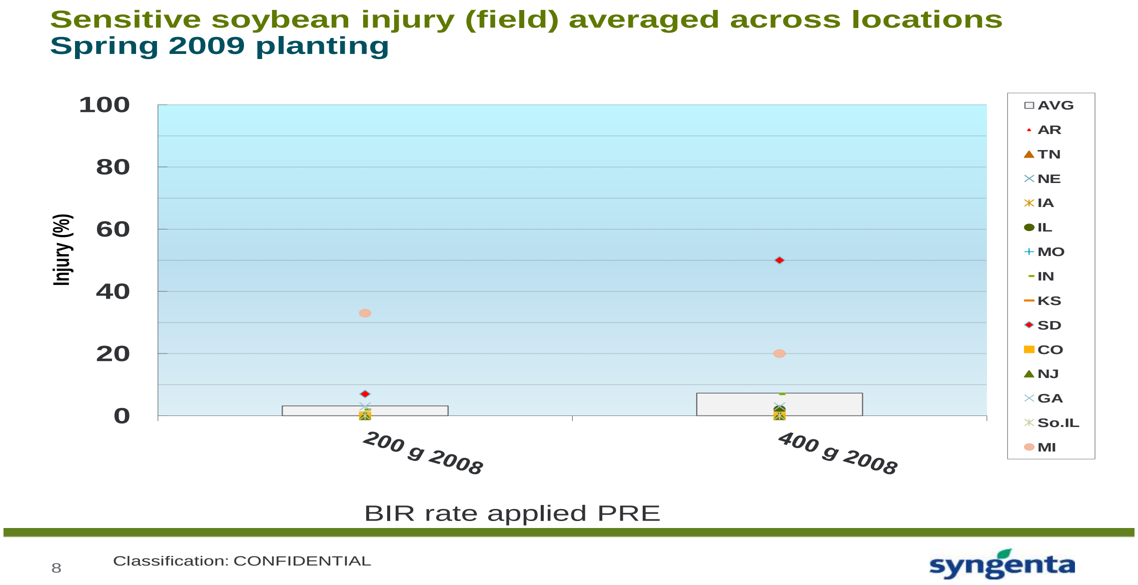
Analysis of Variance (ANOVA) statistics was used for individual field trials and soil bioassays in order to identify differences in herbicide residues. ARM (Agricultural Research Manager®) software will be used to collect, manage, and provide statistical analysis of both the greenhouse and field trials for the duration of the study. Note that the means of the injury data followed by the same letter do not significantly differ (P=.05, Student-Newman-Keuls) in the tables and graphs of this research paper.

Results and Discussion

Year One – Results of Field plantings and soil bioassays for 2009

Soybean injury caused by bicyclopyrone (BIR) residues in the soil and averaged across the 15 trial locations was 3 and 7 percent for the 200 and 400 g ai/ha rates for BIR applied in 2008 (Figure 4). Three trial locations had significant injury from BIR residues at the 400 g application rate, Kansas (20%) South Dakota (50%), and Michigan (20%). South Dakota and Michigan also had significant injury from residues left by the 200 g application, 7 and 33%, respectively. All other trial sites were free of any significant sensitive soybean injury in 2009. The average and tolerant soybeans varieties did exhibit some injury symptoms from BIR residues. Only the sensitive soybean data were discussed in this paper since little to no injury was observed with the average and tolerant soybeans.

Figure 4.

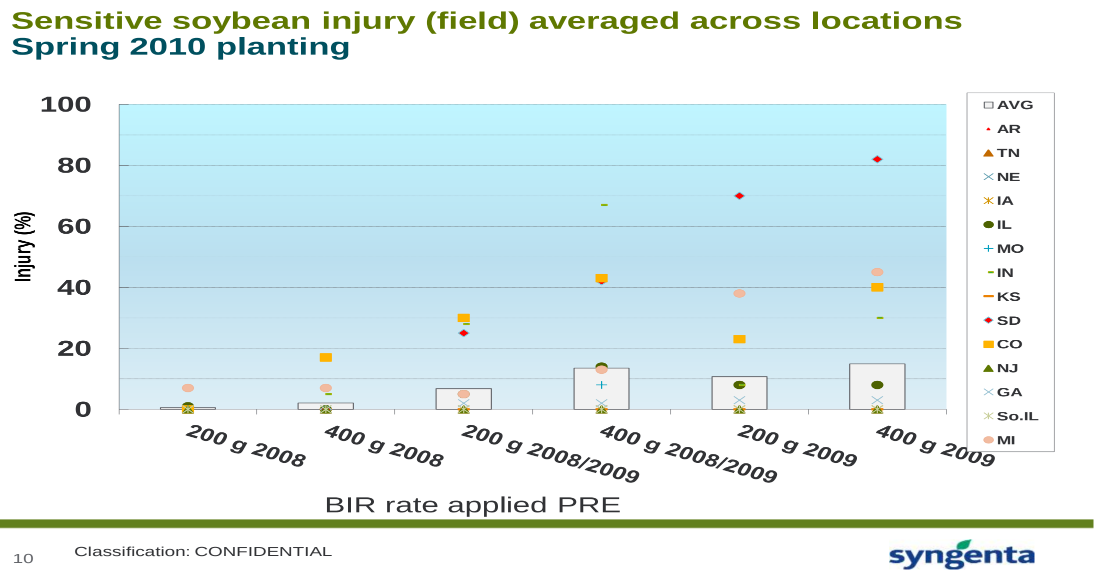


Soil samples taken in the spring of 2009 from just two of the fifteen locations had BIR residues great enough to cause injury to red clover. Residues were not in quantities that would cause injury to sensitive soybeans. The two locations were Michigan and Tennessee.

Year Two – Results of field plantings and soil bioassays for 2010

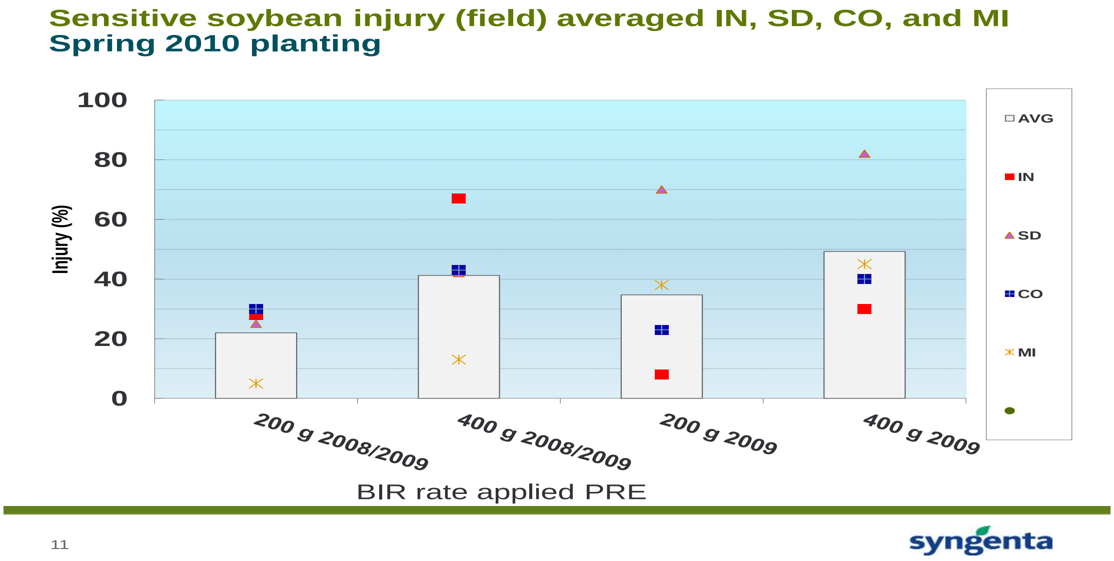
Field planted soybean injury caused by BIR residues in the soil and averaged across the 15 trial locations was less than 15% (Figure 5). Locations where sensitive soybean injury was greater than 20% were South Dakota, Michigan, Indiana, and Colorado (Figure 6). The tendency of BIR accumulating in the soil was not evident in the 2010 soybean rotation data from Colorado, Michigan or South Dakota, but was observed in the data from Indiana where more soybean injury was observed the treatments applied two years in a row (2008 and 2009) versus the single application made in 2009. The bioassay of the 2010 Indiana soil samples reflects the same trends.

Figure 5.



Sensitive soybean injury (field) averaged across locations
Spring 2010 planting

Figure 6.



Sensitive soybean injury (field) averaged IN, SD, CO, and MI
Spring 2010 planting

No BIR residues greater than 20% were detected in 2010 soil bioassayed from Tennessee, Georgia, Colorado, North Carolina, Arkansas, Southern Illinois, Nebraska, New Jersey, Iowa, Missouri, and Kansas. BIR residues were not detected in the Colorado bioassay either, even though soybean injury was observed in the field. Note that the soil pH of Colorado is high (7.5) and soybeans suffered from iron chlorosis since high soil pH is conducive for making iron unavailable for root uptake by plants. A slight dose response from the residues in relation to rate applied can be observed from the data but it is unclear if the iron chlorosis exacerbated the injury from BIR.

Red clover, an indicator plant species used for detecting HPPD herbicides in soil samples, is highly sensitive to BIR residues in the soil. Levels as low as 3 ppb can be detected with red clover, causing about 5 percent injury in the form of bleaching of the leaves. The amount of BIR residues in the soil producing this low level of injury in red clover would not cause any injury to soybeans in the bioassay. The sensitive soybean variety can detect BIR residues down to about 10 ppb in soil samples. Usually, when about 70-80 percent red clover injury is observed in the bioassay, the sensitive soybean variety begins showing symptoms of BIR residues in the soil (about 5% injury – mainly chlorosis and/or smaller trifoliates).

The possibility of BIR accumulating over multiple year applications was evident in 2010 Indiana soil samples from taken from the 0 – 10 cm depth. The red clover injury was 90+ % and sensitive soybeans averaged 30% when grown in soil taken from the 400 g application made in 2008 and 2009, however, only about 45% red clover injury and no soybean injury were observed with BIR applied at 400 g in 2009 only. Similar trends were observed with the 11 – 20 cm soil samples but not to the same intensity (data not shown). The Illinois samples showed similar results but to a lesser degree with red clover data (data not shown). The Indiana 2010 bioassay correlated well with the field data.

Bioassay of the South Dakota 2010 soil samples resulted in red clover injury from residues left by the 200 g application made in 2008 and 2009 (11-20 cm depth), and 200 and 400 g applications made on 2009 only, in the 21-30 cm depth. No red clover injury was observed from the soil sampled at 0 – 10 cm deep (Table 3). No soybean injury was observed in the bioassay of the South Dakota samples yet significant injury was observed in the field. One hypothesis regarding this is that BIR had moved out of the top 30 cm of soil where the sample cores were taken. No BIR residues were detected in the top 10 cm of soil but were detected in soil taken from the lower depths, which alludes to leaching.

Table 3. 2010 South Dakota Bioassay: red clover injury

Level	Name	Rate (g ai/ha)	Level	% injury 21DAA*
0 -10 cm depth	A13765	200	treated in 2008 &2009	0 c
0 -10 cm depth	A13765	400	treated in 2008 & 2009	0 c
0 -10 cm depth	CHECK UNTREATED		untreated check	0 c
0 -10 cm depth	A13765	200	treated in 2009	0 c
0 -10 cm depth	A13765	400	treated in 2009	0 c
11 -20 cm depth	A13765	200	treated in 2008 &2009	40 a
11 -20 cm depth	A13765	400	treated in 2008 & 2009	0 c
11 -20 cm depth	CHECK UNTREATED		untreated check	0 c
11 -20 cm depth	A13765	200	treated in 2009	0 c
11 -20 cm depth	A13765	400	treated in 2009	0 c
21 -30 cm depth	A13765	200	treated in 2008 &2009	0 c
21 -30 cm depth	A13765	400	treated in 2008 & 2009	0 c
21 -30 cm depth	CHECK UNTREATED		untreated check	0 c
21 -30 cm depth	A13765	200	treated in 2009	8.3 b
21 -30 cm depth	A13765	400	treated in 2009	6.7 b

*means of the injury data followed by the same letter do not significantly differ (P=.05, Student-Newman-Keuls)

Red clover was injured from residues in the 0-10 and 11 – 20 cm depths of the 2010 Michigan samples but not in the 21 – 30 cm samples. The red clover injury in soil taken at the 11-20 cm depth occurred with the 400 g rate applied in both 2008 and 2009 and no injury was observed with 400 g applied in 2009 only. Again, this shows the possibility of accumulation possibility of BIR when high rates are used (Table 4). Another reason for differences between bioassay and field injury levels is the random sampling method used. Since only four cores per research plot were pulled, variation could simply be “luck of the draw”. In addition, the 30 cm soil cores were sectioned into three depths some that residues could more easily detected and herbicide movement could be evaluated. The roots of a soybean plant can reach a depth of two meters and can then take up herbicide residues way beyond the boundaries of a soil core.

Table 4. Bioassay of 2010 Michigan soil samples: red clover

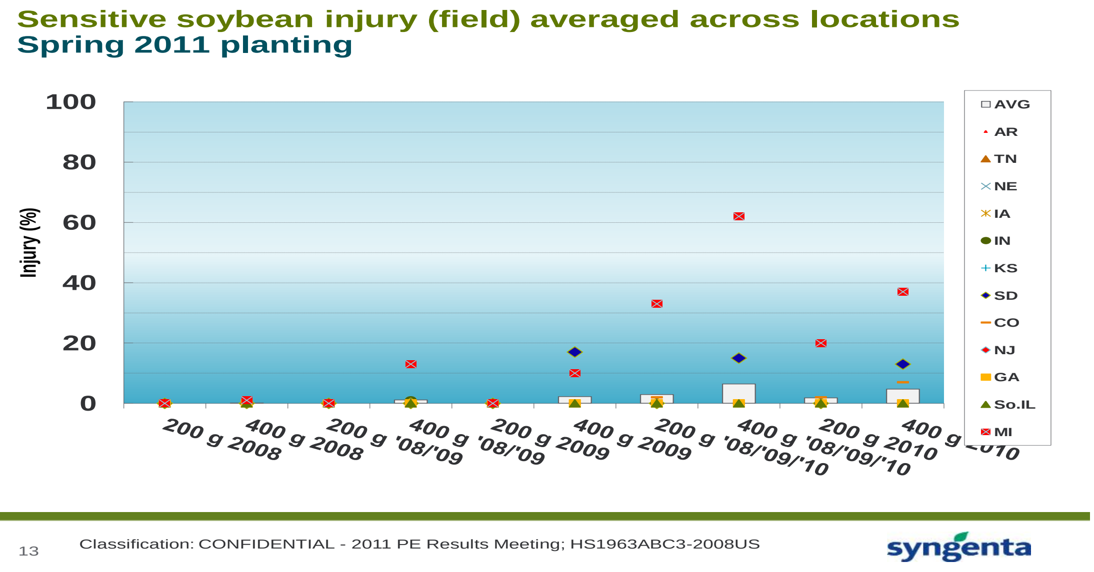
Level	Name	Rate	Level	Injury (%) 20DAA*	
0-4 inch depth	A13765	200	treated in 2008 &2009	63.3	a
0-4 inch depth	A13765	400	treated in 2008 & 2009	23.3	c
0-4 inch depth	CHECK UNTREATED		untreated check	0	d
0-4 inch depth	A13765	200	treated in 2009	53.3	b
0-4 inch depth	A13765	400	treated in 2009	63.3	a
4-8 inch depth	A13765	200	treated in 2008 &2009	10	d
4-8 inch depth	A13765	400	treated in 2008 & 2009	23.3	c
4-8 inch depth	CHECK UNTREATED		untreated check	0	d
4-8 inch depth	A13765	200	treated in 2009	0	d
4-8 inch depth	A13765	400	treated in 2009	0	d
8-12 inch depth	A13765	200	treated in 2008 &2009	0	d
8-12 inch depth	A13765	400	treated in 2008 & 2009	0.1	d
8-12 inch depth	CHECK UNTREATED		untreated check	0	d
8-12 inch depth	A13765	200	treated in 2009	0	d
8-12 inch depth	A13765	400	treated in 2009	0	d

*means of the injury data followed by the same letter do not significantly differ (P<0.05, Student-Newman-Keuls)

Year Three – Results of Field plantings and soil bioassays for 2011

Only two field sites reported soybean injury greater than 10% in 2011, Michigan and South Dakota. Of these two, only Michigan showed accumulation of BIR in the soil as a result of multiple year applications (Figure 7).

Figure 7.



Sensitive soybean injury (field) averaged across locations
Spring 2011 planting

13 Classification: CONFIDENTIAL - 2011 PE Results Meeting; HS1963ABC3-2008US

syngenta

The 2011 soil bioassays detected no significant red clover injury in samples from Georgia, Colorado, Kansas, Southern Illinois, South Dakota, North Carolina, Arkansas, and Iowa. The remaining seven sets of samples, Tennessee, Nebraska, New Jersey, Illinois, Missouri, Michigan, and Indiana caused some injury to red clover of at least 20% depending on treatments applied in the field, but no soybean injury. Indiana soil sampled from both the 200 g and 400 g applications made in 2010 caused about 35% red clover injury (0-10 cm depth). No other clover injury was observed (data not shown).

Minor red clover injury was observed in the Nebraska bioassay. This injury appeared greater in the first evaluation; however, this may have been due to uneven germination and growth since symptoms were more of a biomass reduction versus bleaching. The red clover injury observed in the late rating was mainly from BIR residues present in the 21 – 30 cm soil layer and ranged from 7 to 22%. No significant red clover injury was observed in the 4 - 8 depth and no injury observed in the 0 - 4 inch depth. BIR appears to have moved down through the soil profile (data not shown).

Some red clover injury was observed from BIR residues left by the 400 g ai/ha applications made in 2008, 2009, and 2010 in the 0 - 4 inch depth of the New Jersey 2011 samples. Red clover injury from the 400 g single application rate made in 2010 was much less than the 400 g rate applied three years in a row, which was 15% versus 62% from the three-year application (data not shown).

BIR residues were detected by red clover (25% injury in the 0 - 4 inch depth in soil taken from the 400 g rate applied in 2008, 2009, and 2010 at the Tennessee location. No other residues were detected at this sample timing (data not shown).

BIR residues detected in the 2011 Michigan samples were high enough in some cases to cause injury to sensitive soybeans. High levels of both red clover and sensitive soybean were observed in this bioassay. The sensitive soybean variety (S02-M9) exhibited 15 and 32% injury from BIR residues left by the 200 and 400 g rates, respectively, that were applied in 2008, 2009, and 2010; and, 5 and 30% injury from the same rates applied in 2010 only (0 – 10 cm depth). BIR residues left by the 400 g rate were also detected in the 4 - 8 inch depth causing 16% sensitive soybean injury from applications made in 2008, 2009, and 2010, and 10% injury from the application made in 2010 only. The data alludes to the possibility of BIR accumulating in the soil over successive years of application, and downward movement as well. BIR was not detected in the 21 – 30 cm samples. The "average" tolerant soybean (S27-C4) did not show any injury at the late rating (Table 5).

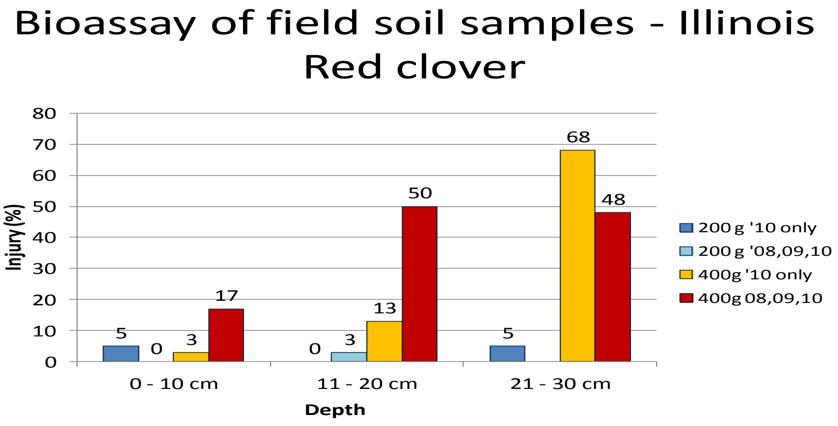
Table 5.

Crop Code							soybean		soybean	
Crop Variety					r. clover		S02-M9		S27-C4	
Part Assessed					PLANT		PLANT		PLANT	
Assessment Data Type					PHYGEN		PHYGEN		PHYGEN	
Assessment Unit					%*		%*		%*	
Trt	MITF	Treatment/Product	g ai/ha	MAF1						
1	0 -10 cm depth	CHECK UNTREATED		untreated check # 13	0	d	0	d	0	a
2	0 -10 cm depth	A13765	200	treated in 08 &09&10 PRE #11	100	a	15	b	0	a
3	0 -10 cm depth	A13765	400	treated in 08&09&10 PRE #12	100	a	31.7	a	0	a
4	0 -10 cm depth	A13765	200	treated in 2010 PRE #14	50	c	5	d	0	a
5	0 -10 cm depth	A13765	400	treated in 2010 PRE #15			30	a	0	a
6	11 -20 cm depth	CHECK UNTREATED		untreated check # 13	0	d	0	d	0	a
7	11 -20 cm depth	A13765	200	treated in 08 &09&10 PRE #11	0	d	0	d	0	a
8	11 -20 cm depth	A13765	400	treated in 08&09&10 PRE #12	45	c	15.7	b	0	a
9	11 -20 cm depth	A13765	200	treated in 2010 PRE #14			0	d	0	a
10	11 -20 cm depth	A13765	400	treated in 2010 PRE #15	90	b	10	c	0	a
11	21 -30 cm depth	CHECK UNTREATED		untreated check # 13	0	d	0	d	0	a
12	21 -30 cm depth	A13765	200	treated in 08 &09&10 PRE #11	0	d	0	d	0	a
13	21 -30 cm depth	A13765	400	treated in 08&09&10 PRE #12	0	d	0	d	0	a
14	21 -30 cm depth	A13765	200	treated in 2010 PRE #14			0	d	0	a
15	21 -30 cm depth	A13765	400	treated in 2010 PRE #15	0	d	0	d	0	a

*means of the injury data followed by the same letter do not significantly differ (P<0.05, Student-Newman-Keuls)

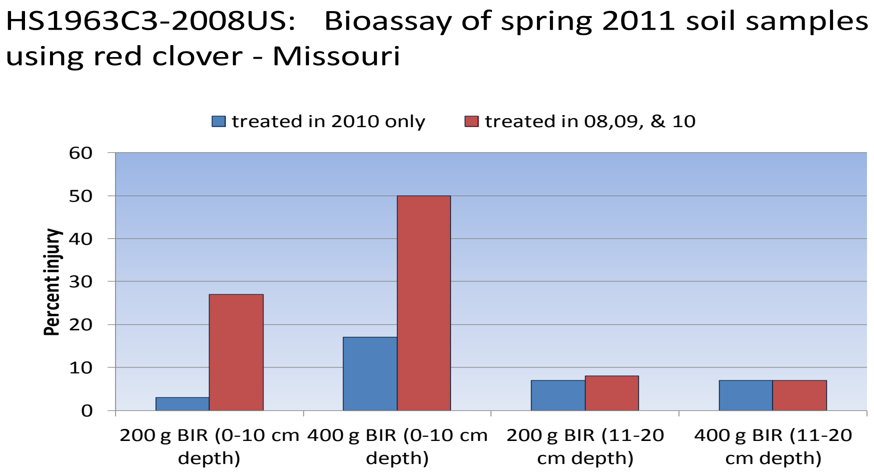
BIR residues left from the 400 g applications in soil samples caused various levels of injury to red clover and depended mainly on soil depth of the sectioned cores from the Illinois location. Clover injury increased with soil depth from 17% in the 0 – 10 cm depth to as high as 68% in the 21 – 30 cm depth (downward movement). In both the 0 - 10 and 11 – 20 cm samples residues from the 400 g rate applied in 2008, 2009 and 2010 caused more clover injury than residues from the 400 g application made in 2010 only, although the reverse is true in the 21 – 30 cm samples (Figure 8). Residues from the 200 g application of BIR did not cause any significant injury. (No soil samples for the 21 – 30 cm depth - 200g treatments).

Figure 8.



The 2011 spring sample timing is the first time significant red clover injury was observed in the bioassay of Missouri samples. More BIR residues were detected with red clover when the 200g and 400 g rates were applied in 2008, 09, and 10 versus the applications made in 2010, only – indicating a potential for BIR accumulation in the soil. As expected, more BIR residues were detected from the 400 g application versus the 200 g applications (Figure 9).

Figure 9.

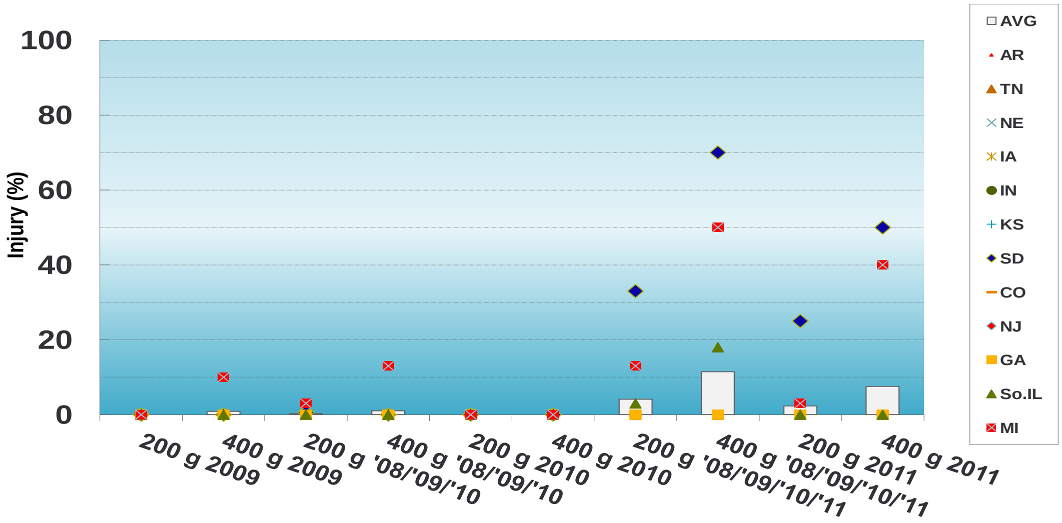


Year Four – Results of field plantings and soil bioassays for 2012

As observed in the soybean results from the previous three years of field data, Michigan and South Dakota exhibited the most soybean injury compared to the other thirteen locations and multiple year applications (2008 – 2011) caused more injury than the single year application made in 2011 (Figure 10). Only the Southern Illinois site exhibited soybean injury of 20% or greater and was caused by the 400g rate applied 2008 – 2011. The single application of 400 g ai/ha made in 2011 did not injure soybeans at the Illinois site. The 400 g rate of BIR applied in 2008, 2009, and 2010 caused nearly 15% soybean injury compared to the 400 g application made in 2010 (no injury). This was two years after the application but is still much less injury than what was observed in 2011 indicating microbial breakdown or dispersion, leaching in the soil profile.

Figure 10.

Sensitive soybean injury (field) averaged across locations
Spring 2012 planting



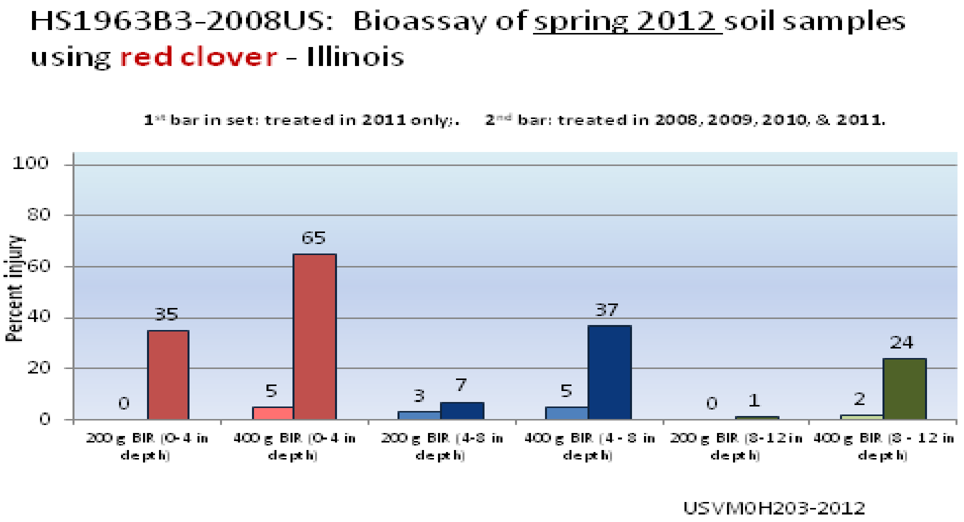
14 Classification: CONFIDENTIAL - 2012 PE Results Meeting; HS1963ABC3-2008US syngenta

The 2012 soil bioassays detected BIR residues in samples from five of the fifteen locations; Tennessee, Illinois, Michigan, and South Dakota. Of these, only the Michigan soil samples caused greater than 10% sensitive soybean injury.

BIR residues were only great enough to injure red clover but not soybeans in soil received from Tennessee. BIR residues left by the 400 g rate in the 0 – 10 cm depth from the four year (2008 – 2011) and the single year (2011 only) caused 22% and 37% red clover injury, respectively. No significant injury observed with lower depths. No trends in accumulating BIR from multiple year applications were evident at the Tennessee site.

BIR residues detected in the 2012 soil samples from Illinois showed that multiple applications (2008, 2009, 2010, & 2011) of BIR at the 200 or 400 g rates left more residues in the soil than a single application made in 2011. Again, alluding to the fact that BIR was not entirely degraded from season to season at the rates applied. Residues were detected in all three depths (red clover data) from the 400 g application rate applied all four years, injury intensity lessened with depth (Figure 11).

Figure 11.



HS1963B3-2008US: Bioassay of spring 2012 soil samples using red clover - Illinois

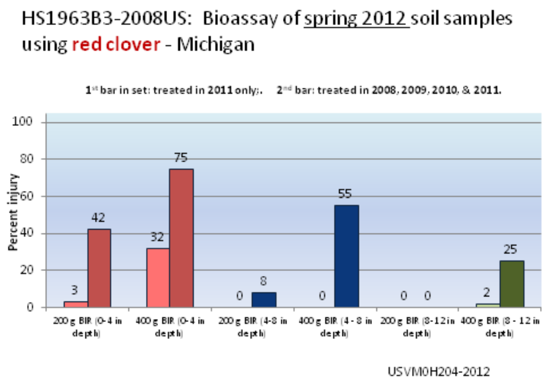
1st bar in set: treated in 2011 only;. 2nd bar: treated in 2008, 2009, 2010, & 2011.

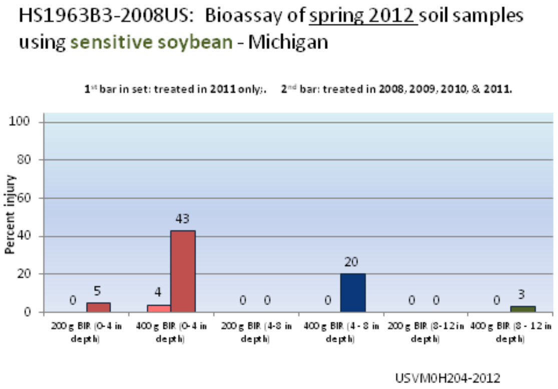
USVM0H203-2012

As observed with the Illinois bioassay, BIR residues detected in soil samples from Michigan showed that multiple applications (2008 - 2011) of BIR at the 200 or 400 g rates left more residues in the soil than a single application made in 2011. This once again alludes to the fact that BIR did not entirely degrade from season to season at this location at the rates applied. Residues were detected in all three depths (red clover data), lessening with depth. Sensitive soybeans were injured by BIR residues as well, with the multiyear applications causing more injury than the single year application (Figure 12 &

13). Residues from the multiple year 400 g applications caused 20% sensitive soybean injury in the 11 – 20 cm depth versus no injury from the single application.

Figure 12 & 13.





Discussion

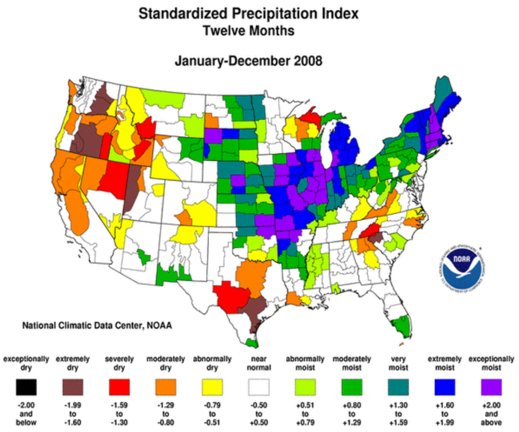
There are three processes that affect herbicide persistence/degradation: physical, chemical, and microbial (Hager and McGlamery, 2000). Soil particle composition is a physical factor that measures the amount of sand, silt, and clay in a relationship known as soil texture (Brady and Weil, 2004). Soil organic matter is an important chemical property of soil that can influence the chemical properties of soil such as pH, cation exchange capacity (CEC), and nutrient status (Brady and Weil, 2004). Microbial processes depend on the type and abundance of soil microorganisms present in the soil and the environmental conditions that they are exposed to (Sylvia, et al, 2005). Physical factors affect herbicide phytotoxicity and persistence through infiltration, leaching, and volatilization (Hager and McGlamery, 2000). Generally, soils high in clay, organic matter, or both, have a greater potential for herbicide carryover because there is increased adsorption to soil colloids, with a corresponding decrease in leaching and loss through volatilization. This “tie-up” results in decreased *initial* plant uptake and herbicidal activity (Dyson, et al, 2002). Thus, more herbicide is held in reserve to be released later, which can potentially injure susceptible future crops (Hager and McGlamery, 2000). An example of delayed release was mentioned in the introduction where an anhydrous ammonia application caused a release of mesotrione (Callisto herbicide) in the field. Some herbicides, like the triazines (atrazine and simazine), are particularly affected by soil pH (also the HPPD herbicide, mesotrione). Small amounts of some herbicides can be sorbed or held onto soil colloids at a high or low soil pH depending on their chemistry. Herbicides in the soil solution are available for plant uptake, hydrolysis, or microbial breakdown. Chemical breakdown and microbial breakdown, two major herbicide degradation processes for sulfonylurea herbicides, are often slower in soils of higher pH (Hager and McGlamery, 2000). CEC (cation exchange capacity: number of moles of + charge adsorbed per unit mass, $cmol_c/kg$), principally a function of clay type and organic matter content, is directly involved in herbicide adsorption. Some herbicides are more available in the presence of certain cations, whereas others may be tied up and therefore unavailable (Hager and McGlamery, 2000). Soil microbes require certain environnemental conditions for optimal growth and utilization of any pesticide. Factors that affect microbial activity are temperature, pH, oxygen, water content, and mineral nutrient supply. Warm, fertile, well aggregated, soil with a soil pH close to neutral is generally most favorable for microorganisms leading to herbicide degradation (Alexander, 1999). Other chemical reactions that affect herbicide degradation are hydrolysis, oxidation, and reduction; however, these do not significantly affect the breakdown of HPPD inhibiting herbicides (Vail, internal communication). Degradation rates of the HPPD inhibiting herbicides generally increase with increasing temperature and increasing soil moisture since microbial decomposition rate increases under conditions of higher temperature and moisture. Cool, dry conditions slow degradation. Overly hot conditions can also slow degradation if the microbes are not thermaphilic microorganisms (Sylvia, et al, 2005). Sunlight or photodecomposition does not play a significant factor in the degradation of BIR (Vail, internal communication). Other important factors for herbicide degradation include water solubility of the herbicide, vapor pressure, and susceptibility to chemical and microbial alteration or degradation. Water solubility of a herbicide helps to determine its leaching potential. Herbicides that readily leach may be carried away or carried to rooting zones of susceptible plants. A benefit to a higher leaching potential is dilution or diffusion into the soil profile (Dharmasri, 2004, internal Syngenta document). This increases the chances for soil microbes to come into contact with the herbicide so that they can degrade it. Low water solubility of a herbicide can also lead to strong adsorption to soil colloids. Thus, dry soils are less likely to leach and may not release the herbicide from its colloids increasing persistence.

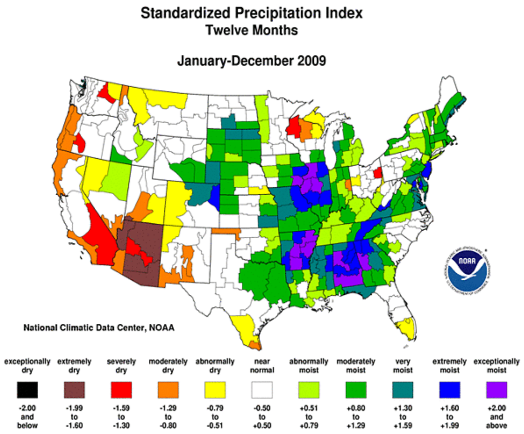
The soybean rotation data collected from the field from 2009 through 2012 support the factors above. Precipitation was acceptable or even greater than the norm at all locations. No carryover was observed at the trial sites in the south, roughly latitude 39 N and southward. The northern most locations, South Dakota and Michigan, consistently exhibited the most BIR residues. Some states, mainly between latitudes 40 – 42 showed intermittent carryover of BIR, dependent upon year. This may have a correlation with temperature (Appendix B) and growing season length (Appendix C). As previously stated, degradation of BIR is mainly microbial driven. Microorganisms need moist, warm soils in order to actively break down compounds. All sites had at least adequate moisture whether it was precipitation or irrigation. The limiting factor is temperature and length on growing season. Cooler temperatures increase north in latitude. As for growing season, the South Dakota and Michigan sites are in or close to 120 – 150 day growing period. The sites with intermittent or slight BIR carryover were in the 150 to 180 day growing period. Sites with no injury had a180+ growing period (Appendix C). The difference between growing periods is roughly 30 days. This time could equal one to two half-lives of degradation of bicyclopyrone and can further explain the decrease in carryover potential southward in latitude. Again, as long as sufficient moisture is available. According to these data, temperature and growing period length along with moisture are more important in the degradation of BIR than soil type, CEC, or pH, etc., on the grand scale of things. Colorado and Nebraska have very close soil characteristics and both were irrigated, however, Nebraska has a slightly longer growing period, thus warmer temps for a longer period and Nebraska also had less soybean injury at its location. Soil type and its characteristics become more important within a temperature, moisture, and/or growing period regime as the case Michigan and South Dakota. South Dakota soil contained high organic matter, CEC, and is a clay loam. Michigan is lower in organic matter and CEC, and is a sandy loam. Michigan generally had more soybean injury and a tendency for more BIR accumulation in the soil versus the South Dakota site, which is likely due to less “tie-up” of the herbicide. The low CECs and organic matter content of sandy loams or loamy sands of the south are less important since temperatures are warmer and growing period is longer – giving the microbes a longer period to utilize BIR. The low CEC of the southern soils can also be an advantage since BIR is more likely to stay in the soil solution making it available to the microbes (as long as sufficient soil moisture is present). The bioassays showed a tendency for BIR to accumulate in the soil after repeated applications and move down through the soil profile. However, the 200 and 400 g ai/ha rates used in this five year research project have recently been lowered to 50 g ai/ha; therefore, carryover risk has been significantly reduced.

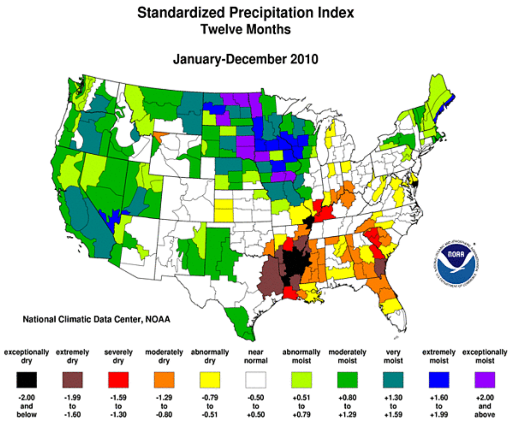
Conclusions

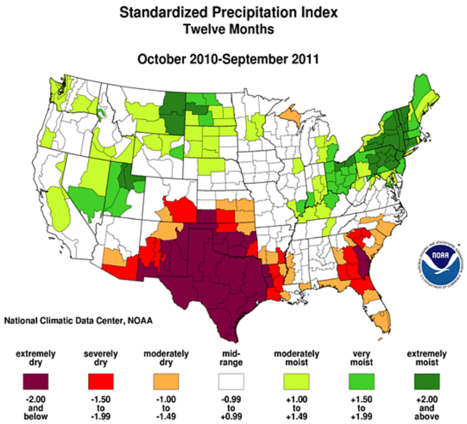
Multiple year applications of BIR applied PRE at 200 or 400 g ai/ha increased the risk of carryover to soybeans compared to single year at some locations. Field trial sites where no observable difference between single and multiple year applications were located mainly in the southern half of the United States below latitude 39 N. Carryover potential correlated well with temperature and length of growing period. Warmer temperatures and longer growing periods decreased the likelihood of carryover as long as sufficient soil moisture is present.

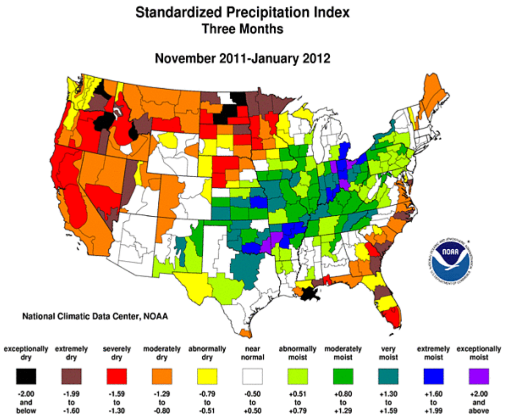
Appendix A: Standardized Precipitation Indexes for the United States 2008 – 2011





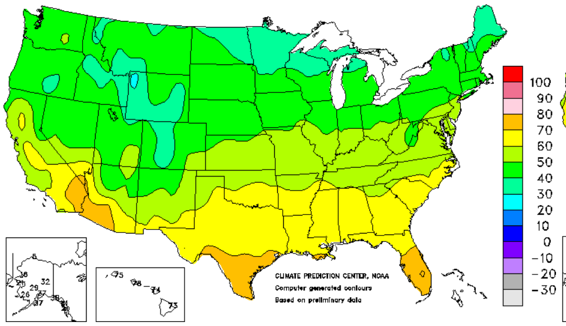




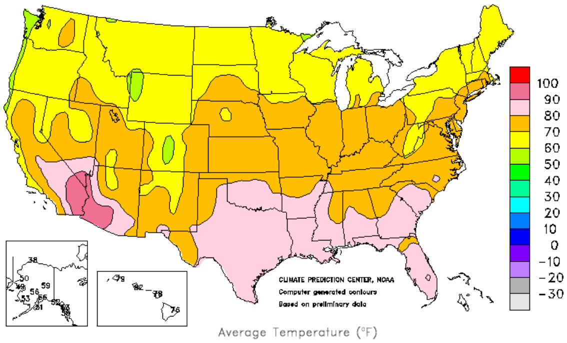


http://www.ncdc.noaa.gov/img/climate/research

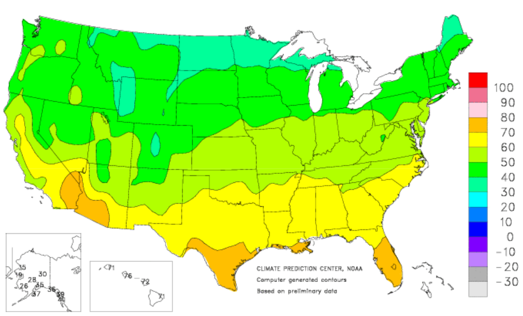
Appendix B. Average Temperatures – United States


Average Temperature (°F)
MAR – MAY 2008

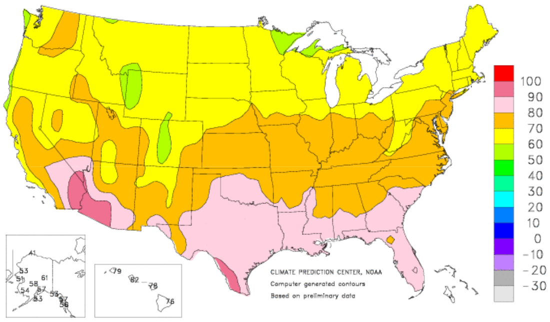
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data


Average Temperature (°F)
JUN – AUG 2008

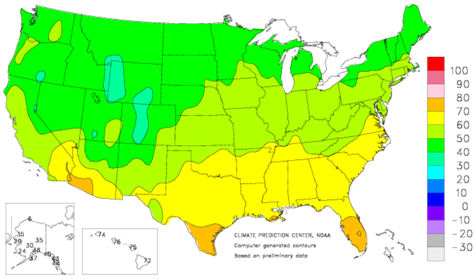
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data


Average Temperature (°F)
MAR – MAY 2009

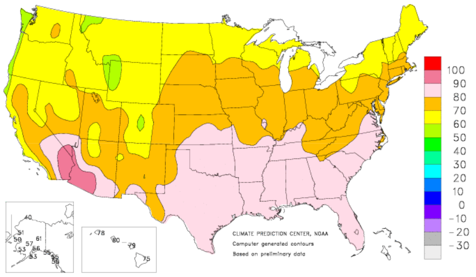
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data


Average Temperature (°F)
JUN – AUG 2009

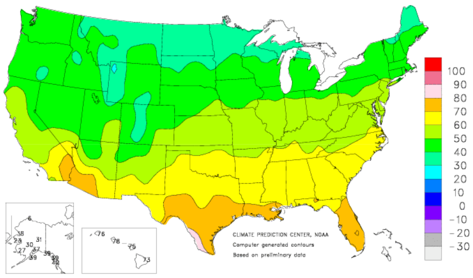
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data


Average Temperature (°F)
MAR – MAY 2010

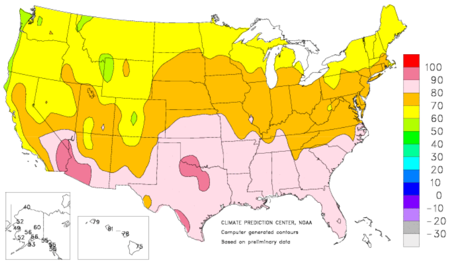
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data


Average Temperature (°F)
JUN – AUG 2010

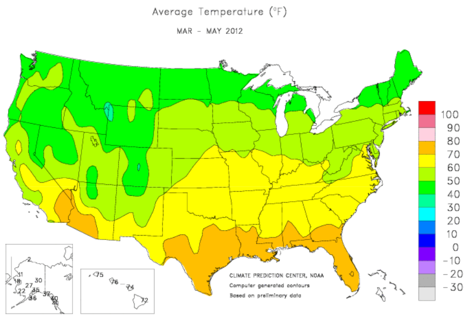
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data

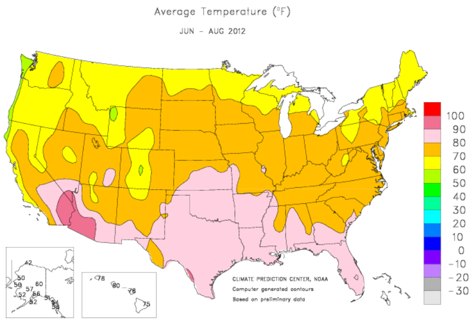

Average Temperature (°F)
MAR – MAY 2011

CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data


Average Temperature (°F)
JUN – AUG 2011

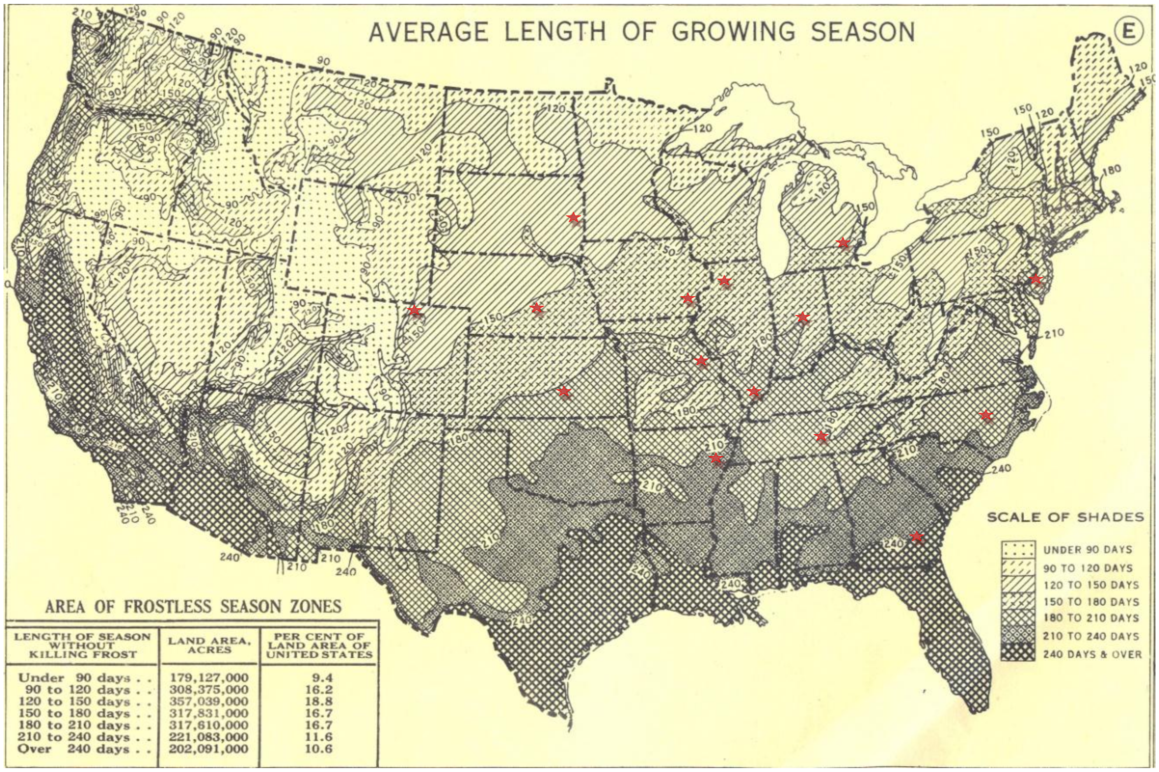
CLIMATE PREDICTION CENTER, NOAA
Computer generated contours
Based on preliminary data





http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/regional_monitoring/archive/us/jjatotp.11.gif

Appendix C. Average Length of Growing Season in the United States



AREA OF FROSTLESS SEASON ZONES

LENGTH OF SEASON WITHOUT KILLING FROST	LAND AREA, ACRES	PER CENT OF LAND AREA OF UNITED STATES
Under 90 days . .	179,127,000	9.4
90 to 120 days . .	308,375,000	16.2
120 to 150 days . .	357,039,000	18.8
150 to 180 days . .	317,831,000	16.7
180 to 210 days . .	317,610,000	16.7
210 to 240 days . .	221,083,000	11.6
Over 240 days . .	202,091,000	10.6

http://www.cartoko.com/content/wp-content/uploads/2010/05/Paullin_1932_pl_3e.jpg

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ftp://ftp-fc.sc.egov.usda.gov/NSSC/Soil_Taxonomy/maps.pdf

http://www.epa.gov/oecaagct/ag101/pestkd.html

http://www.cartoko.com/content/wp-content/uploads/2010/05/Paullin_1932_pl_3e.jpg