Experimental Design and Statistical Analysis: *Bt* Corn, Lignin, and ANOVAs

Part I—"Abstract"

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This case is based on a recent publication by Saxena and Stotzky entitled "*Bt* Corn Has a Higher Lignin Content Than Non-*Bt* Corn" in the *American Journal of Botany* (Sept. 2001, vol. 88, no. 9, pp.1704-1706).

Read the abstract of their paper:

> "*Bt* corn has been genetically modified to express the Cry1Ab protein of *Bacillus thuringiensis* to kill lepidopteran pests. Fluorescence microscopy and staining with toluidine blue indicated a higher content of lignin in the vascular bundle sheaths and in the sclerenchyma cells surrounding the vascular bundle in all ten *Bt* corn hybrids, representing three different transformation events, studied than of their respective non-*Bt* isolines. Chemical analysis confirmed that the lignin content of all hybrids of *Bt* corn, whether grown in a plant growth room or in the field, was significantly higher (33-97% higher) than that of their respective non-*Bt* isolines. As lignin is a major structural component of plant cells, modifications in lignin content may have ecological implications."

Questions:

1. What does the *Bt* in *Bt*-corn represent and why was it used to genetically modify corn plants?
2. What is lignin? Why is it an important plant chemical?
3. What was their research question?
4. Based on the abstract above, reconstruct their experimental approach. What did they do? How did they do it? What did they compare, and how do those comparisons help them answer their research question?
5. What questions do you have about their research protocol that the abstract does not answer?

Image Credit: European corn borer, *Ostrinia nubilalis*, an insect species introduced to North America and belonging to the family *Pyralidae* in the order *Lepidoptera*. Photo by Keith Weller, from the collection of the USDA Agricultural Research Service.

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Part II—"Methods"

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Read Saxena and Stotzky's description of the methods they used (information concerning the soil used is removed):

> "...Seeds of ten different *Bt* corn hybrids (Table 1), representing three transformation events (*Bt*11, MON810, and 176), and their respective non-*Bt* isolines were planted (3 seeds/pot) and the pots were kept in a plant-growth room at 26 ± 2ºC, with a 12-h light-dark cycle... Seeds of eight *Bt* hybrids (Table 2) and their non-*Bt* isolines were also planted in a Haven sandy soil in another site in East Marion, Long Island, New York, USA... Plants were irrigated but not fertilized. The plants were harvested after 97 d of growth in the plant growth room and after 90 d of growth in the field. Uniform free-hand sections of fresh corn stems between the 3rd and the 4th node from the surface of the soil (thickness was ~11 mm for plants grown in the plant growth room and ~18 mm for field-grown plants) were examined for lignin by fluorescence microscopy at 400 nm (Hu *et al.*, 1999) and by staining with 0.01% toluidine blue (Sylvester and Ruzin, 1994). The content of lignin of the same portion of the stems (oven-dried, ground, and passed through an 80-mesh sieve) was determined by the acetyl bromide method (Hatfield *et al.*, 1999)."

Questions:

6. Based on the abstract above and on this description of the methods, modify your reconstruction of their experimental approach. What did they do? How did they do it?
7. What questions do you have about their research protocol that the abstract and methods sections do not answer?
8. Does it matter that the average thickness of the stem sections differed between the growth room and the field-grown plants? Should lignin content be reported as an absolute value, or as a percentage of the total sampled biomass?
9. Why did they test differences in lignin content in both the growth room and in the field? Why might the two experiments yield different results? What can they conclude about lignin based on performing both of these experiments that they could not conclude with a growth room test alone, or with a field test alone?

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Part III—"Results—Figures"

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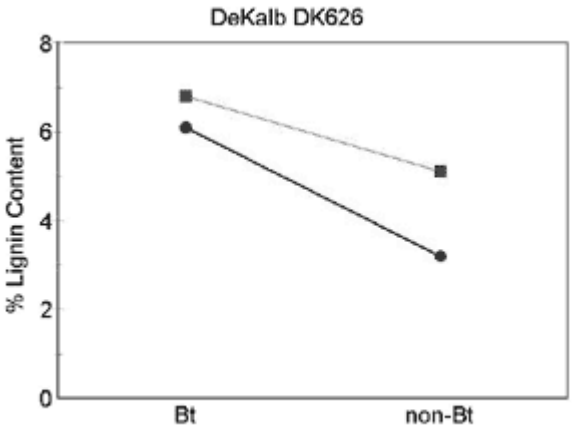
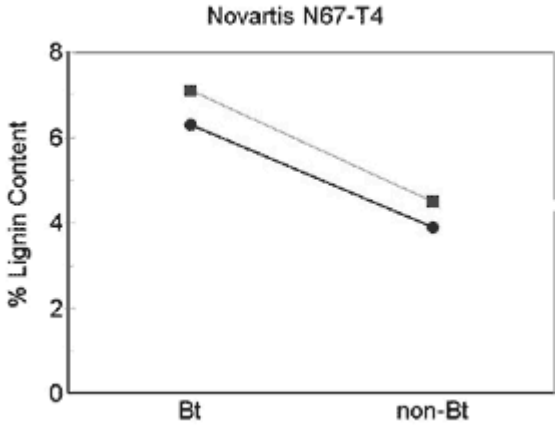
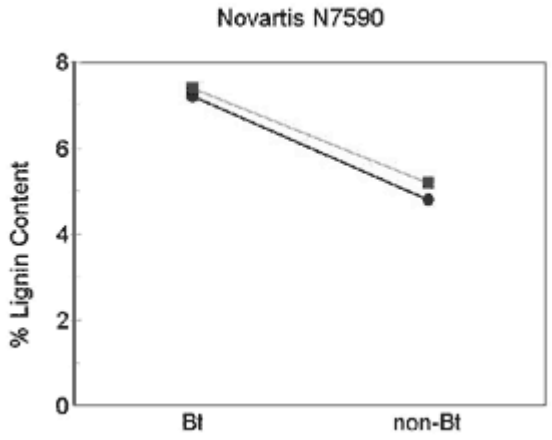
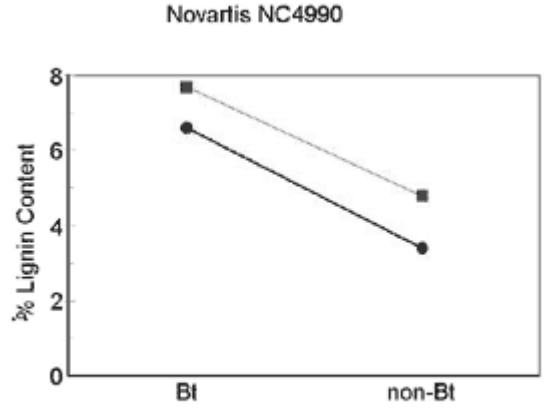
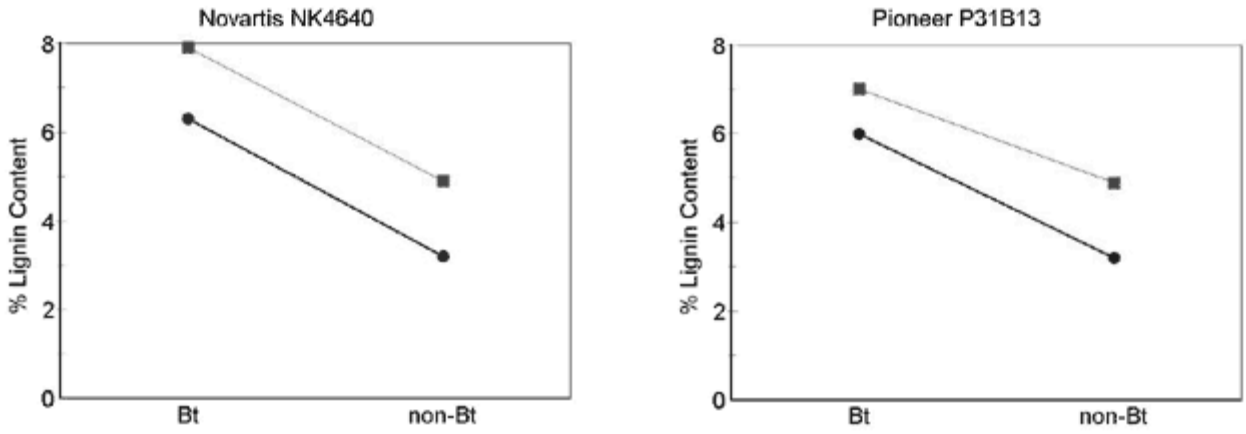
The six graphs below show the mean lignin content for the six isolines of corn for which data was reported for both field and growth room trials for both *Bt* and non-*Bt* varieties. The red line represents the field trials and the blue line represents the growth room trials. The *Bt*-modified corn is on the left, and the non-modified corn is on the right. Lines are included for purposes of enabling greater visual clarity of the differences and interactions, and do not imply that intermediate values could exist. These graphs were constructed from the data provided in the paper in Tables 1 and 2.









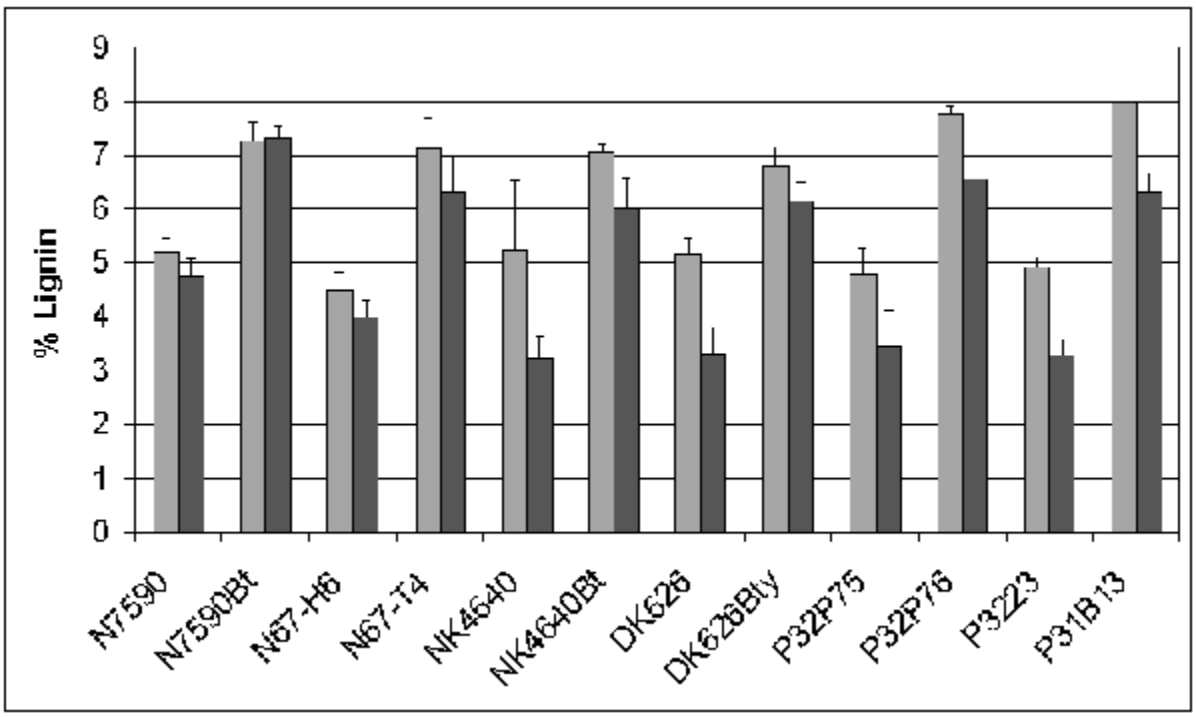


Questions:

10. Why did they test multiple varieties of corn?

11. a. Based on the six graphs, what do you conclude about differences in the percent lignin content between the growth room and the field? Between *Bt* corn and non-*Bt* corn? Between the six varieties of corn? Is it appropriate to make generalizations, or is there evidence that generalizations cannot be supported by the data?

11. b. Based on the bar graph below what do you conclude about differences in the percent lignin content between the growth room and the field? Between *Bt*-corn and non-*Bt*-corn? Between the six varieties of corn? Is it appropriate to make generalizations, or is there evidence that generalizations cannot be supported by the data?



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Part IV—"Results—Tables"

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Here are two tables of results from the paper:

Table 1. Lignin content (% ± SEM) in different hybrids of corn, grown in a plant growth room...

		Bt Corn		Non-*Bt* Corn		
Company	Event	Hybrid	% Lignin	Hybrid	% Lignin	P
Novartis	*Bt*11	N7590*Bt*	7.2 ± 0.10	N7590	4.8 ± 0.14	0.00001
Novartis	*Bt*11	N67-T4	6.3 ± 0.25	N67-H6	3.9 ± 0.15	0.00175
Novartis	*Bt*11	N3030*Bt*	7.0 ± 0.22	N3030	4.4 ± 0.22	0.00003
Novartis	*Bt*11	NC4990*Bt*	6.6 ± 0.18	NC4880	3.4 ± 0.7	0.00020
Novartis	*Bt*11	NK4640*Bt*	6.3 ± 0.14	NK4640	3.2 ± 0.12	0.00001
Novartis	176	Maximizer	4.0 ± 0.15			
Pioneer	MON810	P31B13	6.0 ± 0.24	P3223	3.2 ± 0.18	0.00032
DeKalb	MON810	DK647*Bt*y	6.2 ± 0.25	DK647	4.4 ± 0.22	0.00174
DeKalb	MON810	DK679*Bt*y	6.6 ± 0.11	DK679	3.8 ± 0.10	0.00005
DeKalb	MON810	DK626*Bt*y	6.1 ± 0.20	DK626	3.2 ± 0.18	0.00006

Table 2. Lignin content (% ± SEM) in different hybrids of corn, grown in the field...

		Bt Corn		Non-*Bt* Corn		
Company	Event	Hybrid	% Lignin	Hybrid	% Lignin	P
Novartis	*Bt*11	N7590*Bt*	7.4 ± 0.15	N7590	5.2 ± 0.11	0.00007
Novartis	*Bt*11	N67-T4	7.1 ± 0.22	N67-H6	4.5 ± 0.14	0.00082
Novartis	*Bt*11	NC4990*Bt*	7.7 ± 0.06	NC4880	4.8 ± 0.22	0.00033
Novartis	*Bt*11	NK4640*Bt*	7.9 ± 0.10	NK4640	4.9 ± 0.09	0.00001
Novartis	176	Maximizer	5.9 ± 0.13			
Pioneer	MON810	P32P76	6.8 ± 0.14	P32P75	4.8 ± 0.22	0.00016
Pioneer	MON810	P31B13	7.0 ± 0.04	P3223	4.9 ± 0.13	0.00007
Dekalb	MON810	DK626*Bt*y	6.8 ± 0.15	DK626	5.1 ± 0.12	0.00085

Questions:

12. Why do you think they tested different varieties of corn in the growth room vs. in the field?
13. Examine Tables 1 and 2. What does SEM mean in the title? How is SEM determined? Why is SEM a poor measure of variability? What should have been reported instead and why?
14. What statistical test do you think they used to produce the P values? What do the P values mean? What information should have been reported but is missing?
15. Overall, do you think there are differences between the three different types of genetic modifications (*Bt*11, 176, MON810)? What evidence can you identify to support your conclusion?
16. What statistical test should have been used on the six varieties of corn for which they have both modified and non-modified varieties tested in both the field and the growth room?
17. The authors also report that "The average diameter of the vascular bundle and surrounding lignified cells in *Bt* corn was 21.5 ± 0.84 um, whereas that of non-*Bt* corn was 12.4 ± 1.14 um." Is this useful information? If so, how? What are possible implications?
18. What kinds of ecological implications might modifications in lignin content have? What research should be done next? What suggestions do you have for other experiments and comparisons that should be made to improve this research paper?