Stat 998, Fall 2013 (Larget)
Honeybee Problem

Attached is the problem description for the "honeybee problem". This was initially assigned as a major data analysis problem in 1983. We will use it for class discussion this semester on **Thursday, September 19**. The data is on the course homepage.

The problem description enumerates five major objectives. Our primary focus will be on #1 and #3. You should devote most of your analytical efforts to these two issues.

Your assignment for **Thursday, September 19** is to:

(a) determine as best as you can what the scientist is hoping to accomplish with this study (considering all 5 objectives);

(b) prepare a list of questions to which you believe that you need answers before doing a thorough analysis of the problem (again considering all 5 objectives);

(c) prepare 1 or 2 "key graphs" corresponding to objectives #1 and #3; and

(d) think about an analytical strategy for objectives #1 and #3;

(e) prepare some brief comments that together provide a useful summary of the main points and your (tentative) conclusions for issues #1 and #3.

Prepare two copies of your answers to this assignment, one to hand in at the beginning of class, and one to use for discussion during class.

Use of insecticide sprays for controlling harmful insects has the unfortunate side effect of negatively affecting honeybees that forage in sprayed areas (e.g. cornfields). The mode of action of some insecticides on honeybees is poorly understood but some researchers feel that biochemical protection may be a way of reducing the damage.

Reports from warm weather climates suggest that the insecticide Permethrin (abbreviated PER) has less effect on honeybees than other known insecticides. However, indications from cold weather locations (like Wisconsin) point to increased bee mortality due to PER at lower temperatures. Based on experience with other insects (e.g. cockroaches) it is thought that antioxidants might serve to counteract the negative effect of PER and reduce mortality. The primary goal of experimentation is to compare several antioxidants to determine which, if any, show promise for improving honeybee survival.

Six treatments plus a control (blank) were used in this experiment. The six are as follows:

(A) Glutathione - an antioxidant used successfully with other insects

(B) Vitamin E - a classic and natural antioxidant

(C) Vitamin E + Glutathione - combination of two treatments

(D) Cleland's reagent (Dithiothreitol) - a classic chemical but not natural antioxidant

(E) Cleland's reagent + Vitamin E - combination of two treatments

(F) Propolis - a natural product derived from plants and used by bees in the hives; an experimental antioxidant

The conduct of the experiment is now described. Bees were shaken out of a single colony on June 3, 1983. On a volume basis, bees were randomly placed in small containers; the volume was chosen so that each container contained approximately 50 bees. The bees were fed a sugar solution for the entire time spent in the container. A total of 84 containers were prepared, 12 for each of seven treatments (including the control).

Treatments were applied by two methods, fed or sprayed. For each treatment, 6 containers were randomly assigned to each group. For the "fed" group, the antioxidants were added to the sugar solution twice daily. For the "sprayed" group, the antioxidants were sprayed on the honeybees twice daily. (The control group was sprayed with water.) Treatment applications were continued for six days. For the single antioxidant treatments (A,B,D, and F above) the treatment concentration was 10^{-2} molar. For the combined treatments (C and E) the treatment concentration was 10^{-2} molar for each antioxidant.

From each group of 6 containers (6 "fed" and 6 "sprayed" for each treatment) 3 were randomly selected to receive insecticide (PER) while 3 received no PER. For those honeybees receiving insecticide, the PER was placed in the sugar solution on day 3.

Except for day 4, all containers were kept at ambient room temperature (about 22^oC). On day 4, all containers were moved to a cold room at 10^oC where they remained for 24 hours before being returned to ambient temperature. As the "spray" treatment was not feasible in the cold room, one application of treatment was skipped on day 4 for the containers in the "sprayed" group. (Hence all "fed" containers received 12 applications and all "sprayed" containers received 11.) The purpose of moving all containers to the cold room was to provide cold weather stress typical of cooler climates.

The containers were monitored on a regular basis. Each time a container was observed, the dead bees were counted and removed from the container. Each container was monitored until less than 10% of the bees remained alive. The recorded data contain the numbers of bees found dead for each container on each day of monitoring. Also recorded are the number of bees left alive when monitoring was discontinued.

The individual containers are indexed on the attached data sheet by a number from 1 to 84. the indices are described as follows:

Containers	
1 - 12	Glutathione
13 - 24	Vitamin E
25 - 36	Vitamin E + Glutathione
37 - 48	Cleland's reagent
49 - 60	Cleland's reagent + Vitamin E
61 - 72	Propolis
73 - 84	Control (Blank)

Within each group of 12, the first six containers received PER and the second six received no PER. Within each such set of six containers, the first three were in the "fed" group and the second three were in the "sprayed" group. The pattern is indicated as follows for containers 1-12.

	PER	fed or sprayed
1	YES	fed
2	YES	fed
3	YES	fed
4	YES	sprayed
5	YES	sprayed
6	YES	sprayed
7	NO	fed
8	NO	fed
9	NO	fed
10	NO	sprayed
11	NO	sprayed
12	NO	sprayed

For container 46, the experimenter observed that 4 bees escaped on day 4. This is noted by an asterisk on the data sheet.

As described earlier the primary experimental goal is to compare the antioxidants to determine which, if any, show promise for counteracting PER. The following five issues indicate some of the researchers' major objectives.

1. Characterize generally the performance of the antioxidants.
2. The mortality between days 4 and 6 is of specific interest to the biochemical mode of action of the antioxidants. Assess and compare the effectiveness of the antioxidants during this time period.
3. Of importance to beekeepers is the economic loss due to reduced honey production when bees die early. One variable sometimes used in this regard is "bee days", the total number of days that bees in a living unit survive. Using "bee days" (or some other approach) assess and compare the antioxidants with respect to premature mortality.
4. Compare the relative effectiveness of providing antioxidants by feeding or spraying.
5. Does there appear to be any inherent toxic effect of the antioxidants themselves.

The following references may provide useful background information.

Miller, T.A. and Adams, M.E. (1982). "Mode of Action of Pyrethoids" in *Insecticide Mode of Action* (J.R. Coats ed.). Academic Press.

Norris, D.M. (1981). "Possible Unifying Principles in Energy Transduction in the Chemical Senses" (Chap. 12) in *Perception of Behavioral Chemicals* (D.M. Norris ed.) Elsevier/North Holland Biomedical Press.

Gojmerac, W.J. (1980). *Beekeeping, Honey, and Pollination.* AVI Publishing Co.

Elliott, M., James, N.F., and Potter, C. (1978). "The Future of Pyrethroids in Insect Control". *Ann. Rev. Entomol.* 1978, 443-469.

Various general pamphlets on honey bees at UW Extension.