Streptomycin effects on chlorophyll in higher plants.
Nancy Ziebur, 1949-1950

Pravasoli, Hutner and Schatz (Proc. Soc. Exp. Biol. & Med. 1948, 69:279-282) showed that certain strains of Euglena gracilis in the presence of streptomycin (STM) undergo a permanent loss of chlorophyll but will continue to grow well if a proper energy source is supplied. Their paper also refers to several of von Euler and Bracco who reported that seeds germinated in STM solutions developed into chlorophyll deficient seedlings - no report beyond the seedling stage.

A. Methods.

1. Streptomycin used in two forms:
   Dihydrostreptomycin, 1.2 g. of which is equivalent to 1 g. STM base.
   Calcium chloride complex, 1.45 g. of which is equivalent to 1 g. STM base.
   The reactions to the two forms were indistinguishable.
   The doses given will be referred to in terms of STM base.
2. Seed treatments
   Used agar plates with .5% agar. STM dissolved in sterile water and added to the sterile warm agar. No contamination with this method.
3. Bud treatments
   Agar caps made in the following fashion: Take about 15 mm. of a jumbo drinking straw (6 mm. diam.), cover one end with a 5mm square of aluminum foil, wind a 5mm. wide strip of scotch tape around tightly bringing the two stieky sides together where the ends meet. This gives a tab, a slit in which is useful to hold a thread from slipping up or down in case it is necessary to tie the straw to the plant. A gel of .7% and 1% STM is used, with .3 ml. in each straw.

   Lanolin suspensions were tried but didn't work well.
4. Injections were made with non-sterile solutions of varying strengths.

B. Experiments.

1. Radished - Scarlet Globe
   a. Seed treatments.
      Seeds sterilized with $HgCl_2$ 2:1000 and deturgent (together), rinsed, soaked overnight in sterile water, resterilized. Placed on agar plates. After two days transfered to test tubes with .7% agar, 2% sucrose, and Randolph and Cox minerals. Some seedlings entirely white, some partly green. Growth poorer than controls and extremely variable. Even if small parts of the treated seedlings remain green, they do not durvive in soil. On sugar medium seedlings never grew more than about 6 inches (however, controls also did poorly in sterile culture. Attempts at quantitative comparisons of radish growth were abandoned because of the variability of the material.
   b. Bud treatment
      Inflorescences or growing tips were treated by squeezing them into agar filled straws, having removed as many buds or leaves as necessary to get a good fit. Not necessary to tie straws on. Straws left on about 5 days or until agar had dried. The leaves and stems inside were invariably

bleached when they expanded, with some green remaining if the leaves were quite large when treated. The effects could also be seen on leaves farther down on the stem. White leaves were often stiff, and always grew more slowly than green ones. Affected stems were redder than normal. When the apical growing point was treated, green side shoots farther down began rapid growth and accounted for most of the further growth of the plant. Flowers arising from treated buds frequently aborted before opening. Some opened into flowers smaller and pinker than normal. Others appeared normal. Pollen from the last two types was applied to normal stigmas and about 50% set seed. Individual flower bags were used. No real control for fertility comparisons was used, but pollen from one inflorescence that was only very slightly pale effected 100% fertilization. It seems likely that pollen fertility is actually reduced by severe STM treatment. Treated flowers were pollinated with normal pollen, but of 25-50 flowers only 2 showed the beggginings of fruit development. One of these aborted, and the other produced two shriveled seeds. Female sterility is unquestionably present. Normal ♀ x STM ♂ and comparable normal x normal seeds were plentiful and should be grown out.

2. Peas - Alaska - Bud treatment
   Similar to that for radishes except that the straws had to be tied on. Several affected pods started growth, presumably self-pollinated, but aborted after reaching a maximum size of about 3/4 inch long. Pollen output of affected flowers seemed more reduced than in the case of radishes; the few attempts to use it for fertilizing normal flowers were unsuccessful.
3. Tomatoes - Bonny Best
   a. Bud treatment. Growing points were killed by as short a treatment as 1 day with 1 % STM. No chlorophyll abnormalities appeared, altho the plants grew somewhat purplish. near treated point.
   b. Injection. 1% or .1% STM solutions were injected into ends of cut-off side branches. No positive effect was shown.
4. Corn
   a. Injections.
      Seedlings in soil, about 3-4 inches high, injected from the top with a solution containing .1% STM and .01% deturgent. Seedlings became bleached, and died in about 3 weeks. Controls injected with deturgent only were ok.
   b. Seed treatments. Same methods as for radishes, after 5 days on STM agar still no effect.
   c. Embryo treatments.
      Seeds sterilized, soaked 2 days, embryos excised and resterilized, then placed on STM agar plates. Effect clear after 5 days and seedlings could be transferred to test tubes as with radishes. Quantitative tests in light and dark were also run to determine whether the affected plants can utilize sucrose.