

# **ILLUSTRATED GUIDE ON HOW TO WORK WITH CORN**

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STANFORD UNIVERSITY

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Pledge your life to corn

## 1. PLANNING EXPERIMENTS

This is the stage at which you must decide what seeds will be planted. Planning should begin in March - April for mid May planting, in October for Hawaii, and in December for the greenhouse.

Time Required to Plan 100 families:

|                                 |                 |
|---------------------------------|-----------------|
| A. outlining                    | 5               |
| B. calculating number of plants | 2               |
| C. planning staggered plantings | 1               |
| D. laying out field             | 1               |
| E. preliminary computer file    | 5               |
| F. use information              | 3               |
| <b>TOTAL ESTIMATED TIME</b>     | <b>17 hours</b> |

### A. Outlining

Use 4-6 column sheets as shown in Figure 1, one per experiment. Assign names to basic experiments and begin listing materials (by previous use name) in appropriate column. Be sure to include testers, back-up versions of important materials, etc. Some of the items listed will be failures from the previous crop listed in your field cards so be sure to check this list (see SF). Also review previous year cards and make sure you have not forgotten anything. Assign family numbers in pencil and leave gaps of -5-10 blank families between experiments.

It is a good idea to write out a flow sheet for each type of experiment you are planning. Figure 2 shows the rationale and initial crosses to construct *Bperu* anthocyanin testers. Each year relevant families are added. These flow sheets will help you remember to plant every stock required for your experiments.

### B. Calculating The Number Of Plants

Carry this list around with you for a week or so and add ideas carefully complete each genotype description. Proofread! Proofread again!! Decide how many seeds will be required (Figure 3) for an appropriate probability of success in the experiment. Then, factor in expected germination (-90% for one year old seed, 80% two year old, etc. based on your experience) and possible losses to predators. Most people over-plant by about 20%. You will probably be happier if you over-plant all testers by 50%. Also, keep in mind that a skilled person can do about 250 pollinations per day, and a team about 600.

### C. Staggered Plantings

To make sure that ears and pollen involved in cross pollinations are ready at the same time, plantings of one or both materials can be repeated at weekly intervals. In most materials pollen is shed for about 7 days (Figure 4), and the ears are receptive for about 7 days also (Figure 5). Usually pollen is shed 1-2 days before the ear on the same plant is ready.

In general, materials with a Northern inbred background (A188, W23, BMS) flower about 60-65 days after planting. Materials from the central Midwest (M020, K55) flower in about 62-67 days and Southern lines (Ky21, KYS) take 70-75 days. Hybrids among plants in a similar maturity zone will flower 5-7 days earlier than either inbred. The most reliable guides to flowering dates are your own records from previous years.

Figure 1

F year preliminary list

| Family # | Derivation     | Expt.           | to do   | #K             |
|----------|----------------|-----------------|---------|----------------|
| 1        | E 110-5 X 78-1 | stock construct | ⊗       | 5 Purple       |
| 2        | E 110-5 X 79-1 | "               | ⊗       | 5 P            |
| 3        | E 110-7 X 80-3 | "               | ⊗       | 5 P            |
| 4        | C 229 X 230-8  | mutable         | by bz 2 | 10 sp          |
| 5        | C 229 X 230-8  | "               | " ⊗     | 10 bz          |
| 6        | E 78-1 ⊗       | stock           | ⊗, sib  | 5 P            |
| 7        | E 79-1 (X)     | "               | ⊗, sib  | 5 P            |
| 8        | A 188 —        | K. Newton stock | ⊗       | 10 cl          |
| 9        | RSCM - Neuffer | stock           | ⊗       | 10 P           |
| 10.1     | E 300 X sib    | bz2 tester      | tester  | 25 bz } week 1 |
| .2       | "              | "               | "       | 50 bz } 2      |
| .3       | "              | "               | "       | 100 bz } 3     |
| .4       | "              | "               | "       | 25 bz } 4      |

etc.

---

#K 265

3-8-84

# FIGURE 2. FLOWSHEET

Stock 2: Rself color Sources in C year: C112 = Rscm-2, C113 = Rsc, C114 = Rsc, C115 = Rscm, C131 = Rscm2. In a few cases Robertson Mu Rscm plants x either r-g or Rscm/Rsc were used in crosses; these families include C208, C209, C218, C226. In these cases careful checks of the final self-color genotype should be made to be sure that Rsc or Rscm was actually incorporated into the stock.

## INBRED & HYBRID TESTERS

Rsc & Rscm

YEAR / FAMILY

| Genotype & Family            | C    | CF          | CH | D                           | DH | E                                     |
|------------------------------|------|-------------|----|-----------------------------|----|---------------------------------------|
| a1 sh2 (NI) C142)            | F112 |             | 15 | 143-repeat                  |    | ✓ 105                                 |
|                              | F113 |             | 16 | 144-repeat                  |    | ✓ 106                                 |
| a1 sh2 (K55) C143            | F112 |             | 17 | <145> repeat                |    | ✓ DH10 = 066x44 107                   |
|                              | F113 |             | 18 | <del>repeat</del>           |    | 066=C143x142 108                      |
| a1 sh2 <del>(K55) C143</del> | No   |             |    | <del>start over</del>       |    |                                       |
| a1 sh2 <del>(K55) C104</del> | No   |             |    |                             |    |                                       |
| a2 bt pr (W23) C67           | F113 |             | 19 | 160-8, 11 = R NJ only       |    |                                       |
| a2 bt pr (K55) C135          | F114 |             | 20 | 031: No yield start over?   |    | C135 wrong stock? E140                |
|                              | F115 |             | 21 | 032: No yield start over?   |    | C135 wrong stock?                     |
| 02 Coop E122<br>E123<br>E124 |      |             |    |                             |    |                                       |
| sh1 bz1 wx (W/K) C101        | M112 |             | 22 | 155-4                       |    | E148                                  |
|                              | M113 |             | 23 | fungus repeat               |    | 142                                   |
| 2. bz2 (W/K) C229            | M112 | <31> repeat |    | DH20                        |    | E155 < insert line for repeat of DH20 |
|                              | M113 | 148         |    | 79-2                        |    | E156                                  |
| • bz2 (K55) C137             | F115 |             | 43 | 138                         |    | E164<br>E159??                        |
| c1 sh1 wx (W/K) C102         | M112 |             | 24 | 171-5 E171                  |    |                                       |
|                              | M113 |             | 25 | 172-5 E172                  |    |                                       |
| su c2 (W/K) C215             | M112 |             | 26 | 410x<br><del>fail 187</del> |    |                                       |
|                              | M113 |             | 27 | fail 188                    |    |                                       |
|                              | M115 | <27>        |    | repeat                      |    |                                       |
| su c2 (W23) C138             | F113 |             | 28 | 194-2 c2 Rsc/R              |    | 189                                   |
| su c2 (NI) C139              | M112 |             | 29 | <del>repeat</del>           |    | 190                                   |
|                              | F113 |             | 30 | 196-6                       |    | 191                                   |
| b r-r pl (W/K) C100          | No   |             |    |                             |    |                                       |
| r-g (W/K) C103               | No   |             |    |                             |    |                                       |
| r-g b pl (W23) C140          | No   |             |    |                             |    |                                       |

How should stock look? Spots?  
what about D197 (c2 AC Rscm)

Figure 3

Calculating # of seed

$$n \cdot \log(1 - \text{fraction}) = \log P$$

$n$  = # of plants

$1 - \text{fraction}$  =  $1 - \text{fraction expected to be correct}$   
= "not required class"

$P$  = probability

Problem Example

How many plants required of 3:1 segregating population to be 95% sure of finding 1 recessive

$$n = \log\left(\frac{3}{4}\right) = \log 0.05$$

$$n = \frac{\log 0.05}{\log 3 - \log 4} = \frac{1.30103}{0.12494} = 10.4 = 11 \text{ plants}$$

Figure 3 cont'd

\* TABLE III

| Fract.<br>expect. | 0.900 | 0.950  | 0.980 | 0.990 | 0.995 | 0.998 | 0.999 |
|-------------------|-------|--------|-------|-------|-------|-------|-------|
| $\frac{1}{2}$     | 3.3   | 4.3    | 5.6   | 6.6   | 7.6   | 9.0   | 10.0  |
| $\frac{1}{4}$     | 8.0   | 10.4   | 13.6  | 16.0  | 18.4  | 21.6  | 24.0  |
| 1/8               | 17.2  | 22.4   | 29.3  | 34.5  | 39.7  | 46.5  | 51.7  |
| 1/16              | 35.7  | 46.4   | 60.6  | 71.4  | 82.1  | 96.3  | 107.0 |
| 1/32              | 72.5  | 94.4   | 123.2 | 145.1 | 166.9 | 195.7 | 217.6 |
| 1/64              | 146.2 | 190.2  | 248.4 | 292.4 | 336.4 | 394.6 | 438.6 |
| 1/3               | 5.7   | 7.4    | 9.6   | 11.4  | 13.1  | 15.3  | 17.0  |
| **2/3             | 2.1   | × 2.72 | 3.6   | 4.2   | 4.8   | 5.7   | 6.3   |
| 1/9               | 19.5  | 25.4   | 33.2  | 39.1  | 45.0  | 52.8  | 58.6  |
| 1/27              | 61.0  | 79.4   | 103.6 | 122.0 | 140.4 | 164.7 | 183.0 |

The numbers in the body of the table are the numbers of individuals which should be raised, in a progeny, in order that a certain type, expected to form a known fraction of the progeny, may be expected to occur, with a chosen level of probability, at least once. For example, suppose on selfing a plant heterozygous for one gene (i.e. Aa) we want to raise a family sufficiently large to contain at least one recessive (aa) in 99 cases out of 100. Recessives types are expected in  $\frac{1}{4}$  of the cases. Then taking the second row of the table (the  $\frac{1}{4}$  row) and the fourth column (probability 0.99) we find that sixteen plants are needed

\*Mather, Kenneth, 1957. The Measurement of Linkage in Heredity. Methuen and Co. Ltd., London 9. Page 143.

\*\*Calculated by Ming Tang Chang 11/6/81

With this information in mind, we usually plant all testers four times, *i.e.* F100.1, F100.2, F100.3, F100.4 (Figure 6 explains notation). The first planting is one week before any materials to be crossed with and will contain about 15% of the seeds. The second planting one week later will contain about 50% of the seed and will coincide with the bulk of the planting of the other materials. The third and fourth planting are one week apart and have 15-20% of the total seed. Any tester out of synchrony with crossing materials can be bulked up by selfing. If hybrid testers are used the relevant inbred testers should be planted for reciprocal crossing and for crossing to hybrid tester as well to maintain the stocks.

If cold weathers comes (<55°F), then each cold day results *in* 2-3 days of no/slow growth in seedlings at cold sensitive periods. The most cold sensitive stages are [1] germination, [2] 6-8 leaf stage during transition to adult leaves, and [3] tassel and ear maturation. Thus, a .1 planting made one week earlier than a .2 planting may flower later, depending on weather conditions. Thus, staggered plantings don't always work out as planned but having lots of tester plants available throughout the season is a great feeling!

#### **D. Laying out The Field**

Basic terms:

row = physical space where seed are planted

range = square or rectangular section of field with wide aisles

around it; *in* our field the corners of ranges are where the sprinkler heads are

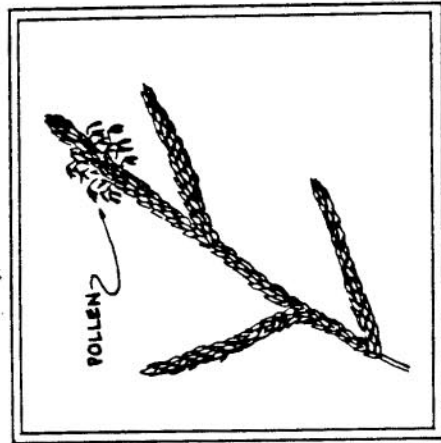
family = group of seeds derived from the same ear

Using a piece of graph paper, note the location of sprinklers and other items *in* the field (Figure 7) that determine the ranges. You will be assigned a specific range(s) for your plants. Number the rows within your range(s); not all ranges have the same number of rows so check this carefully to make sure you have enough space. Then, sketch out where your individual families will be: using the assigned number of kernels per family on your planning sheet, lay out a planting diagram.

If there is room, plants are 8' inches apart *in* rows 36 inches apart. To fit more into the field, we can use two close rows, then a spacer row: 18" (plants), 18" (plants), 36", 18" (plants), 18" (plants), 36", etc. At the edge of most ranges there is a wide aisle, don't plant in the aisle. The aisle space is critical during pollinating season for getting quickly from one place to another. If you are assigned ranges that lack obvious aisles, leave 3 - 4 feet between ranges anyway. Be careful that you and a neighbor don't plant 4 rows 18" apart at edge of range: you won't be able to get to the interior plants easily during pollinating.

Hints:

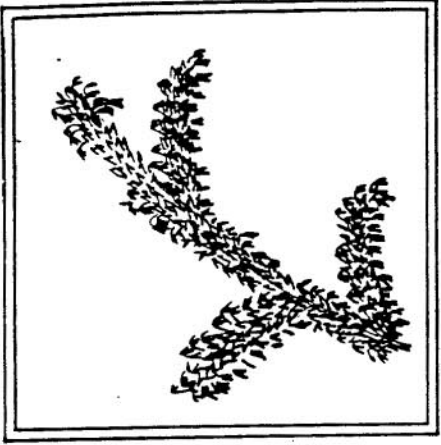
- 1) Plant left to right down each row, don't serpentine unless it is important for your experiment; mixture of serpentine and straight planting will be very confusing to helpers.
- 2) If possible, don't split families: try to keep all in the same row.
- 3) Try to minimize long walks; it may be helpful to split testers into families within each experiment rather than have one large block.



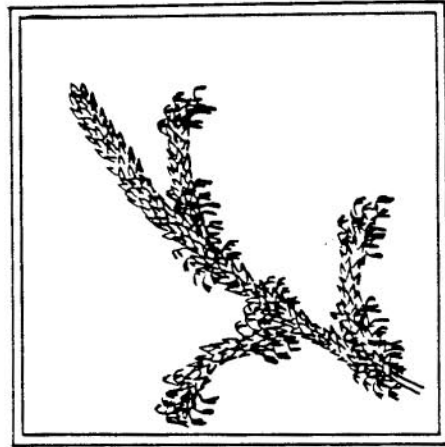
DAY 1



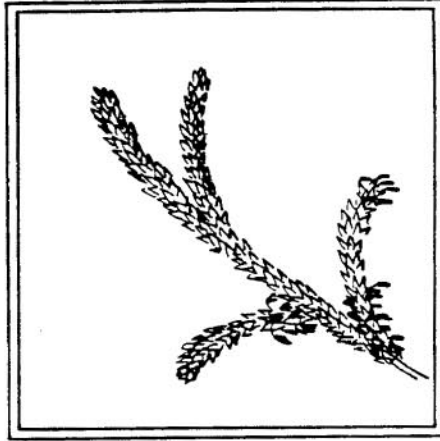
DAY 2



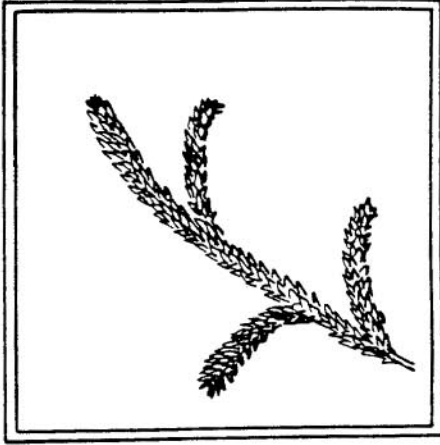
DAY 3



DAY 4



DAY 5



DAY 6

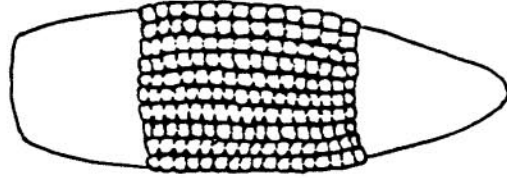
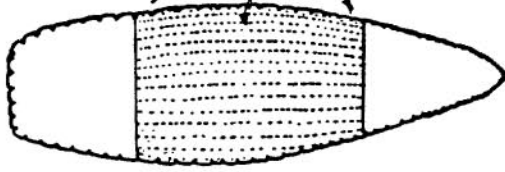


FIGURE 4. PATTERN OF TASSEL MATURATION

AREA OF NEW SILK GROWTH

DAY 1

SILKS GROW FROM HERE FIRST

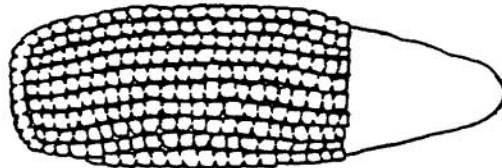
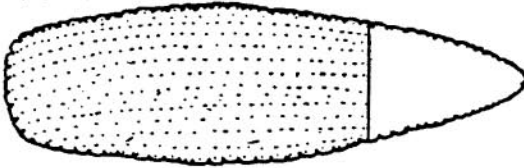


IF YOU POLLINATE JUST THESE SILKS, YOU GET AN EAR..... LIKE THIS

AT HARVEST

DAY 2

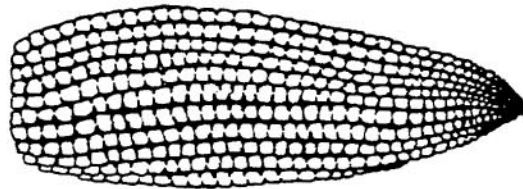
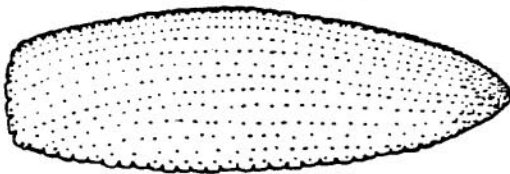
THEN BASE



AT HARVEST

DAY 3

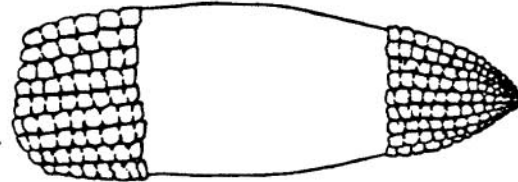
THEN TIP



AT HARVEST

DAY 4

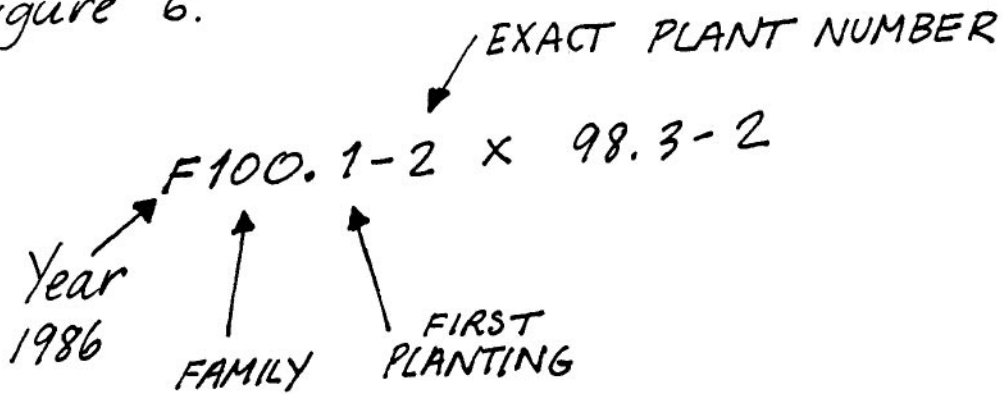
EXTREME TIP



AT HARVEST

FIGURE 5. EAR MATURATION

Figure 6.



A Year 1981

FIGURE 7

RANGE #1

| RANGE #1         |           |          |            | Row |
|------------------|-----------|----------|------------|-----|
| F1 (10K)         | F2 (5K)   | F3 (20K) | F4 (10K/5) | 1   |
| F4 (5K)          | F5 (10K)  | F6 (10K) | F7 (15K)   | 2   |
| BLANK            |           |          |            | 3   |
| F8 (40K)         |           |          |            | 4   |
| F9 (20K)         | F10 (20K) |          |            | 5   |
| BLANK            |           |          |            | 6   |
| F11 (40K)        |           |          |            | 7   |
| F12 (100K - 40)  |           |          |            | 8   |
| BLANK            |           |          |            | 9   |
| F12 cont'd 40K   |           |          |            | 10  |
| F12 - cont'd 20K | F13 (20K) |          |            | 11  |
| BLANK            |           |          |            | 12  |
|                  |           |          |            | 13  |

~ 40 PLANTS PER ROW

## E. Preliminary Computer File

This section assumes that you are already somewhat familiar with the IBM-PC and the Cardbox programs. If not, read chapter 9 on the computer. There are several purposes for the preliminary computer list.

- 1) to proofread computer input before printing permanent "bluecards"
- 2) to have a place to jot down initial germination records
- 3) as inspiration for additional experiments

We use the CARDBOX program to organize our records on the IBM PC. Figure 8 shows the standard format for a family card in the BLUECARDS format of Cardbox. Cardbox allows you to reformat data in many different ways (examples given later on). Anne Britt has also written a Hypercard stack for the Macintosh for handling corn records for Mac users. At the University of Missouri, field cards were traditionally printed on blue card stock, hence the nickname "bluecards." We now print these cards on buff, green, pink, yellow or green stock with or without lines.

Typically the new year's records are kept as a separate file with the letter of the year as its name (1981 = A, 1982 = B, etc. current year file is HYEAR). Greenhouse records are given double letters (FF) and Hawaii crops with year and H (FH). FF and FH are the winter (1986-1987) crop after the F (1986) year.

Under ideal conditions the preliminary file is created before any seeds are planted; this is always true of Hawaii crops, but for summer and greenhouse crops it is unfortunately all too easy to postpone creating the file until after planting. In any case, the file is established in the spring; see chapter 9 for the detailed "how to."

Basic Procedure for entering new families into an existing file, i.e. HYEAR. Open the HYEAR file, and go to last entry. using the Aadd (create next card please command) you will get a blank card. Enter the Year F Family 1, and Family Number then save the card. Make 20 - 50 new cards and then proofread that you have them in the correct numerical order. Then return to the first new card you created and begin adding the specific information on each on the outlining list or seed envelopes (Section 2A). This is a good time to very carefully proofread all the derivation information and genes. For example, be very careful to distinguish between homo zygotes (BZI) , heterozygotes (Bz1/bz1) and cases where you don't know (BZI/-). The more information you put on the card, the more useful your records will be. Try to specify exactly what kinds of seed are required: cl (colorless), pu (purple), mo (mottled), sp (spotted), ye (yellow), bz (bronze), etc.

At this point you should have a final list of families. We find it is a good idea to leave a few blank families at the end of every experiment to fill-in with last minute ideas.

Now, print out the HYEAR card list on white paper for proofreading, as an aid in pulling seed, and for initial field notes. If holes are punched at the top of the sheets they can be attached to the rings of a clip board. For printing out you should consult the computer chapter.

**Important note:** The spacing for the bluecards is a few lines less per group of 3 compared to standard 8 1/2 x 11 paper. If you want exactly 3 records per sheet of paper, you have to adjust the printing parameters slightly.

FIGURE 8 and 9

|            |                                     |                   |
|------------|-------------------------------------|-------------------|
|            | F# D49                              |                   |
|            | Yield                               | 7 EARS<br>} SAVED |
| Year D     | Family 49                           | D498              |
| Derivation | 35:341-2 x -1                       | D49-2 X -3        |
| Experiment | observe, paramutation               | D49-3 X 99-1      |
| Genes      | R-v2/R-g Sh Wx / sh wx x Sib        |                   |
| Nucbkgd    | Neuffer                             | Cytoplasm Neuffer |
| No. seeds  | 5                                   | Date planted 3/27 |
| Purpose    | observe plants, sib to expand; cros |                   |
|            | 1 -2 by Bperu                       |                   |
| Obs        | mottled seed, purple or red (Pr?)   |                   |
|            | All 36                              | Keplant 5/15 26   |
| Use        | D498 = E100, F93                    |                   |
|            | D49-3 x 99 = E101                   |                   |

REDLINE IN RED PEN OR PENCIL

## **F. Use Information On Prior Year Cards And In Computer**

Now that you have an accurate list of all things to be planted, it is time to log in the USE information on prior year records. This information is critical for "search forward" questions - that is, when you want to trace the fate of material from some long ago generation up to the present day. Derivation information on each card allows you to "search backward," of course, but sometimes taking an old record and tracing it can show up ideas that you had but failed to pursue to completion. Ah, nostalgia.

Traditionally USE information is entered in red ink and is called "red lining" (Figure 9). This operation has to be done twice: on the previous year bluecards and on the computer records. If either a field card or computer file gets full, create a second card for that family. The cardbox format file called REDLINE will compactly organize the information you need to carry out this operation; if you format your HYEAR file, for example, in REDLINE, then select year A+ (A, AA, etc.) and print you will get a numerical list of all of the A year records that must be redlined; then select year B+ (B, BB, BF), etc. This will provide you with an easy to use checklist.

## **G. Summary**

The main advice is to be as organized as possible. Try to start early and keep all scraps of paper. Xerox anything important. In a pinch you can even xerox all of your seed envelopes to make a preliminary list. Be sure to make back-up copies of your current year file ~- after every addition. For example, keep the working file on a diskette and the back-up version on the hard disk. Be sure that you always add to the same file. After you enter the USE information be sure to make a new backup of your permanent file.

**Figure 10. Checklist for a Summer Crop**

| <b><i>Task</i></b>                                    | <b><i>Target Date</i></b> | <b><i>Completed</i></b> |
|---|---------------------------|-------------------------|
| 1. Rationale for Experiments written Preliminary List | March 1                   |                         |
| 2. Preliminary List                                   | April 1                   |                         |
| a. assign family members                              | April 1                   |                         |
| b. designate seed properties and number               | April 1                   |                         |
| c. include Hawaii materials                           | April 15                  |                         |
| d. include greenhouse materials                       | April 15                  |                         |
| 3. Plan Field   | April 15                  |                         |
| 4. Plan Staggered Plantings                           | April 15                  |                         |
| 5. Layout Field Plan                                  | April 25                  |                         |
| 6. Make Preliminary Computer File                     | April 25                  |                         |
| 7. Log use information on cards                       | May 15                    |                         |
| 8. Log use information on computer files              | May 25                    |                         |

## 2. PULLING SEED

This section is concerned with obtaining the seeds required for your experiments. The quality of this step depends on your work in stage 1 -- planning experiments. As soon as your preliminary list (either outline or computer list) is ready, about April 15th, you can begin making seed envelopes.

Time required for 100 families:

|                             |                   |
|-----------------------------|-------------------|
| Writing Seed Envelopes      | 3                 |
| Obtaining Seed              | 4                 |
| Seed Treatment              | 1                 |
| Storage of Seed Envelopes   | 0.25              |
| <b>TOTAL ESTIMATED TIME</b> | <b>8.25 hours</b> |

### A. Preparation Of Seed Envelopes

Using a stamp, label each seed envelope (Figure 11) making sure that the top of the envelope (flap end) is at the top relative to the stamp. The minimum information to put on is family number, derivation and seed number and type. This information is required to get the proper seeds. It is also wise to fill in the other categories if you are working from an outline sheet. If you are working from a computer list, all of the information is asked for by the bluecards format.

If you are paranoid, xerox the seed envelopes.

### B. Removing Seeds From Cobs

Using the seed envelopes as your guide, get each type of seed from seed storage. Previous year materials are filed by year (A, B, C, etc.) and then numerically by female parent: AI06-1 x 22 would be filed in an A box in a bag with numbers around 106. Materials more than 3 years old are usually shelled while younger materials are still on the cob.

In general the best seeds are in the middle of the cob, although they are easier to get off at either end of the cob. If the end kernels are well formed, use them. If central kernels (or specific phenotypes are desired) use a letter opener to pop particular kernels off. If other kernels come off you may be able to just discard them. If material is precious or you are in doubt about the kernels' value, place the ear and loose kernels in a glassine ear bag. Double check that the correct number and phenotype of kernels were taken. If there is concern about germination, you may want to put in a few extra kernels now and you will already have seed ready for replanting. Note on envelope if seed should be started in peat pots.

When seed envelopes are filled, line up in numerical order in a narrow box or plastic tray. Put sequential (staggered) plantings in separate envelopes and label appropriately: 100.1, 100.2, etc. The dot then number signifies sequential planting of the same material. Then, seal each filled seed envelope. Put a paper clip on unfilled envelopes in a group of families that are already filled: the paper clip is a reminder that there is still something to do. Most unfilled families arise when you cannot find the designated ear or it has too few seeds.

FIGURE 11. STAMPING SEED ENVELOPES

Year \_\_\_\_\_ Family \_\_\_\_\_  
Derivation \_\_\_\_\_ X \_\_\_\_\_  
Experiment \_\_\_\_\_  
Genes \_\_\_\_\_  
Nuclear Background \_\_\_\_\_  
Percentage \_\_\_\_\_  
Cytoplasm \_\_\_\_\_  
Number of seeds \_\_\_\_\_  
Date Planted \_\_\_\_\_  
Purpose (Crosses to be Made)

Year \_\_\_\_\_ Family \_\_\_\_\_  
Derivation \_\_\_\_\_ X \_\_\_\_\_  
Experiment \_\_\_\_\_  
Genes \_\_\_\_\_  
Nuclear Background \_\_\_\_\_  
Percentage \_\_\_\_\_  
Cytoplasm \_\_\_\_\_  
Number of seeds \_\_\_\_\_  
Date Planted \_\_\_\_\_  
Purpose (Crosses to be Made)

As you pull the seed write the use information on the ear tag. This is an easy step to forget, but it often helps later on particularly if there are several ears of the same type and you want to verify which ear was used. This also serves as a crosscheck that you pulled seed from the correct ear, and allows you to proofread another time.

### **C. Seed Treatment**

Usually fungicide is put on the seeds before planting. Wear a mask and gloves to minimize contact. Do not touch your eyes. Do the fungicide treatment in an area that is easy to clean and safe for others (i.e. away from food supply).

First, transfer a small amount of fungicide from the can to a seed envelope or to a squirt bottle with a large nozzle. Then, pop open each seed envelope and either squirt in a small amount of fungicide or use a weighing spatula or the end of a small stake to pick up small amount and lightly dust onto the kernels in each envelope.

### **D. Storage Of Seed Envelopes**

When planting is finished (Section 3) keep the seed envelopes. Some will have additional seed in them, handy for replanting. If of a particular genotype is required you already have an envelope.

At the end of the planting period, file the envelopes in the cabinet at the field laboratory for future reference.

### **E. Summary**

Refer to checklist (Figure 12).

#### **Figure 12. Checklist for Seeds**

| <b><i>Checklist</i></b>    | <b><i>Target Date</i></b> | <b><i>Completed</i></b> |
|----------------------------|---------------------------|-------------------------|
| Write out seed envelopes   | April 25                  |                         |
| Obtain Seed                | May 1                     |                         |
| Write use info on seed tag | May 1                     |                         |
| Seed Treatment (in field)  | May 15                    |                         |
| Any last minute ideas?     |                           |                         |

### 3. PLANTING

This section is concerned with the actual physical layout of the field, seed planting and initial record keeping.

Time required for 100 families:

|                                  |                   |
|----------------------------------|-------------------|
| Travel to/from Field             | 1.0               |
| Painting and Labeling Stakes     | 2.0               |
| Placing Stakes in Field          | 2.0               |
| First Planting - 10 Families     | 0.5               |
| Second Planting - 80 Families    | 4.0               |
| Third Planting - 10 Families     | 0.5               |
| Germination Records and Computer | 3.0               |
| Transplant 50 Pots               | 2.0               |
| Checking Plant Phenotypes        | 2.0               |
| Printing Field Cards             | 1.5               |
| <b>TOTAL ESTIMATED TIME</b>      | <b>18.5 hours</b> |

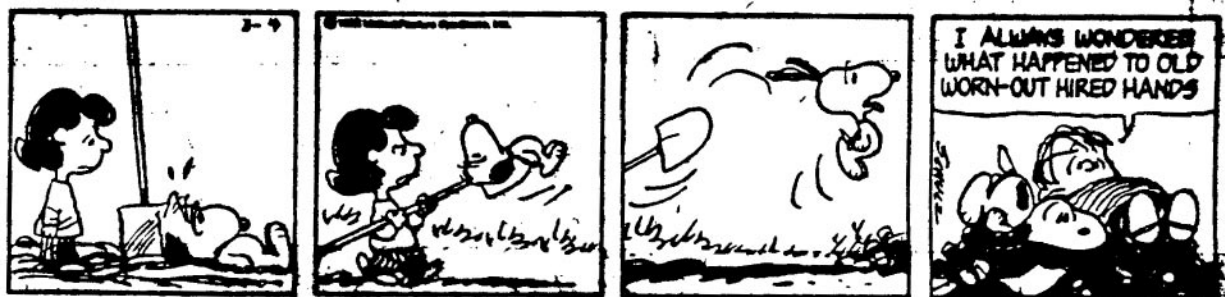
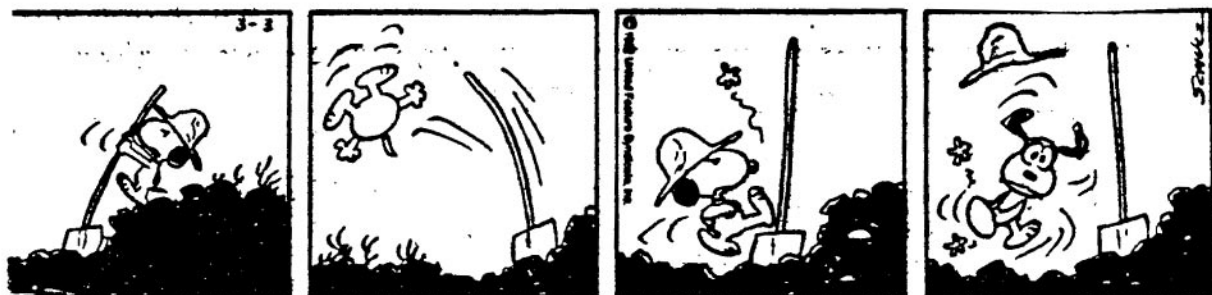
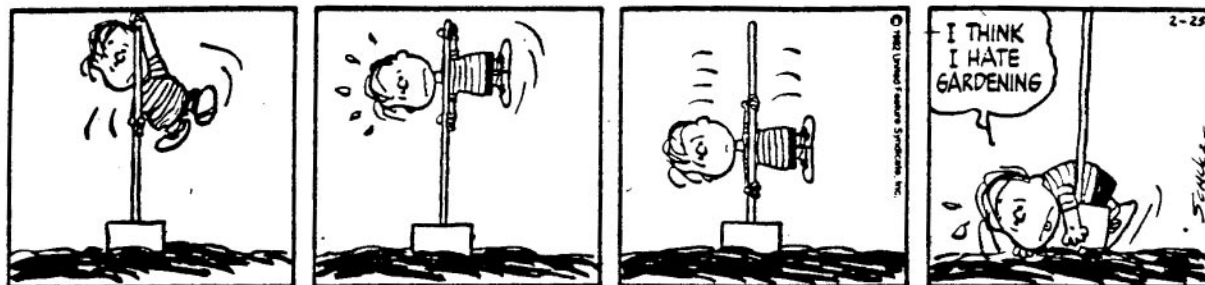
#### A. Stakes and Field Design

To mark families use 12" painted wooden stakes. It's a good idea to spray paint stakes different colors for different experiments. Mark stakes with a high density marker (MARKSALOT) in large numerals. write the family number on BOTH SIDES OF EACH STAKE. It is important to mark the number of kernels (10K, 25K, etc.) on one side of the stake.

Stakes We have a stake for the beginning of each family and end of every row (i.e. end 17 means end of family 17). All of the family stakes should be written before you go to the field. The family stake gives the family number and number of kernels; if you are "double planting" 4 inches apart put DP # kernels so you will remember to leave room in the field for one-half this number of seed. If you have a detailed field plan you can write the end stakes in advance: the end stake has the word END at the top and the family number (Figure 13). Many people find it easier to write the end; stakes in the field after laying out all of the family stakes: carry a MARKSALOT pen and a bucket of all the different colors of stakes with you to do this.

It's best to plant the field "left to right" in each range rather than serpentine, but having different beginning and end stakes makes a serpentine pattern possible. If a family is longer than the row, write a third stake with the family number and the word MORE (Figure 13) to place at the beginning of the next row. If a family extends into the next range, write GO TO RANGE! on the END stake.

# FIELD SEASON STARTS SOON



If at all possible plant the field in exact numerical order. It is confusing to have families out of order, and such "out of order" families can be forgotten or be the source of mistaken pollinations. Put in "dummy" stakes for blank families to prevent confusion (Figure 13). Be sure to write separate stakes for different planting dates of the same material (150.1 = first planting, 150.2 = second planting, 150.3 = third planting, etc.).

Laying out Field Thoroughly hoe the tops of all rows that are to be planted to disrupt any weeds. Some weed control can be achieved by irrigating about 10 days before planting to stimulate weed seed germination and then eliminating weeds by hoeing and by arranging to have your area sprayed with Round-Up. Because Round-up is a nonselective herbicide, it must be used with great care in the corn field to prevent the disaster of wiping out someone's seedlings. Only the field manager should apply herbicides.

Before planting any seed, place about 1 range worth of stakes out using your field plan and the kernel number on each stake as a guide. In our typical layout plants are spaced 8" apart (10 plants in 6 feet of a row). If you measure out space for typical family sizes (5, 10, 20) very carefully in the first few rows you can line up subsequent rows with reasonable accuracy. As you finish a row, place or write the appropriate END stake.

It's a good idea to recheck the stake layout before planting a single seed. Particularly check:

(1) Order of families

(2) Appropriate rows left open as aisles; in our high density planting scheme (Figure 7), rows are 18" apart; rows 1, 2, 4, 5, 7, 8, etc. are planted and rows 3, 6, 9 are left blank.

(3 ) No stakes in the aisles separating ranges

## **B. Planting**

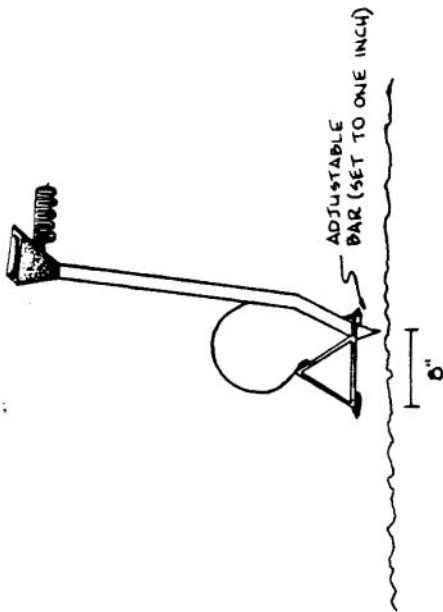
Consult the drawings to see the *corn planters shuffle* (Figure 14). Basically, pick up an envelope, check the family number against the stakes, and drop one seed at a time into the Narrow Bit Hand Jab Planter. Push tip of planter about 1 inch into soil and rock forward. This motion delivers the seed through a "trap door. The; front of the planter makes an impression in the soil about 8 inches forward, the right distance for the next seed. Use this impression for placing the planter into the soil. As you plant, use your foot to tamp down soil, or walk back over each row when down planting. If seed need a different planting depth, adjust the sliding ring at the base of the planter to determine how deep the planter pushes into the soil.

While planting watch that the planter springs shut properly after each seed is planted. Clean off soil and small rocks. It's best not to plant if the soil is wet enough to cause mud to enter the planter

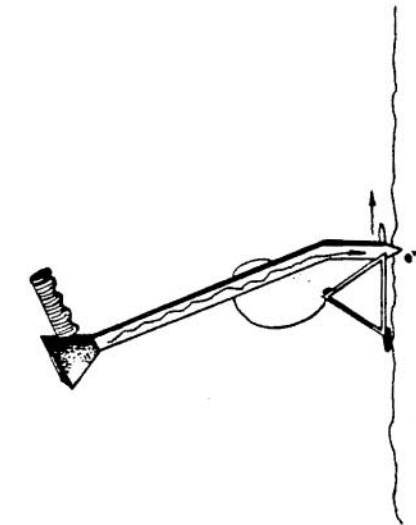
Predators are a substantial problem. After planting, cover everything important with bird netting. pin the netting down with extra stakes and/or rocks.

Safety tip As this is likely to be your first long day in the sun, be sure to use sun screen, sunglasses, a hat, and appropriate clothing. Do not get dehydrated -- Drink at least one large glass of water per hour! Be careful not to get fungicide in your mouth or eyes.

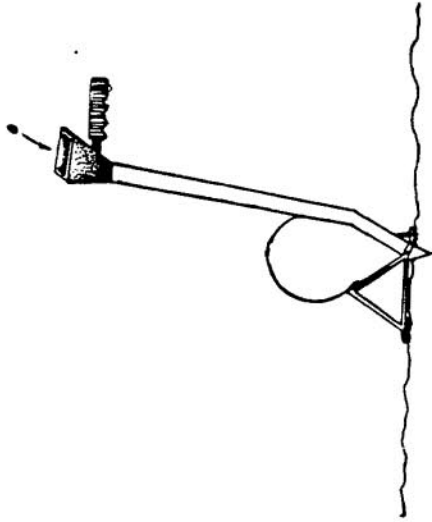
FIGURE 14. THE SHUFFLE



PUT PLANTER POINT INTO SOIL  
 BAR NEAR POINT SETS DEPTH TO ABOUT ONE INCH  
 DROP SEED INTO PLANTER



NOW ROCK FORWARD  
 TRAP DOOR OPENS AND SEED IS PLANTED  
 TIP OF PLANTER MAKES IMPRESSION EIGHT INCHES AHEAD  
 USE FOOT TO TAMP DOWN SOIL  
 MOVE PLANTER EIGHT INCHES AHEAD USING SOIL MARK AS GUIDE



### **C. Pot Planting and Transplanting**

Some materials are very precious or sensitive to cool weather (all shrunken, brittle or sugary types) and will give better germination in small peat pots in the greenhouse. Organize pots in a plastic tray and mark every pot with a 2 or 4 inch stake. Germination will take 5-7 days, but the plants should be grown up to the 3-5 leaf stage before transplanting; by this time there is a good root system.

To transplant dig or hoe a hole or trench about 4 inches deep in moist soil. Break up pot or remove soil ball completely and set plant in place. Water thoroughly. Water lightly for next 2-3 days. It's a good idea to make a list of transplants so you can find them quickly in the field for watering.

The best time of day to transplant is late afternoon so the plants have a chance to recover overnight. The best days to transplant are right before a watering day: then you need to hand water the transplants at the time of transplanting, the next day they are watered automatically, and they should be checked on the third day.

### **D. Germination Records**

Seeds in the field will germinate -10-14 days after they received their first watering. Initially each plant looks like a rolled up dollar bill stuck in the ground. A few days later the first two leaves unfurl. This is the best stage to take germination records and record seedling phenotypes. At the dollar bill stage corn is often ripped off by birds; squirrels like to take the seed before it emerges.

If % germination is low or predators get the seed, you can partially replenish the families by planting in pots and transplanting. If replants are started on day 15, the pots will be 7-10 days behind the field grown material; so the replants are likely to be quite late, really a second planting. This can be a disaster for important testers. Take this into account when planning the number of testers.

Also, families at the edge of the field are more likely to be hit by predators or to be under-watered. To minimize this edge effect, consider planting "border rows" of sweet corn or extra testers.

### **E. Printing Field Cards**

After taking germination records add this data to the cardbox file. It is usually sufficient to note the date, the number of plants germinated/number planted: 6/16 5G/10. This is a good time to correct typos, rectify errors, and fill in any blanks you found on the original printout of the file. When your records are as complete as possible, it's time to print out the field cards. Although called blue cards (Figure 15), print on your favorite color of card stock. Check format for printing; carefully align cards on tractor feed with appropriate tension and make sure large holes in card are on the left. Then print.

If you want double side printing you can individually print the cards or do individual odd families, flip the cards and then do even numbers.

About 100 cards fit comfortably on a set of rings convenient for a field apron. Add a discard card(s) to front and back of set as covers. Between experiments place one or more cards as spacers and for making pollination check lists (Figure 16). Put tape tabs on these cards for easy access. Everyone has their own style for keeping track of pollinations, but some kind of summary/check list is very important.

## F. Additional Data Collection

Now is a good time to make photographs of seedlings or to start special records (leaf number, height, color, etc.) on specific plants. If brief, all this will fit on your bluecards. If lengthy plant diagrams are required or design a specific data collection sheet to fit your needs (Figure 16). Best to use a mechanical or soft pencil for recording data.

Photographs provide invaluable records. To minimize the busy background of the field use a large blue poster board or a beige trash bag as a background for B+W or color photos in the field. Shoot bags and stakes make good labels for photos. Some people find it important to have a "color card" of green, purple, pink, etc. colors in corner of each photo to gauge how well plant photo matches reality. Carefully store photos and negatives with dates. Note that photographs exist on the appropriate family cards.

For extensive notes consider dictating into a portable tape recorder. Each mini-reel on our hand-held model holds about 20 min dictation and require about 1 hour to transcribe.

## G. Isolation Plots.

For bulking up a stock and for some large experiments it is convenient to let the wind do the pollinating: no shoot bags and no cutting back. For bulking up, essentially no work is required. For experimental crosses, the female plants in such a plot must be detasseled to ensure an outcross. Plant 3-4 female rows (detasseled) then one male row. Males will be self-pollinations.

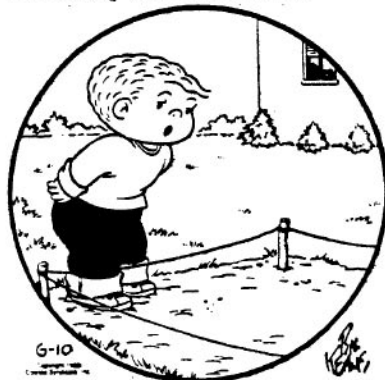
Such plots have to physically separate from all other corn (300 yards or more) or temporally segregated (one week gap at least). We have facilities on Searsville Road and Pasteur Dr. for 1/4 acre isolation plots at each site. Our major problem is predation: in 1983-1984 we lost -50% of material even with permanent netting around the plots.

If outcross isolation plots are used they must be detasseled and checked every day until every female parent has been detasseled. One escape can ruin dozens of ears.

## H. Summary

Planting is a fun stage, because all the planning finally results in ACTION! It's great when the seedlings emerge and you have real plants to look at. Like most aspects of corn genetics, a good planting season requires organization, see Figure 17 Checklist.

**The Family Circus** □ Bill Keane



"I think those seeds I planted were blanks."

Figure 16. Pollination Checklist

FAMILY #  
↓  
INDIVIDUAL PLANT #s

CROSSES TO DO <

| F1     | 1 | 2 | 3   | 4    | 5 | 6 | 7 | 8   | 9 | 10  |
|--------|---|---|-----|------|---|---|---|-----|---|-----|
| ⊗      |   |   | 7/2 | 7/31 |   |   |   | 8/1 |   | 8/1 |
| to r-g |   |   | 8/2 | 7/31 |   |   |   | 8/1 |   | 8/1 |

FILL IN DATES AS CROSSES ARE COMPLETED

| F2                | 1    | 2    | 3    | 4    | 5    |
|-------------------|------|------|------|------|------|
| ⊗                 | 7/22 | 7/28 | 7/29 | 7/30 | 7/30 |
| by 8 <sup>p</sup> | 7/22 | 7/28 | 7/30 | 7/30 | 7/30 |

Figure 17. Checklist for Planting Season

| Checklist   | Target             | Completed |
|---|--------------------|-----------|
| Pre-treat Field for Weeds                                       | April 15           |           |
| Stakes Made Out and Placed in Field                             | May 10             |           |
| Treat Seed with Fungicide and Plant                             | May 10, 17, 24     |           |
| Plant Pots of Weak Materials                                    | May 15             |           |
| Take Germination Records<br>(10 days after each planting)       | May 20, 27, June 3 |           |
| Print Field Cards   | June 10            |           |
| Proofread Field Cards Carefully &<br>Correct Errors on Computer | July 1             |           |

#### 4. FIELD MAINTENANCE

This section is concerned with keeping the field in good shape during the growing season and during the off seasons. A good reference is Modern Corn Production which contains pictures of plants infected by different pests and under nutrient limitation. There's even a photo of what lightning damage looks like! The manager of the Plant Growth Facility takes care of most of these items, however, if you want to set up your own field, you will need to know these things.

The time required for field maintenance can be very high if the field is in poor shape. Assuming 100 families occupy 3 ranges here is a rough breakdown of time:

| <b><i>Task</i></b>  | <b><i>Time</i></b> | <b><i>1986 Cost<br/>at Stanford</i></b> |
|---|--------------------|---|
| October Disking of Field (Webb Ranch)   | 1.0 hr             | \$10                                    |
| Application of 50 lbs Elemental Sulfur (Webb)                                       | 0.0 hr             | \$10                                    |
| Seeding In a winter Legume Crop By Hand/Machine                                     | 2.0/0 hr           | \$10                                    |
| Spring Disking, Fertilizing, Plowing (Webb Ranch)<br>and Turning Under Weeds Nearby | 2.0 hr             | \$175                                   |
| Early watering to Encourage Weed Seed Germination                                   | 0.5 hr             | -                                       |
| First Round-up Spray  | 1.0 hr             | \$10                                    |
| First Hoeing at Planting Time   | 1.0 hr             | -                                       |
| Second and Third Hoeing (Undergrads)  | 6.0 hr             | \$50                                    |
| Webb Ranch Crew Hoeing -- July 4  | 4.0 hr             | \$25                                    |
| Side Dressing of Nitrogen Fertilizer  | 1.0 hr             | \$10                                    |
| Spot Spraying For Ants and Weeds  | 2.0 hr             | \$10                                    |
| Picking Rocks out   | 1.0 hr             | -                                       |
| Turning Water On/Off 40 Times   | 40.0               | -                                       |
| Hand Watering Transplants   | 3.0 hr             | -                                       |
| Spot Watering   | 5.0 hr             | -                                       |
| Miscellaneous   | 5.0 hr             | -                                       |
| <b>TOTAL ESTIMATED TIME</b>   | <b>78.5 hrs</b>    | <b>\$315</b>                            |

## A. Plowing

At Stanford this is handled by the Webb Ranch. (Call Tom Hubbard at (415)854-4951 in March to arrange for them to come to plow under the winter legumes and disk the field.) Soil depth should be evened out and manure and other materials incorporated as early as possible. If rain delays the initial field preparation, Webb's people will prepare seed bed and set-up rows on the same trip in late March or April. Have rows set up 18 inches apart. Make sure soil is well broken up by the Webb crew.

## B. Watering

The upper field has underground copper pipe with 6' risers and rainbird sprinklers. For the lower field plastic pipe has to be laid out, each year. The water supply is from Searsville Lake and is non-potable. It can smell strongly of sulfur.

Prior to planting any corn seed, the field should be watered weekly to encourage weeds to germinate. Most weeds in our climate set seed with variable dormancy: one-third of the seed may germinate after the first watering and a declining percentage with each subsequent watering. If you can water 4 times, for example, 50% of the weed seed carry-over or introduced in manure may germinate. These weeds can be eliminated with herbicide.

Once corn is planting, the field should be watered to 3-4" penetration twice per week to promote seed germination. Because not all seeds are planted at the same time this twice weekly schedule usually lasts until late June, or about 3 weeks after the last planting. Then the field should be watered thoroughly once per week -- delivering 1-2" of water with soil wet to 10-12". spot water or soak problem areas and remember to hand water transplants.

with the exception of *Adhl* nulls it is difficult to over-water. It is easy to underwater certain spots particularly as plants get tall and block the rainbirds. The initial symptoms of drought are dull, rolled upper leaves. Some materials like our c2 tester are particularly sensitive. Double planted (4" apart) materials are very sensitive to water stress, and a soaking hose may be required to water them more often or more deeply than the bulk of the field. Last watering is 3 weeks after the last pollination.

## C. Weeds

Our major weeds are radish, amaranth, hedge bindweed, mustard, grass, crabgrass and common purslane., Crabgrass is the hardest to get rid of, but early in the season the amaranth causes the most problems because it grows taller than the corn. Weeds early in the season can shade and choke out the corn. Always hoe just before you plant to break up surface soil for better planting and to eliminate young weed seedlings. Weed at least every 2 weeks or as often as necessary to achieve excellent control. Even late in the season, hedge bindweed and other tenacious weeds can lower yield substantially as well as make it difficult to do your work. Do not rely solely on chemical weed treatment: hand weeding is the most effective because it works IMMEDIATELY!

Rogue corn from last year's seeds can also be a problem. Some is easy to spot, because seedlings are not in a row. Some will germinate in this year's rows. Remove any plants that are *very early, an unusual color, or different height* compared to the rest of the family.

Everyone who plants seed should also plan on weeding before planting and at two week intervals. Undergrad helpers start about June 15th and they spend about 2 hrs per range on weeding each week. We usually call in Webb's crew to do a thorough weeding about 4th of July.

Their weeding is very complete -- in fact, it is important that the corn is of reasonable (12" size) before they arrive and that the crew be instructed to leave the tillers on the plants.

Spot weeding and/or Round-up spraying is likely to be required about August 1st to get late germinating weed seeds. Continue these treatments as necessary until September.

## **D. Pests**

### *1. At Planting Time*

Birds take seedlings and squirrels steal seeds. Bird netting slows them down. Also cracked corn treated with bitters, quinine or hot sauce may deter them. The predators usually start at the edges of the field so that material is the most vulnerable. Aggressive cats are by far the best rodent and bird control program.

### *2. Early Plant Growth stage*

Watch out for pale green bugs with black spots, about the size of a ladybug. These are adults of the corn ear worm. Best control is squashing on sight. Once you get over the yuck response you'll find it easy to kill these slow-moving bugs. Our main worry is that a large population will colonize the ears so, in addition to squashing the bugs, shoot bags should be treated with dipel (Bacillus thuringensis spores). These bacteria produces delta crystal toxin protein, a compound lethal to the green bug. Because the females lay only a few eggs on each ear, dipel-treated bags put some of the ears help with population control, but using all dipeltreated~bags is the only method to protect every ear.

Any plants showing signs of systemic corn smut (wrinkled leaves and pustules on prop roots and leaf margins) should be cut out and discarded in the dumpster before spores are shed. Wash your hands thoroughly after touching a smutty plant.

Ants will bring aphids to sweet corn and to weakened plants to colonize the tassel. They don't really do much damage but are a bit of a mess during pollen collection. A spray of Isotox or diazinon can be applied to aphids; spraying should be done only by trained personnel. In California, certification by the state is required for a person to use many agricultural chemicals.

### *3. Pollinating Season*

Keep a sharp eye out for smut on tassels: pick it and seal in bags for disposal. Carefully wash your knife if it touches smut or the soil. soil-borne spores transferred to the ear are a major problem in our field. One smut gall can cover one-third of an ear!

### *4. Late Season*

Fusarium ear rot is the worst problem late in the season. It attacks sweet ears (shrunken, sugary, brittle). This fungus grows very rapidly on the ear about 3 weeks after germination. There is no known control except pulling husks back so that sunlight suppresses the fungus.

## **E. Wind**

When the field has been extremely well watered and the wind picks up in the late afternoon its not uncommon for plants to blow over. This is particularly true after they've begun to elongate rapidly; in other words when they're about four to five feet high and the stem is only two to three feet tall. The plants can be prone and actually touching the ground.

DON'T PANIC. within a week most healthy young plants right themselves by producing prop roots (also called adventitious roots). These "roots" are actually stem tissue that differentiates into roots from the basal nodes. Some varieties of corn produce prop roots automatically even without stress, and these plants are almost impossible to knock down in the wind. Many of the varieties that we use (see Chapter 12) have few prop roots or they'll just have prop root primordia that you can see as little knobs around the base of the plant but the roots will not have grown out and touch soil.

Sometimes it's not convenient to wait for the plants to right themselves or you're worried that they'll still be leaning into the aisles or the plants are already shedding pollen. Prone plants can be staked up but this should be done extremely carefully so as not to break anymore vascular tissue than you have to. When the plants blow over a fair amount of vascular tissue is broken on the side away from the ground and when you push the plant back up there is a tendency to over push and then break vascular tissue on- the side that was facing the ground. This is worse for the plant then not moving it at all so the plant should really be staked up in stages, first pulled up off the ground just maybe six or eight inches the first day and then gradually pulled back up in an upright position. This procedure would compliment what the plant is doing itself by making prop roots.

## **F. Fertilizer**

Consult a plant physiology book or *Modern Corn Production* for pictures of the various nutrient deficiency syndromes. The Webb crew incorporates manure into the field each fall and a mixed fertilizer into -the soil when they set up the rows in the spring. If your plants are yellow by the 4 - 5 leaf stage, it is most likely that no fertilizer was incorporated in that area of the field. Ask the facility manager for help in adding fertilizer. We usually side dress with nitrogen at least once, about six weeks after planting. spot applications in problem areas can occur at any time.

If nitrogen fertilizer falls on a leaf it will cause a burn spot or severe yellow streaking if the fertilizer is wetted by fog or dew and runs down the leaf into the whorl.

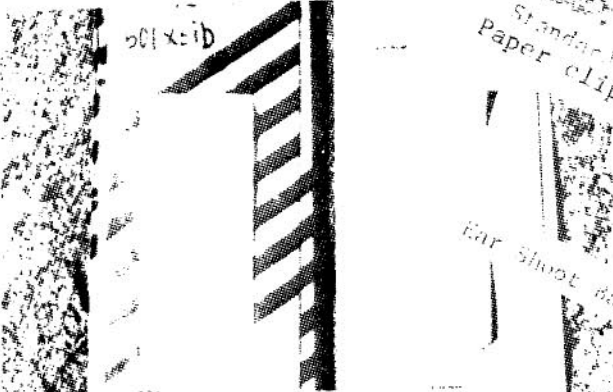
Elemental sulfur is applied to the Stanford field each fall. This treatment helps sterilize the soil, killing fungi, nematodes, etc. to a depth of about 3 inches, where most pest live. Killing is by soil acidification, a soil amendment in Northern California where alkaline clay is the major soil type. Sulfate is also a limiting nutrient, and the oxidized sulfur adds this nutrient to the soil.

If you set up your own field, it is a good idea to talk to local farmers and the agricultural extension service about soil improvement appropriate for your area.

## **G. Summary**

Field management can be a tricky proposition. We have few problems compared to many places. The routine maintenance outlined above keeps things from getting out of hand, but local expertise is very important.

All Bags Have a TONGUE SIDE  
that is Placed Next to Stem



Glassine Bag for Collecting  
Pollen Cleanly.



Corn Geneticist Ready to Go.

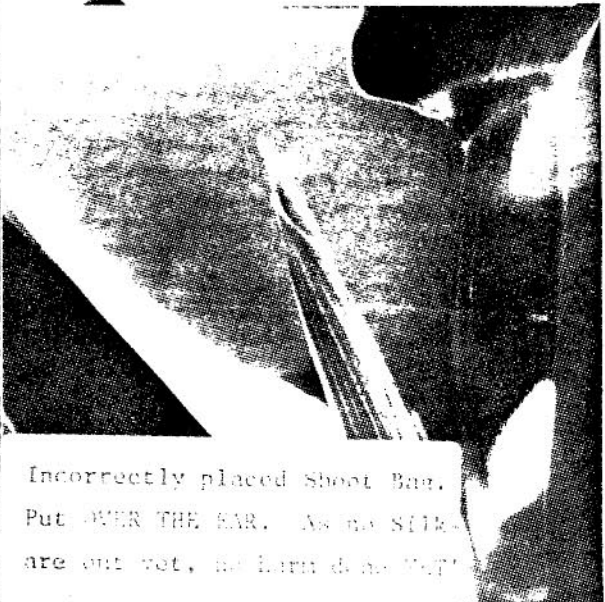
Use Sunscreen.



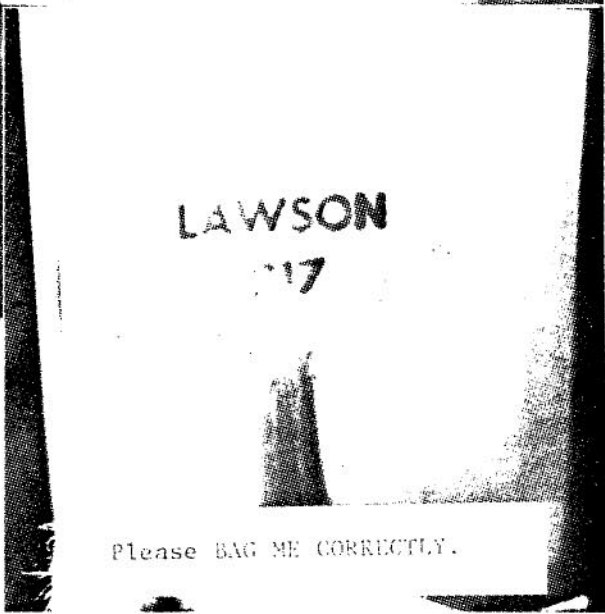
Striped Bag for Special Uses.  
Note Date and Cross Info



Use Striped Bag When You Use  
an Ear in Someone Else's Plot  
and for Late Pollinations.



Incorrectly placed Shoot Bag.  
Put OVER THE EAR. As the SILK  
are out rot, no harm done. BUT



Please BAG ME CORRECTLY.



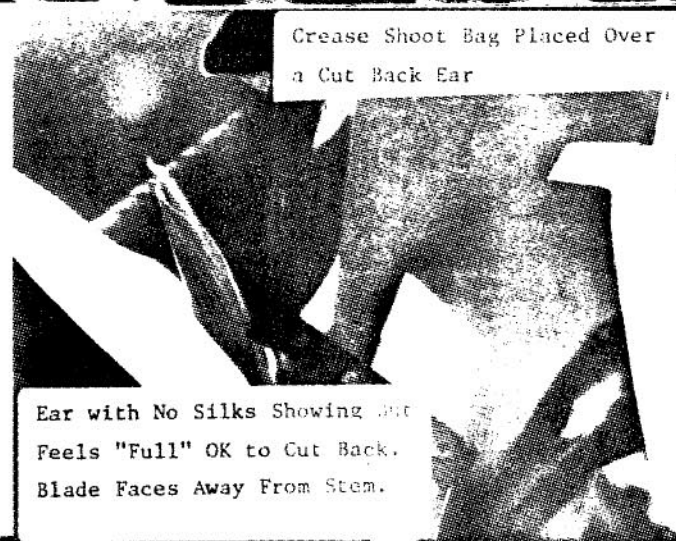
CORRECT SHOOT BAGGING  
Bag Top Two Ears Only  
Write PC on Shoot Bag if Any  
Silks Are Showing!



Entire Population of Silks  
Grown Out. A Little Old.

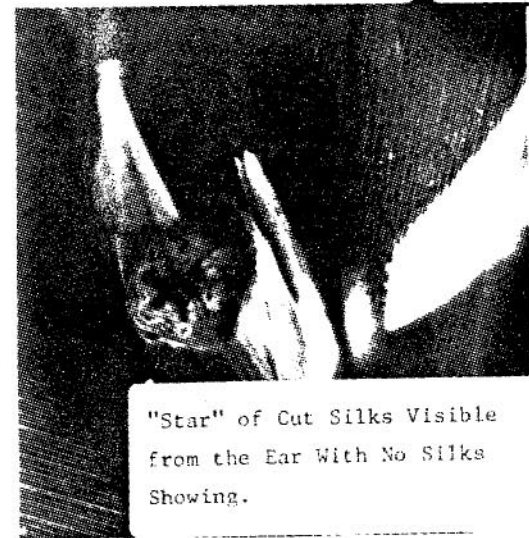


Part of Silk Population Has  
Grown Out. Excellent for  
Cutting Back.

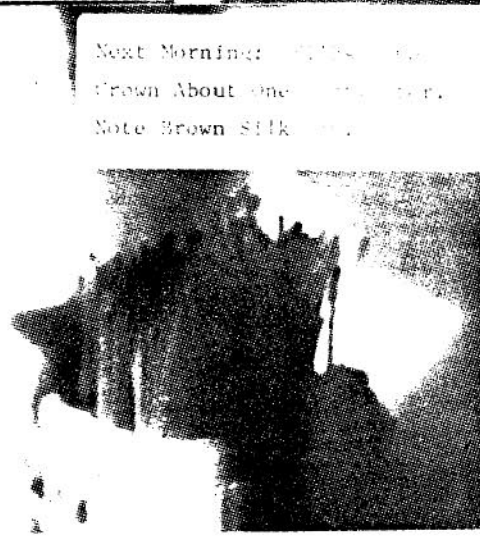


Crease Shoot Bag Placed Over  
a Cut Back Ear

Ear with No Silks Showing Out  
Feels "Full" OK to Cut Back.  
Blade Faces Away From Stem.



"Star" of Cut Silks Visible  
from the Ear With No Silks  
Showing.

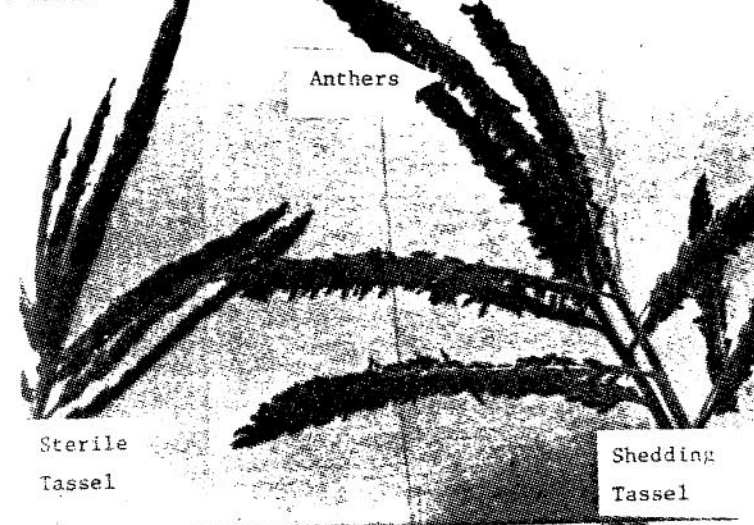


Next Morning: Silks Have  
Grown About One Inch Long.  
Note Brown Silk

Check BLUECARDS and FIELD  
CHECKLISTS as You Cut Back  
and Write Bags

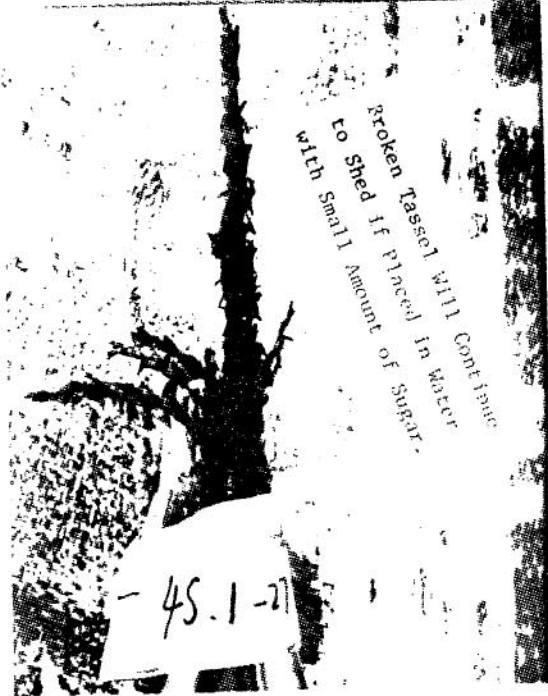


To Bag Tassel, Crease Brown  
Bag, Then Slip Over Tassel  
Held in Place.



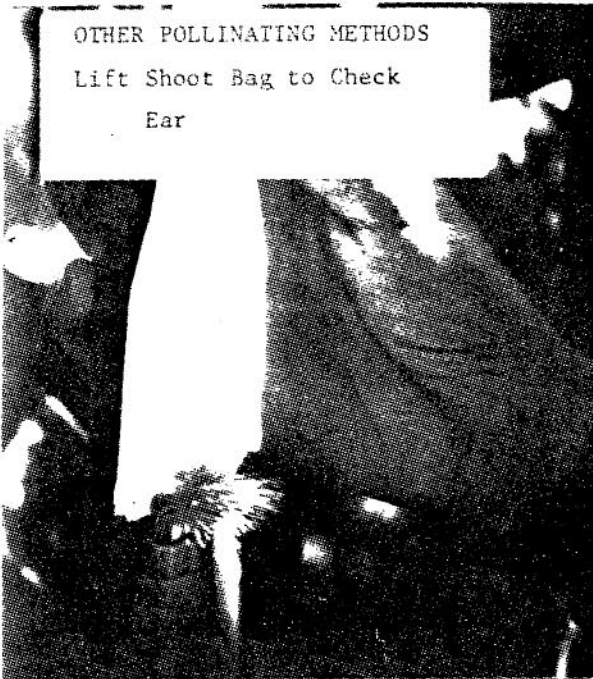
Sterile  
Tassel

Shedding  
Tassel

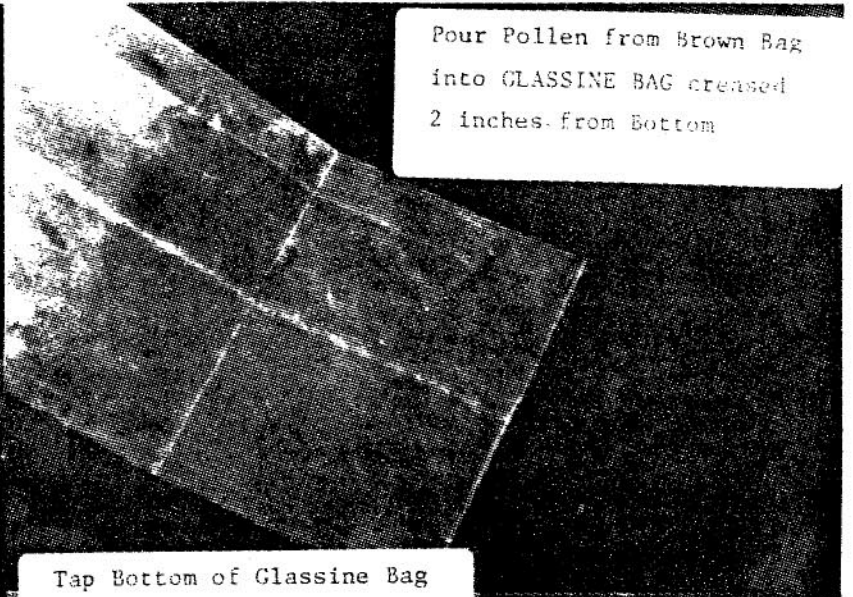


OTHER POLLINATING METHODS

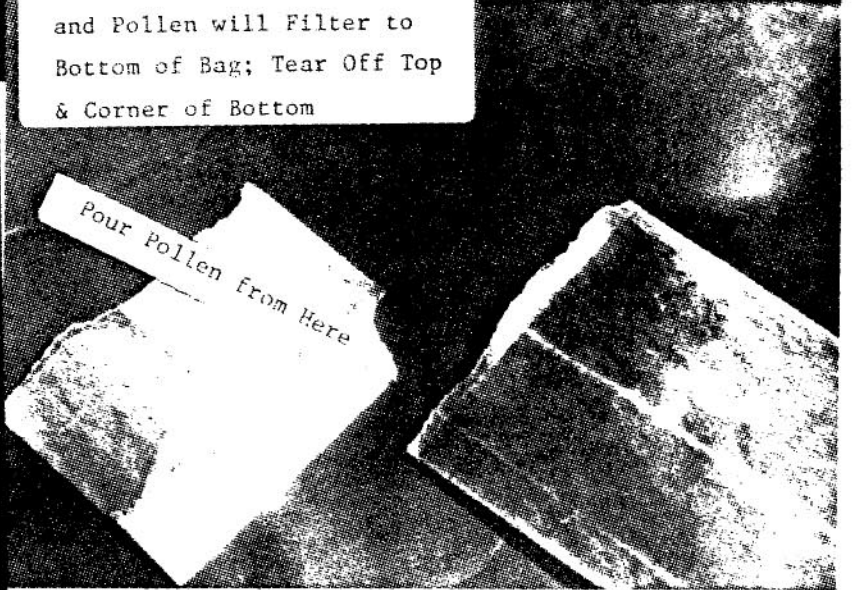
Lift Shoot Bag to Check  
Ear



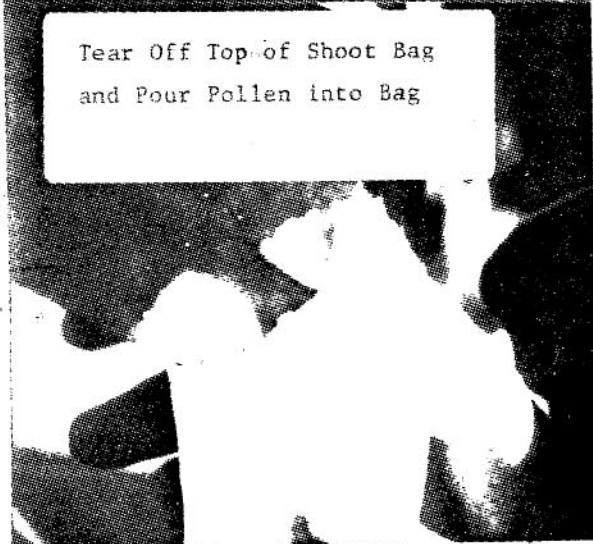
Pour Pollen from Brown Bag  
into GLASSINE BAG creased  
2 inches from Bottom



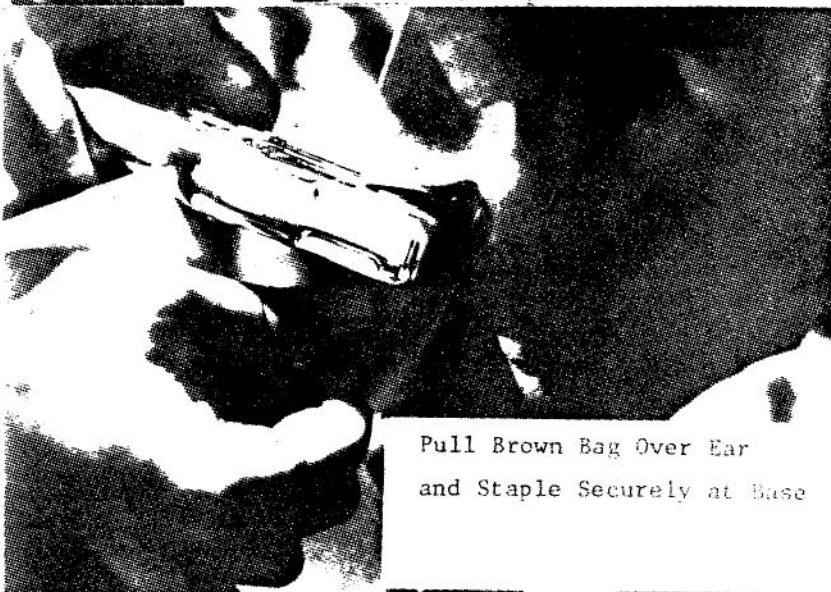
Tap Bottom of Glassine Bag  
and Pollen will Filter to  
Bottom of Bag; Tear Off Top  
& Corner of Bottom



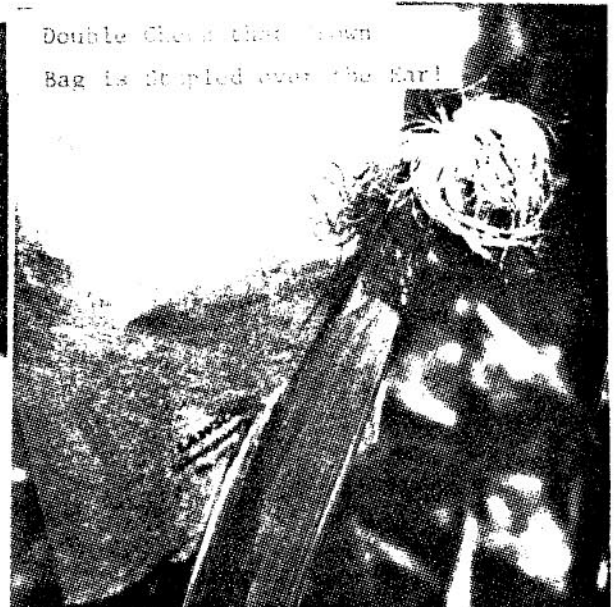
Tear Off Top of Shoot Bag  
and Pour Pollen into Bag



Double Check that Brown  
Bag is Stapled over the Ear!



Pull Brown Bag Over Ear  
and Staple Securely at Base



## 5. POLLINATING SEASON

Well, this is it: your plants are ready for their crosses. In this section we'll cover the steps in executing your experiments successfully. This section is devoted to the "hands on" of performing the crosses. It is a hectic but rewarding part of corn genetics work. Your organization efforts earlier in the season will be put to the test now: will plants be ready at the appropriate time? Do you have enough tester? It is realistic to expect that 90% of your crosses will work but hope for 100%!

One person can easily handle 100 self pollinations per day and could manage 250 while a team of two could readily manage 250 and might be able to handle 1000. If multiple (10-20) pollinations per pollen collection are performed then one person could do 250-500 and a team could handle 500-1,500. Because plants don't know about weekends, keep in mind that you may have to work every day for weeks at a hectic pace.

Time required for 1000 self pollinations in two plantings of 500 plants each. The work will occur over a 15 day period.

|  |               |
|--|---------------|
| Rechecking and Labeling Family stakes*           | 2             |
| Shoot Bagging Over 15 days, Starting 10 days     | 10            |
| Before Pollination Begins* Numbering Each Plant* | 6             |
| Prepare Final Pollination Checklist              | 3             |
| Cutting Back and Writing Bags                    | 15            |
| Setting Up Bags*                                 | 10            |
| Pollinating                                      | 15            |
| Miscellaneous                                    | 5             |
| <b>TOTAL ESTIMATED TIME</b>                      | <b>71 hrs</b> |

In our field the \* items are usually completed by undergraduate helpers = 28 hr.

### A. Shoot Bagging

Shoot bags are small white bags (Figure 18) placed over immature ears before silks emerge. Proper shoot bagging is absolutely critical to perform controlled crosses and hence is essential for successful genetic research. This task is often assigned to undergraduates, and they must be carefully trained and supervised. Shoot bagging should be checked daily.

Shoot bags can be put on as soon as the uppermost ear shoot is a few inches long - even while it is hidden within the leaf sheath although it is more conveniently done when the immature ear shoot is 2-4 inches above the ligule of the leaf (Figure 19). *Because of apical dominance, the uppermost ear will give the best seed set; the second ear may or may not set seed. Lower ears are very unlikely to set seed at all.* Thus, it is somewhat ironic that the lowest ear shoot emerges first, followed a day or two later by the next most apical, etc. Consequently,

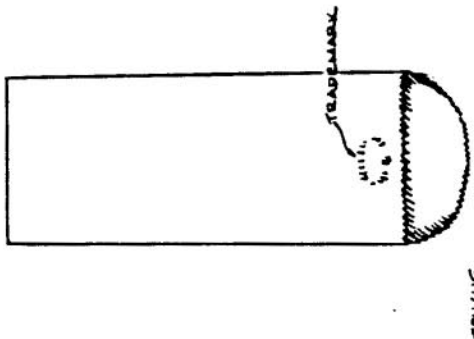
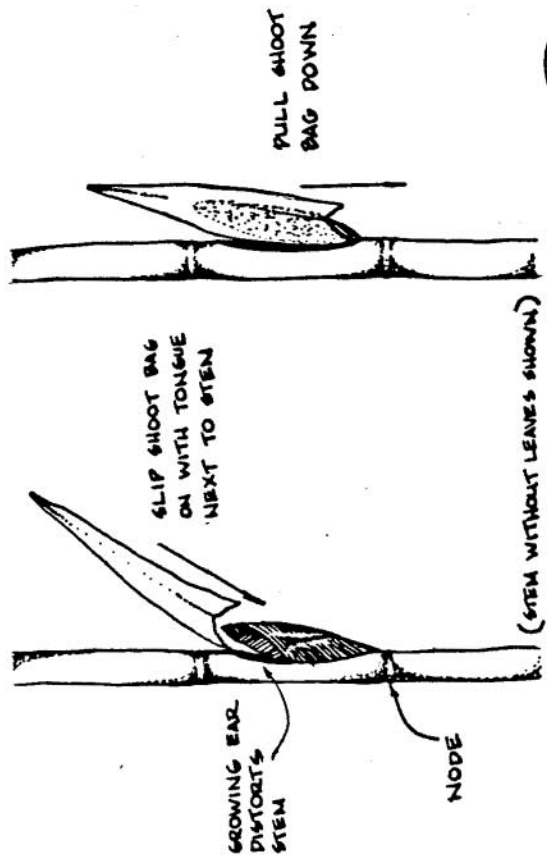


FIGURE 10. SHOOT BAG



See Day

FIGURE 19. BAGGING EAR

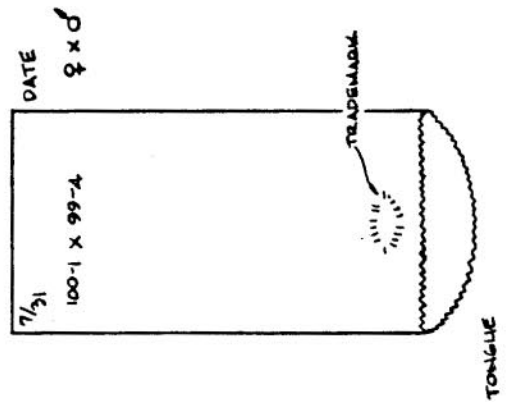
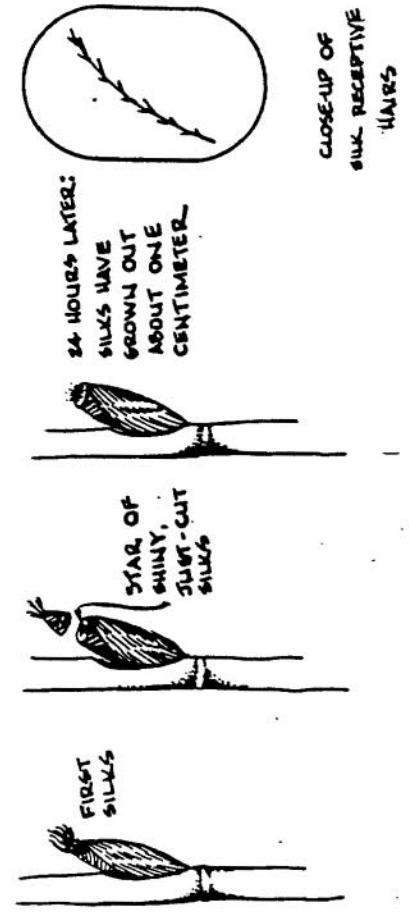


FIGURE 21. BROWN BAG



See Day

FIGURE 22. CUTTING EAR

## Figure 10. Advanced Shoot Bagging

1. Rip leaf off at the ligule when ear just barely visible. Make sure you rip off leaf subtending the top ear.
2. Slice sheath around ear with side of shoot bag
3. Slip shoot bag — tongue toward stem — into groove cut into the sheath.

it is important to recognize the upper ears and shoot bag them. Once shoot bags are placed on a plant it is easy to skip over that plant, missing the later emerging and most important ear shoots.

Proper recognition of the topmost shoot is very important. Clues include:

1. Topmost shoots usually emerge -5-7 nodes below the tassel.
2. Shoots often cause a bulge in the stem.
- 3 . You can gently pull back the sheath and peek in to see if there is a shoot.

Shoot bags have a "tongue" side that should be placed on the stem side of the emerging shoot. The bag should be pulled securely down over the shoot and wedged between the sheath and stem. The shoot bag edge can be used as a knife to cut into the sheath tissue and then the bag can be slipped further (and more securely) over the shoot. Confident and experienced shoot baggers often strip off the blade of the leaf subtending the shoot (Figure 20), and use the shoot bag knife method to attach the shoot bags.

If corn ear worm is a problem, dipel-treated shoot bags offer a low cost, effective worm control. Dipel is conveniently put into the bags with a long, cotton-tipped applicator. These bags can be placed in a block over critical materials or used throughout the field. A typical undergraduate can dipel-treat about 250 bags per hour. We often have students do this spring quarter, accumulating 10,000 bags before summer starts.

As mentioned previously too many shoot bags can be a problem; this "Christmas tree" syndrome in which each shoot is decorated, often results in the mistake of missing the topmost, and best, ear. A second mistake is placing the bag in front of or behind the ear shoot rather than over it. Shoot bags can also be dislodged by plant growth or wind.

If a few silks emerge on an unbagged shoot, cut the ear back several centimeters and mark the shoot bag PC for possible contamination. If many (>20) silks are visible or if the silks are longer than a few centimeters too much contamination may have occurred to warrant saving the ear. If a second ear is visible, remove the upper ear and the second ear will mature in 1-7 days. Keep in mind that silks grow 1-3 cm per day while pollen grows 1 cm per hour, effecting fertilization about 24 hr after landing on the silk. Thus, a 3 em silk may have been pollinated the day before and fertilization may have already occurred.

Each shoot bagger should take responsibility for a group of plants, although people may enjoy working in teams. The responsible person should recheck shoot bagging daily or every other day. A good way to do this is to walk through the field "backwards" from family #100 to #1, and check that every plant has a shoot bag on the uppermost ear or does not need one. Special care is required for dwarf plants and certain genotypes in which the silks emerge while the ear is very small and still hidden in a sheath. A good rule is that a plant with a visible tassel is ready to shoot bag; a plant shedding pollen must be "dissected" to find the shoot.

## **B. Numbering Plants and Tagging**

When pollinating it is very convenient to know precisely what plant you are working with. An easy way to accomplish this is to use a MARKSALOT pen to write the plant number on the shoot bag or stem about the position of the ear. We label the first plant in the family with the family number as well. Record the number of adult plants on your field cards as an aid to planning crosses.

To draw attention to particular plants, shoot bags or 3 x 5 cards can be folded around the stem below the tassel and stapled in place. These tags are a convenient place to write the genotype or to single out particular plants as male parents.

## C. Pollination Checklists

By now you should have an accurate count of the number of plants in each family and can refine your original pollinating plans. We make up a "check off" list for every family (Figure 16), as part of the final planning. As pollinations are set up, the box corresponding to each cross is checked or better yet the date is logged in. From this information you can easily record the average days to pollination - information useful for next year's planning. Dates also help you find material for timely harvesting. This is also a good opportunity to think of additional stock constructions and crosses to complete based on what genotypes will be ready at the same time.

## D. Cutting Back and Writing Bags

Pollen for crosses is collected in brown bags (Figure 22) similar to lunch bags. Each bag is labeled with the date across the top and then the cross: female parent x male, *i.e.* 101.1-3 x 99-4. [The year is implied but will be recorded on the harvesting tag.]

Preferred method Write bags while cutting back. The day before pollination, you walk through the field checking which plants are ready to go. Most people write a list of all of the female plants that are ready on an old brown bag. Then match ready ears with appropriate pollen parent ready at the same time. Then write the complete cross and next day's date on the brown bag.

Other methods write only the male parent on the bag while you set up, then write the female parent [1] as you pollinate or [2] at some later date. You can be sure that female plants don't move! The disadvantage to this method is that you can use the wrong female parent quite easily, *i.e.* by glancing at a pollinating plan in the wrong column.

Silk emergence An ear is ready when a few silks have already emerged from the husks, but ears a day or so younger as well as about 5 days older are OK. The top of the husks are cut off with a knife; this should expose a cross-section of about 250 silks; the silks will grow out 1-3 cm overnight and be ready to pollinate the next day (Figure 22). Silks emerge first from the kernels on the middle of the ear, then the next day from the base of the ear, and on the third day the silks grow from the kernels on the tip of the ear. The silks grow through the whorl of husks and take 2-4 days to emerge. consequently, when you cut off the top of the husks there should be silks from all kernels. This cutting back allows pollination of all silks. If cut too early, only silks from the middle of the ear are present. When an ear is old many silks will already have emerged from the husks, then after cutting back most will grow only a little but the youngest silks from the tip of the ear will probably grow 2-3 cm.

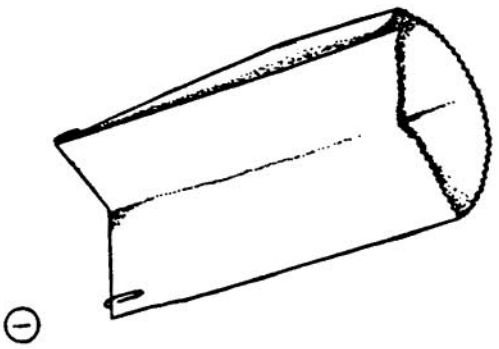
Choosing where to cut husks to expose the most silks without nicking the top of the ear takes some practice and experience with individual families. In general, ears are below the prophylls (Figure 22) so it is usually safe to cut back at that point; you can always cut again. And don't worry if you clip the tip of the ear, because only 10-20 kernels are affected, if that many.

Within 10 minutes of cutting back the tips of the cut silks will turn brown. These silks will be non-receptive to pollen for about an hour, possibly from shock. As the silks begin to grow new receptive areas are exposed; small hairs spaced about 0.2 mm apart along the silk are the receptive surfaces, and there are thousands of them on each silk.

Two things can go wrong when cutting back:

1) An ear is cut back and no silks are found -- this occurs if the ear is too young or female sterile. Not much can be done except to wait.

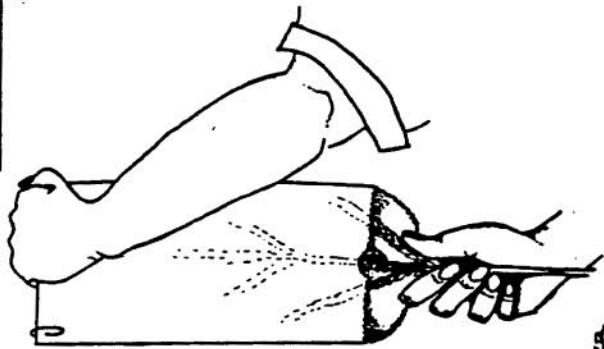
FIGURE 23. BAGGING TASSEL



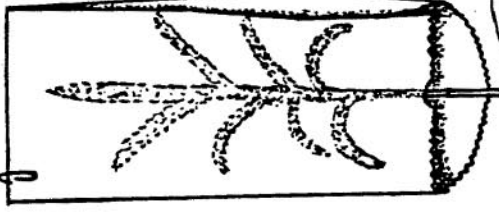
①

TO BAG TASSEL;

CREASE BROWN BAG, THEN SLIP OVER TASSEL HELD IN PLACE

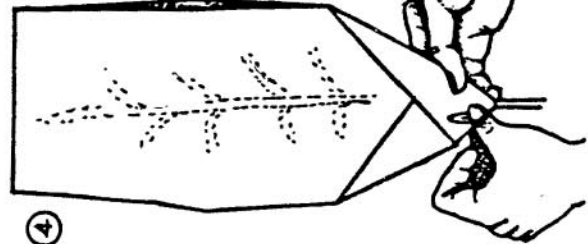


②



③

PULL BAG FAR DOWN (OKAY TO RIP OFF TOP LEAF)



④

FOLD OVER BASE OF BAG, AND PAPERCLIP FOLDS TOGETHER

Sandy



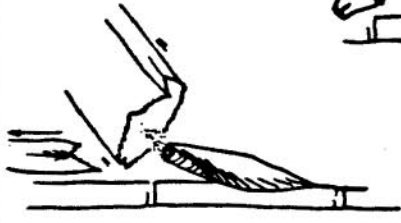
USE EYES AND EARS TO DETERMINE IF POLLEN IS SHED

GOOD POLLEN : PALE YELLOW

: SOUNDS LIKE SALT IN BAG



TAP TASSEL BAG BRISKLY AGAINST YOUR PALM. LISTEN...



LIFT SHOOT BAG OFF, POUR POLLEN ON

REMOVE BAG AND LOOK



POLLEN SHOULD BE PALE YELLOW AND DRY

TEAR OFF TOP OF SHOOT BAG, AND POUR POLLEN ON

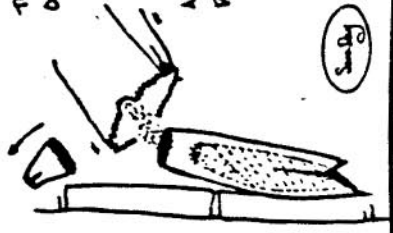


FIGURE 24. POLLINATION

Sandy

2) The next morning no silks have grown out -- this occurs about 1% of the time. The situation can be rescued by slicing the husks lengthwise to expose the silks from the side if it is important to pollinate that day. otherwise, wait a day. It is best not to cut an ear back a second time.

A few hints on preparing bags and ears efficiently:

- 1) Have undergrads put a paper clip on each tassel bag in advance.
- 2) Prepare all ears in an experimental plot but just keep track of how many tester ears are required for reciprocal crosses, then cut back the required number of tester ears in one pass through the tester group.
- 3) Date ear shoot bags in large blocks of testers just cut back so they are easier to find the next day.
- 4) After cutting back, crease top of ear shoot bag into a 900 fold as a sign that cutting back has been done.
- 5) A team with one person cutting back and writing on shoot bags, and the second person writing tassel bags will be much more efficient than two people working alone. Person with knife must be very careful. *Some people put a Band-aid on knife-hand thumb in advance, because the most common wound is slicing through ear shoot into your own thumb as you hold the ear.*
- 6) While cutting back check neighboring plants for appropriate shoot bagging.
- 7) Put bags in numerical order by pollen parent to make setting up easier.
- 8) Clip bags together that go to the same male parent to make it easier on whoever is setting things up.
- 9) Make a daily tally of number of pollinations.

Strategy For large plantings, we find it most convenient to alternate pollination and cutting back between two plots; each day we can concentrate getting ready in one plot while pollinating the other.

## **E. Collecting Pollen and Pollination Techniques**

Setting Up: In Palo Alto we usually set up the brown bags the afternoon before. Alternatively, bags can be put up *early in the morning before any pollen is shed*. This job is usually done by undergraduates. Setting up involves:

- 1) Finding the plant -- a cross check on the bag writing.
- 2) Carefully placing the brown bag on the tassel, folding the bottom of the bag around the base of the tassel and then paper clipping the bag in place (Figure 23).
- 3) If there are multiple pollinations from one male,
  - a) Put the bag on the tassel that involves the longest walk for the pollinator.
  - b) Place the additional bags around the stem near the ear and paper clip in place.

FIGURE 25

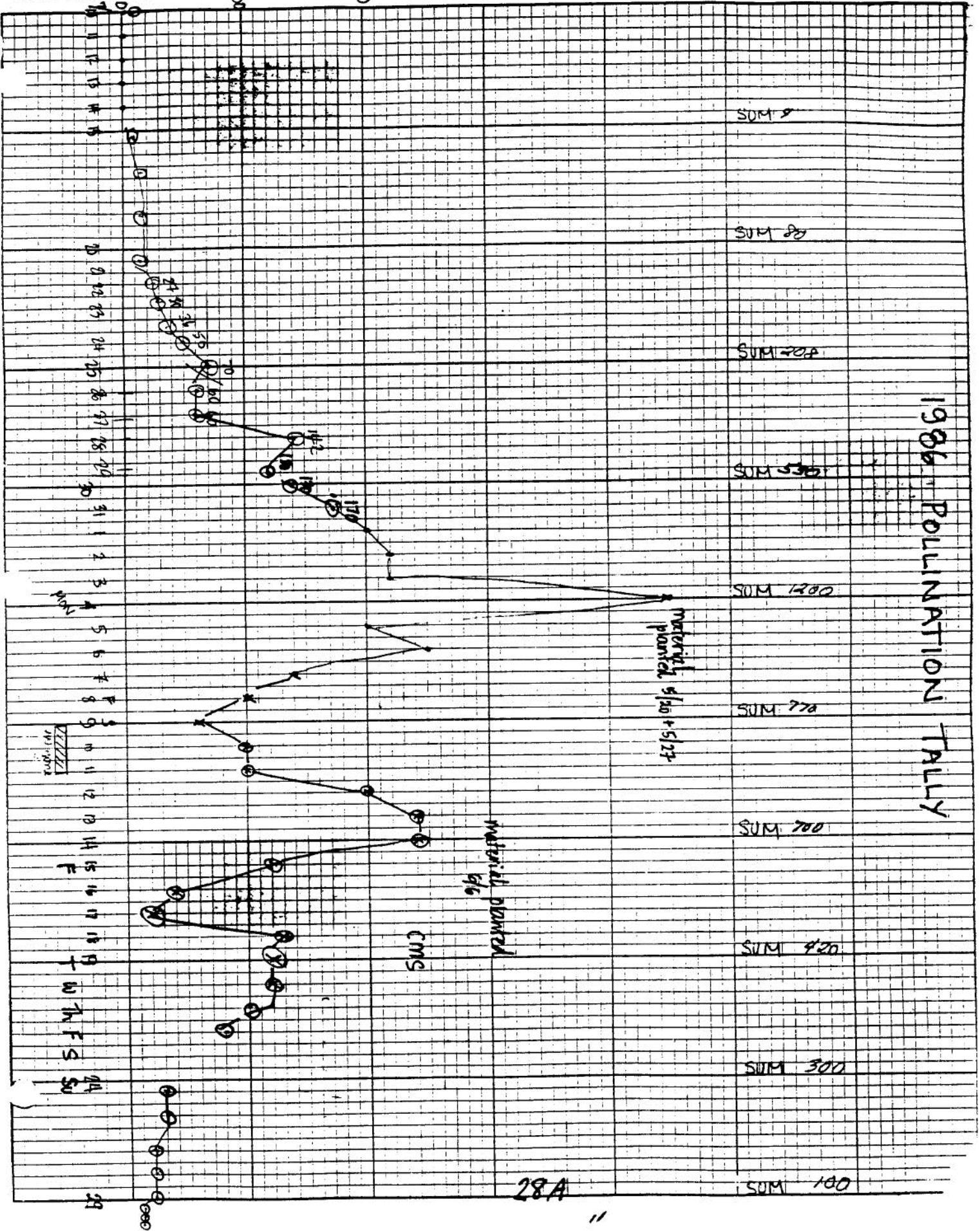
CHAMPION LINE NO. 810-3  
 CROSS SECTION - 10 SQUARES TO INCH

200

300

400

500



1986 POLLINATION TALLY

SUM 28  
 SUM 208  
 SUM 536  
 SUM 1280  
 SUM 770  
 SUM 700  
 SUM 920  
 SUM 300  
 SUM 100

28A

"

# FIGURE 26 CHECKLIST

RANGE 3 Lower Field

Responsible Person Sally

|               | Dates                                     |
|---------------|---|
| Weed          | 6/22 6/29 7/4 (Webb) 7/21 8/3             |
| Nitrogen      | 6/22 7/18 families 5-8                    |
| Round-up      | 8/1                                       |
| Remove Rocks  | 7/1                                       |
| Shoot bag     | 8/1, 8/3, 8/4 8/6 8/7 8/10 8/11 8/14 8/17 |
| Ants?         | spray families 7, 12, 14 8/2              |
| Smut?         | 12:1-4 (8/2) bad 14.5 (8/2) 21-5 (8/2)    |
| Number Plants | 8/1 8/4                                   |

## Late Families

5 shoot bag 8/15  
 8 " " 8/18

Pollen Shed In our climate pollen shed starts in bright sunshine about 30 min after the morning fog lifts, about 9:30 about AM and lasts about 3 hr. Pollen is shed again from about 5-8 PM. During these periods, collect pollen by:

- 1) Remove paper clip from tassel bag and let old pollen fall out.
- 2) Holding tassel base with one hand for reinforcement, tilt bag and tap gently with the other hand to release more pollen.
- 3) Then lift bag off the tassel and crease gently along the tongue side of the bag.
- 4) Pop the bag open and tap to agitate pollen into a line in the crease -- often pollen gets trapped in the folds of the bag so tap hard if pollen is limiting.
- 5) Carry pollen to the appropriate ear.

Good pollen is pale yellow and does not clump together -- it is very dry. Old pollen is dark yellow and somewhat sticky.

Pollinating Methods There are several techniques for pollinating. If you are really rushed and have plenty of pollen you may just lift the shoot bag off and quickly slip the brown bag over the ear, tapping some pollen onto the ear as you go (Figure 24). Pull the bag firmly down over the ear, and pull the wings of the bag around the stem. Then staple the "wings" of the brown bag together encircling the stem of the plant.

With more limiting pollen you may want to rip off the top of the shoot bag to form a funnel into which you pour the pollen. The staple bag in place.

With plenty of time and pollen, pollinate, then replace shoot bag on ear, then bag. Shoot bag contains dipel which will kill corn ear worms. Also shoot bag will restrict growth of "new" unpollinated silks, keeping the~ from emerging from the bottom of the brown bag.

If there are a large number of pollinations to do from a single

male, it is very convenient to transfer the pollen to a glassine bag (Figure 25). Fold the base of the glassine bag up towards the top making a sharp crease; empty the contents of the brown bag covering a tassel into the glassine bag. Now tap the bottom flap on the bag and watch all of the fresh pollen flow into the bottom of the glassine bag leaving anthers and lumpy pollen behind the crease. Now tear across the crease discarding the chaff. Tear off a corner of the bottom part and you have a great container similar to a salt shaker for delivering the pollen to ears.

A common concern is, how much pollen do I need? Basically if you can see it, there's enough. Each anther contains about 2,000 grains so even one shedding anther should have enough pollen for an ear. Too much pollen is more likely to be a problem: some plants produce a teaspoonful and this will cause a traffic jam when all the pollen germinates on the silks. If you have a lot of pollen tap a small amount directly onto the silks, put the shoot bag back on or crimp the top, then put the brown bag on the ear.

You can check to make sure pollen has arrived on the silks; if you have sharp vision you can see the pollen as a kind of yellow dust on the silk hairs. A successful pollination depends a lot on silk quality: prime silks are about 2-3 cm long and pale green and fluffy like a small brush.

## *Emergency Measures for Various Problems*

Not enough pollen. Tap the bag again bag as pollen sometimes gets trapped in the folds of the bag. Make sure the tassel is shedding. You can dissect an emerged but not yet shedding anther and smear the still wet immature pollen on the silks. Single anther pollinations yield from 0 - 200 kernels.

Forgot to bag tassel? Pollen is viable for at least 6 hr in California so you will have to bag and wait to make sure there is no contamination from stray pollen.

Will there be pollen tomorrow? Pollen is shed first from the middle of the central spike (Figure 4) then the tip and base, the middle of the branches and then the bases and tips. Remember that each set of glumes encases 2 florets each with 3 anthers; the two florets mature one day apart. A good tassel will shed for about 7-10 days. The best pollen shed will be on days 2 - 5.

What if there are aphids on the tassel? Icky, but no problem.

I've just broken the tassel off. Place it in a glass of temperature water containing a teaspoon of room sugar. Pollen shed will continue as normal; this method even works with tassels broken off and found a day later.

I'm getting plump anthers in the bag but no pollen. Dissect anthers to make sure they are fertile and wait a day. A pocket microscope (about \$50 from scientific supply house) is useful for classifying pollen. Good pollen is pale yellow and round; bad pollen is shrunken, not plump.)

The tassel looks fried. It may be sterile, but a few anthers may be exerted so check carefully. The tassel has kernels and silks. This is tassel seed and the kernels are a drain on the plant so break them off.

Help, all the pollen is gone. If there is a tiller it will shed about the time the main tassel finishes. Check for a late floret on the main tassel near the base of the main spike.

An important tassel has smut. You should discard the affected area; you can rescue a branch in a glass of water.

Silks look dead. Pollinate any way and pollinate the second ear as a back-up.

Forgot to cut back the ear. Slit down the side to expose more silks, then pollinate.

Shortage of tester ears. You can double pollinate by placing a card to divide grown out silks into 2 groups, i.e. side toward stem and away from stem. Be sure to label brown bag carefully, i.e. inner half = X x Y, outer half = A x B.

You need to use 2 ears on a plant. It is best to pollinate the lower ear first to give it a head start of a day or two then the upper ear.

Do early evening pollinations work? If pollen is shedding you're OK, at least in Palo Alto.

What if the stem breaks? If break is above the ear shoot you may be OK, but if it is at the ear node yield is likely to be zero.

Help, I just pollinated the wrong ear. Cut it back again and hope the silks grow out again.

Pollinations in someone else's field. If you need to use ears in someone else's plot ASK FIRST, then cutback and put your name on the creased shoot bag. When you do the pollination use a color striped bag (Figure 26) so this ear will not be harvested along with the rest of the plot. Striped bag will help you retrieve ear at harvest.

Special Pollinations. If certain ears need special handling such as early harvest for tissue culture or husking for kernel color development you may want to use a color stripe bag (Figure 26) for easy retrieval. *Overuse of color stripe bags defeats their purpose so be frugal.*

As pollinations are completed, you may detassel to eliminate potential sources of contamination and to focus on plants needing attention. It is a good idea to detassel most tester plants in large blocks to reduce contamination. Removal of immature tassels is easy: just pull and they pop out. Old tassels have to be cut off.

When a family is completed mark it on the check list so you can skip over it.

Keep a sharp eye on dwarves and late plants to make sure they are handled when ready. Some families have special requirements; for example, *an bz2* dwarves require gibberellin acid treatment of the tassel to promote pollen shed. Some dwarves are female sterile or nearly so. When you receive seed, be sure to ask about any special pollination requirements.

## **F. Accurate Records and Getting Ready for Next Season**

As the pollination season draws to a close, start jotting down ideas for the next season on spare cards in your field book. List materials that failed and must be repeated in the Hawaii or greenhouse nursery.

After finishing pollination, it is a good idea to walk through the field and make sure that the bags match the plants. If you have delayed writing female parents on the bags, do it now.

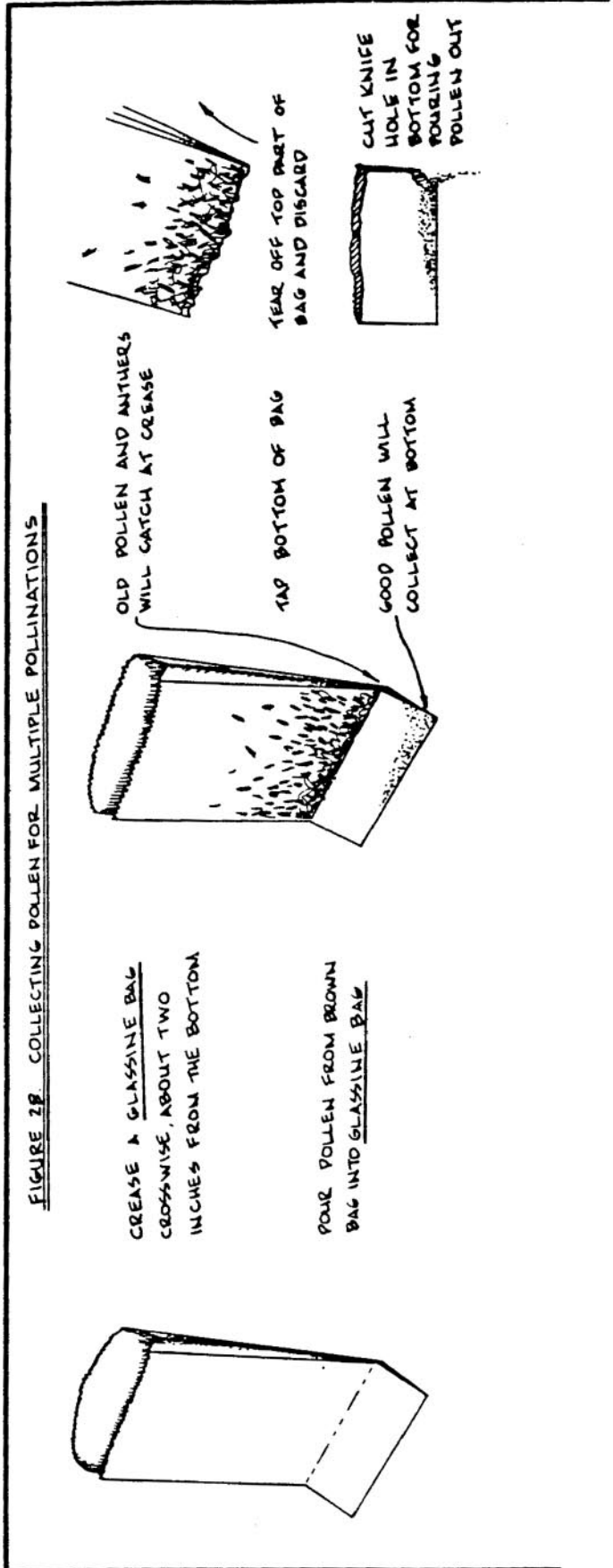
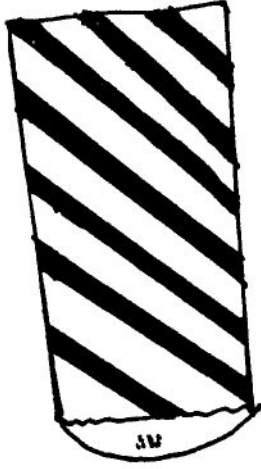
Many people write ear harvest tags at this point by reading bags into a tape recorder and then transcribing at the lab; this will save time and reduce transcriptional errors at harvesting. Ear tags with tractor feed sides can now be purchased so a computer record of crosses completed can be set-up, then printed out on continuous tag stock. There is usually 2-3 weeks between the last pollination and first harvest so quite a few tags can be prepared in advance. Furthermore, failed ears generate unused tags so you have an automatic record of crosses that failed.

## **G. Summary**

Pollinating season is the busiest time of the year. All of the activity depends on accurate shoot bagging and daily assessment of ear readiness. As illustrated in Figure 27, some days in 1986 were really busy for one person and a helper. .

Figure 28 is a suggested checklist for each worker assigned to a plot.

Figure 27  
Color Stripe Bags





*Harvest finger-sized ears of corn when silks first show. Whole ears—including cobs—are tasty. Note the close spacing shown below: you can crowd plants because ears are not expected to size up*



## *Baby corn . . . you can crowd it*

**Although it's easy to grow baby ears of corn like the ones served in Chinese restaurants, it's practical only if you have considerable garden space. Plants are big (they are common sweet corn), but the ears, harvested when the silks first appear, are tiny.**

You can increase your yield by spacing plants close together in the row. Because ears aren't expected to reach full size, plants can stand 5 or 6 inches apart instead of the customary 12. Except for spacing, grow just as you would any corn. You need not worry about planting in blocks for pollination. If you want to freeze your crop, plant hybrid seed; ears will all be harvestable at the same time. □

## 6. HARVESTING

This section describes the steps in harvesting ears, recording ear phenotypes, tagging and drying ears for permanent storage, and entry of yield data on the computer. Harvesting generally occurs 45 days after pollination. The plants will be brown and brittle, and the ground should be hard as irrigation stopped 3 weeks or so before harvest. It is generally the best plan to wait until a group of families is ready (about 100-200 plants) before harvesting an area to prevent picking some materials too early.

There are 2 basic harvesting methods: [1] stripping ears for phenotype scoring and discard and [2] harvesting for tagging and storage. In the first case, 2 people are much more efficient than one person; in the second method, one person can solo very easily. For both methods you will need:

1. 2. 3. 4. 5.

Your field cards for reference

Large number of onion sacks

Gloves

Knife

Clipboard with harvest list printout for quick scoring in the field (Figure 29)

Harvesting is reasonably easy, because you have a lot of leeway in when you do the work: any time of day, any day from -45-55 after pollination. It is a good idea, however, not to fall behind, because rain in late fall is very disruptive and later harvest ears are subject to ground squirrel predation and fungal attack especially by Fusarium.

Time required to harvest 1,000 ears over a 3 week period:

Travel to/from Field scoring 500 Ears (team of 2)

Time Required to Harvest and Tag 500 Ears

Laying Ears Out for Drying

sorting Ears After Drying and putting in  
Ears in Storage

Recording Yield on Field Cards

Recording Yield on Computer

Miscellaneous

TOTAL ESTIMATED TIME

FIGURE 29. CLIPBOARD AND SCORING SHEETS

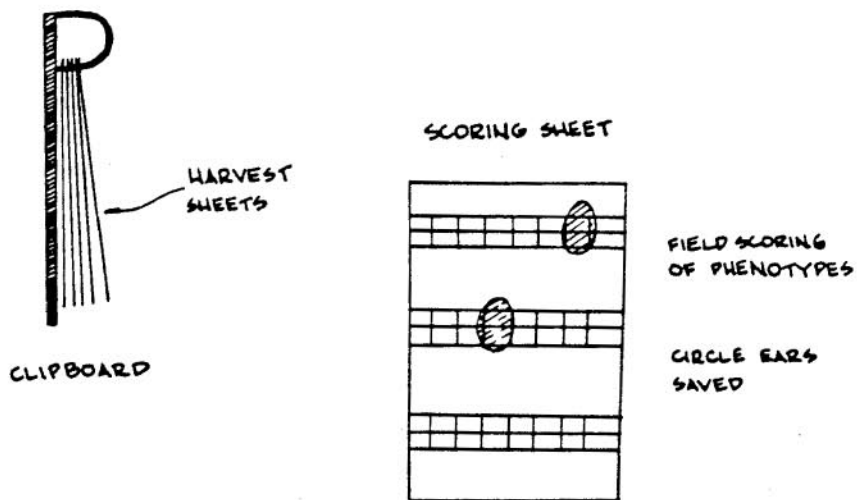
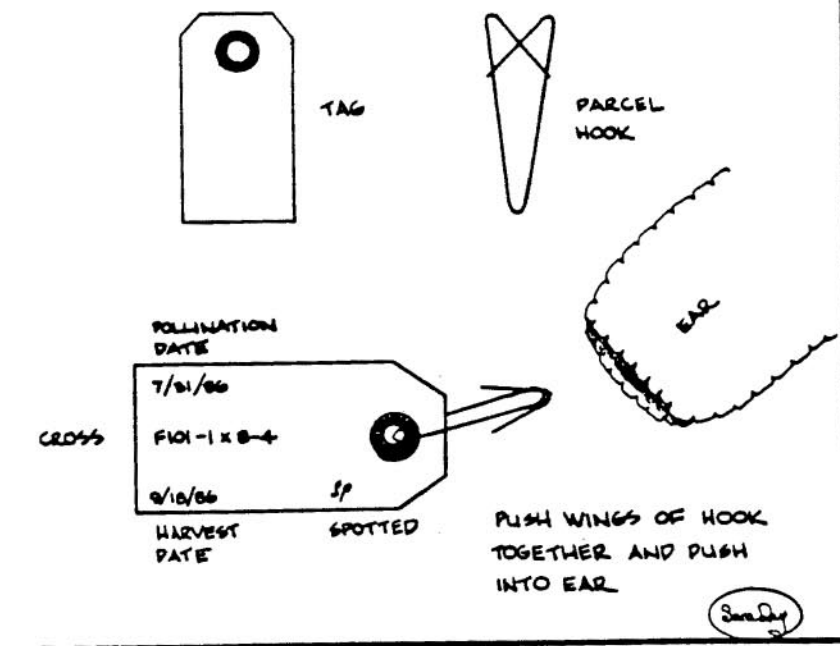


FIGURE 30. HARVEST TAG



|     |    |  |
|-----|----|--|
| 2   |    |  |
| 5   |    |  |
| 15  | hr |  |
| 1   | hr |  |
| 3   |    |  |
| 2.5 |    |  |
| hr  |    |  |
| 2.5 | hr |  |
| 2   |    |  |
| =   |    |  |
| 33  |    |  |

| Family 99     | B peru    |           | a2 bt pr?   |           |      |
|---------------|-----------|-----------|-------------|-----------|------|
| plant no.     | 1         | 2         | 3           | 4         | etc. |
| (X)           | <i>pu</i> | <i>pu</i> | <i>pa:d</i> | <i>cl</i> |      |
| <u>to a2</u>  | <i>pu</i> | <i>cl</i> | <i>pu:d</i> | <i>pu</i> |      |
| <u>to r-g</u> | <i>pu</i> | <i>pu</i> | <i>pu</i>   | <i>cl</i> |      |







# FIGURE 32. HARVSCOR. FMT SCORING SHEETS

F# H264 M14 x +/-Les8 W23  
 Scoring P=purple cl=colorless bz=bronze M=mottled  
 Other codes: \_\_\_\_\_

|        | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|--------|---|---|---|---|---|---|---|---|---|----|----|
| (X)    | : | : | : | : | : | : | : | : | : | :  | :  |
| to r-g | : | : | : | : | : | : | : | : | : | :  | :  |
| to     | : | : | : | : | : | : | : | : | : | :  | :  |

Circle ears to keep. All others assumed tossed.  
 Conclusions:

F# H265 M14 x +/-Les9 W23  
 Scoring P=purple cl=colorless bz=bronze M=mottled  
 Other codes: \_\_\_\_\_

|        | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|--------|---|---|---|---|---|---|---|---|---|----|----|
| (X)    | : | : | : | : | : | : | : | : | : | :  | :  |
| to r-g | : | : | : | : | : | : | : | : | : | :  | :  |
| to     | : | : | : | : | : | : | : | : | : | :  | :  |

Circle ears to keep. All others assumed tossed.  
 Conclusions:

F# H266 M14 x +/-Les10 W23  
 Scoring P=purple cl=colorless bz=bronze M=mottled  
 Other codes: \_\_\_\_\_

|        | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|--------|---|---|---|---|---|---|---|---|---|----|----|
| (X)    | : | : | : | : | : | : | : | : | : | :  | :  |
| to r-g | : | : | : | : | : | : | : | : | : | :  | :  |
| to     | : | : | : | : | : | : | : | : | : | :  | :  |

Circle ears to keep. All others assumed tossed.  
 Conclusions:

CUSTOM SCORING SHEETS GENERATED  
 IN CARDBOX

Figure 31. Checklist for Harvesting.

| <b><i>Date</i></b> | <b><i>Families Harvested</i></b> | <b><u><i>Date Drying Finished</i></u></b> |
|--------------------|----------------------------------|---|
|--------------------|----------------------------------|---|

Zero yield materials ... get ready for Hawaii immediately:

Zero yield materials ... get ready for greenhouse crop:

This is a running list of what you've done and a reminder not to leave families drying too long.

## 7. Sorting and Storing Ears

Ear storage has the same requirements as a library -- you want easy access to each item and to have related items grouped together. Also both work best if a single storage system is used, not a mixture of "Dewey decimal" and "library of congress." For ears the most fundamental piece of information is the female parent in each cross. consequently, sorting the ears by their unique female parents is the easiest way to store everything, every year, no exceptions! Whatever method you decide on, stick with it because you will have years of ears to deal with, and it will become impossible to remember that in 1985 you stored the *bronzel* tester (female) with the male parent "for convenience" this one time.

Time required to store and sort 1,000 ears:

|   |        |
|---|--------|
| Sorting Into Boxes and Bags                                 | 5      |
| Recording Yield on Cards and Computer (if not done before)  | 5      |
| Moving Boxes to Cold Storage                                | 1      |
| Detailed Scoring of Phenotypes on 100 Ears                  | 5      |
| Detailed scoring of 20 Families in A Sandbench (400 plants) | 3      |
| Miscellaneous   | 2      |
| TOTAL ESTIMATED TIME  | 21 hrs |

### A. Boxes and Internal Divisions

We use plasticized boxes used for fur storage; the boxes are shipped unassembled in stacks of 25 tops, 25 bottoms. These boxes are sold in lots of 1,000 so either purchase a lifetime collection or share with several other groups: each box holds ~200 ears. Assemble boxes using a heavy duty stapler. On the bottom put the flaps inside the box to make the bottom as small as possible; put flaps outside on the top so it fits easily onto bottom. After stapling use wide plastic or duct tape to seal over the staples to prevent ripped fingers.

As you begin sorting the seeds, attach temporary labels to tops and bottoms with post-it notes to time tape. No need to close boxes at this point if you have the space to leave things spread out do it. As boxes fill, sort ears into brown flat-bottom lunch sacks cut about 5 inches tall. About 10-12 bags fit in one box. Shuffle ears around so bags are essentially full and are in numerical order. Because you have harvested whole families on the same day, this is actually pretty easy to do. Eventually you will have all the ears in bags, then finish arranging the bags into boxes, and label each bag with the family numbers it contains. Close the box, and attach a more permanent label (time tape or stick-on label) to the box lid and close-up.

Finally, add a few moth balls to each box to suppress moths. Then tape a sheet of paper to inside of lid titled BORROW LIST with columns for name of borrower, date and ear. Pencil an m (for moth) and p (paper) on the box tag so you'll know you're done those tasks, then move boxes to cold storage.

## **B. Shelling vs. Storing on the Ear**

Removing kernels from the ears makes much more compact storage, however, you lose information on the position of the kernels. In general, it is OK to shell testers, inbreds and bulk populations of any type. Shelling can be done with:

[1] a single ear sheller (kept in gray file in Room 251 and at the field) by pushing the ear through the sheller; do this over a box so you can catch all of the kernels

[2] or with a hand-crank Black Beauty corn sheller (kept in seed storage facility) in which more muscle power is used but you don't get bruised fingers from pushing on the kernels. Catch the kernels in a drawer placed alongside the sheller.

For all materials be sure to clean the shelling equipment and box/drawer carefully between genotypes.

Because we are very short of cold storage, our usual practice is to keep materials on the ear for about 3 years. After that, most ears are partially shelled and ~50K samples are kept of each. Also, some testers and intermediate crosses in stock constructions can be tossed. Usually three boxes of ears storage will reduce to one box of shelled storage, with some materials tossed altogether.

## **C. Additional Data Collection**

Using a scoring sheet of some kind (Figure 32) record precise ear phenotypic ratios and/or other information, for example, ratios obtained from sandbench tests, tests for fertility, mutability, etc. It is a good idea to make a master list of all of the scoring to be completed, i.e. count ratio of spotted:bronze kernels on all ears families 100-200, 100K per ear, then have all helpers sign-up for materials already completed. If possible, try to collect all data by 1 March of the following year so you have it in hand before you begin planning the next year's crop in detail.

## **D. Cold Storage**

Corn seed viability is increased by storage in cool, dry conditions. A rule of thumb is that the % relative humidity + temperature in of should equal less than 100. Presently we are using an old cold room kept at about 450F and about 30% relative humidity. Under these conditions seed viability should decrease no more than 5-10% per

year. Some exceptions are [1] the K55 background which loses 25% viability per year and is effectively gone in 3-5 years of storage and [2] many shrunken/brittle/sugary genotypes which also decline in germination percentage at a quick rate.

The key for the cold storage room is kept on the back of the door in Room 266B. Be sure to sign out for the key when you take it, and return the key promptly when you are finished. Frequent users should obtain their own key.

Bug-infested plant material of any type should be kept out of the cold storage room. Place a few mothballs in all boxes of freshly harvested material to suppress wildlife. The naphtha in mothballs is a known cause of liver cancer so inhale as little as possible. It is preferable to purchase balls not flake naphtha, as the flakes sublime quickly and increase personnel exposure. The mothballs sublime over a year or so, and the entire cold storage room smells -- therefore it is dangerous to your health. We used to replace the mothballs annually, but now we just add

fresh mothballs to each new box as it is placed in storage. This should suppress/kill all insect larvae in the fresh corn, and reduces the amount of carcinogen exposure.

If you open a storage box and moths fly out YOU HAVE A PROBLEM. Capture one insect for close examination and try to identify the species; if not obvious, send to state inspectors. Check all boxes near the infected box to determine if larvae or adults are present. Always carefully check all shipments from Hawaii for insect damage. We have already had one disaster in which rice moth larva hatched in our corn, because the Hawaiian Growers forgot to gas our corn. The treatment was to store all of our corn at 40C for a week in a humid cold room, resulting in a substantial loss of viability. Another sign of insect infestation are "drill holes" in corn seed filled with powdery fine material -- this means that insects have bored into that kernel. Discard affected ears if possible or inspect and save only good seed.

Humidity control in the storage room is partially maintained by an air dryer on the top of the cold room, but is primarily accomplished by an inexpensive home dehumidifier. This dehumidifier must be emptied about once a month.

Please keep the storage room as clean as possible. Sweep up seeds often. If you drop an ear and seeds pop off, don't use them unless you see the seed still moving ... unless you know that the floor was absolutely clean.

Reminder: It is a good idea to review what you have in storage about 3 years after the crop year. Stock constructions may be finished and you can toss intermediate crosses, or shell examples into a few envelopes. New testers and inbreds are more desirable, and you may wish to discard older material. Usually you can shrink the storage about 30-50% at this stage, although it is psychologically very difficult to actually throw out an ear, particularly a tagged ear. Saving a 25-50K sample in an envelope is an intermediate step and a good task for an undergraduate helper.

### **E. Germination Checks**

For important materials such as testers and inbred lines, it is important to know if you have enough stock on hand. Also, if there is a disaster in the storage facility -- high humidity, high heat, or both -- a random sample of germination percentage should be made to determine what the impact is. There may be plenty of seed, but will they germinate? A good job for undergraduate helpers is to test the percent germination of key stocks every 2 - 3 years. Pull 30 seed and place in a plastic petri dish or in a sandbench, add water, and count the number of seedlings in 5-7 days. Both of these tests over-estimate field germination under variable environmental conditions. When germination falls below 50%, it is time to reconstruct the stock.

### **F. Summary**

Sorting and storing provide you with your permanent collection of ears, and allow you to collect data in the future. As with all of the other tasks associated with maintaining your genetics program, organization and planning may take time but the payoff in ease of access is substantial. The best advice is to complete all aspects of sorting and data collection each year, *before* you pull seed for the next crop.

## 8. WINTER CROPS

To more rapidly carry out experiments, two or even three crops per year are possible. We usually have staggered greenhouse crops and one planting in Hawaii. Preparation for these plantings follows more or less the same procedures as for the summer crop in terms of details. The major difference is that for most people everything is done on a smaller scale, because there will be far fewer plants. This means it is more difficult to conduct complicated crossing programs in which more than a few plants have to be ready at the same time. Consequently, many people use the winter crops primarily for selfing, test crossing and seed increases. Experiments must be more tightly planned than usual because of space limitations and because many genotypes flower unexpectedly early or late when grown in pots or Hawaii.

### A. Greenhouse

In 1988, the greenhouses and all other plant growth facilities are managed by Mary Alice Andrews. Figure 33 is a reprint of the space reservation form currently in use. Mary Alice assigns space, and you should sign-up early to insure that you have the kind of space you need.

Facilities available:

*Roof top greenhouses* -- space is always very limiting in these greenhouses; very precious stocks and plants receiving treatments with microbes or chemicals not cleared for usage at the field will be given priority in this space. Pesticides, however, cannot be used in these greenhouses because some of the roof top greenhouses are used to grow insects. Routine plantings should be maintained at the field.

*Individual glass greenhouses* -- there are three of these; they are about 250 ft<sup>2</sup> usable space and will hold up to 200 plants in large pots or in the ground plantings. The temperature and light regimes are reasonably good except near the outside walls. These greenhouses are very useful for "isolation experiments" in which you want to exclude most kinds of pollen.

*Large greenhouse bays* -- standard for most experiments and offering higher and more balanced light for the plants; temperature control and air circulation is superior to the smaller houses.

Some general information about the greenhouse crop:

1. Stressed plants shed pollen early and may not produce an ear. Use largest available pots if you want ears and don't crowd the pots to close together plants.
2. Crowded plants get very tall and you will need a step stool to reach the tassels.
3. If plants get too close to lights, pollen may be fried.
4. Plants are generally very weak, possibly from a lack of wind ... stems often break at ground level or near the ear. Stake all weak plants.
5. Days to pollination and ear maturity similar to field-grown plants in most cases.
6. Contaminating pollen is a big problem: you will be covered with pollen, so be careful.
7. silk growth is often slow, so allow 2 days after cutting back and/or pollinate important ears on two successive days.
8. with few exceptions, second ears are a complete wash out.
9. Aggressively attack pest problems.
10. Dipel all shoot bags.

### Figure 33. GREENHOUSE GUIDELINES

1. Consult Mary Alice as you BEGIN your plans to use greenhouse space. This will help me plan ahead to allocate space, and might benefit you to get a grower's viewpoint. At this time fill out a workrequest form and return same.
2. Pesticides will be applied by licensed staff. Your plants will not be treated without your knowledge, but you are urged to cooperate in a spraying program. The greenhouse bulletin board has information on dates sprayed and chemicals used. It will also be posted on the door the day of application. If you will be using any chemicals on your plants be sure to consul~ with Mary Alice first.
3. Sanitation is the most important aspect in avoiding insect and disease problems. Many people will be using this facility... please clean up after yourself! And note:
  - a) No clutter allowed.
  - b) Any unwanted plant debris should be REMOVED from the greenhouses as soon as possible. Do not discard materials under benches etc. Take them OUT and deposit them in the trash. Dead plants will not be tolerated without explanation.
  - c) Hang the nozzle of the hose up OFF the floor when you finish using it, do not drop it in the dirt or leave the nozzle on the ground.
4. Please sign the guest book whenever you visit the greenhouse facility. This will be used to tell us just how much we are used, by whom, and why! So please don't forget!
5. If you run into problems with insects/disease/running over re: time of completion etc. please notify Mary Alice immediately.
6. Be considerate of others! Please return tools. If you use the last of something, tell maa. Suggestions are welcome. If you need help just ask.

GREENHOUSE WORK REQUEST FORM

Please note: I will assume that I can water, fertilize, groom, spray for insects, etc. unless you tell me otherwise. NOW IS YOUR CHANCE! If your plants need special treatment, TELL ME! If you already know your plants will look abnormal WARN ME! I asked for all of the following information because I need it!! PLEASE BE COMPLETE! Thanks for your cooperation. Mary Alice.

Today's Date \_\_\_\_\_

Your Name \_\_\_\_\_

Starting Date \_\_\_\_\_

Number of plants \_\_\_\_\_

Harvest Date \_\_\_\_\_

Brief Description of Project \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Special Requests/Needs/Comments \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_

REMAINDER TO BE FILLED OUT BY MARY ALICE

date ss \_\_\_\_\_

date of harvest \_\_\_\_\_

Treatments/Problems/Comments \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

## **B. Hawaii**

Aloha. You are now to the section that represents one of the truly great fringe benefits of corn genetics: the required trip to Hawaii each winter.

We routinely have a winter crop in Hawaii, planted about November 15, for pollination in January, and shipment back to Palo Alto in early April. Figure 34 shows current materials concerning the Hawaiian Research Foundation and their financial and field arrangements. Although it seems expensive to grow corn in Hawaii, it will cost about \$1 per harvested ear for all plant-related costs, and about \$1,000 for one person to be there for 2 weeks (about another \$0.50 per ear). These costs are less than we end up paying at Stanford on a per ear basis, considering the high cost of our labor.

The major difficulty with the Hawaii crop is that you entrust the planting to other people and do not arrive until shortly before pollination time. Thus far, the Hawaiian Research Foundation staff has been very unreliable in getting materials planted on time, sometimes even skipping small populations altogether. Their general field maintenance is also poor, and their reports by telephone or mail about what is going on are often unreliable. However, yield in Hawaii is usually excellent, and the working conditions are very nice.

Our laboratory sends one shipment of seed to be planted in one or two plantings; about 6,000 plants (about 2/3 of an acre) is now typical. We collect the seed by approximately November 1, and then mail it to Hawaii with a request for November 10 planting date. We are lucky to get things planted by 15 November. Seed are planted by hand at the rate of 23 per 15 foot row: one family of 23, two families of 10-12, four families of 5-6 per row. All seed packets for families to be planted in a single row are banded together with the 12" stakes for those families. This minimizes errors on the other end and allows mixing of different sized families with a minimum of difficulty. Alternatively, all single family rows, 10 plant families and 5 plant families are grouped so that the planter plants pure rows, then two packets per row, then four. Main advice is to label everything very carefully because you will not be there to supervise planting it!

*F. Johnson - Handle by  
hypocrite*

RECEIVED

AUG 7 1984

HAWAIIAN RESEARCH, LTD.

PRESIDENT'S OFFICE

POST OFFICE BOX D  
KAUNAKAKAI, MOLOKAI, HAWAII 96748  
TELEPHONE (808) 553-5070

July 23, 1984

Stanford University  
Stanford, CA 94305

Dear Sirs:

I am trying to do some planning for our land needs for the '84-'85 season and, if at all possible, I'd like an estimate on what you anticipate your needs will be.

The sooner we know what your plans are the better we will be able to prepare for the coming season.

We hope to be of service to you again this season, and looking forward to hearing from you in the very near future.

Sincerely,

*Barry O'Keefe*

Barry O'Keefe  
Nursery Manager

# HAWAIIAN RESEARCH, LTD.

CUSTOMER Stanford

POST OFFICE BOX D  
KAUNAKAKAI, MOLOKAI, HAWAII 96748  
TELEPHONE (808) 553-5070

SEASON 1987 - 1988

## Daily & Week Average Heat Units

Month NOVEMBER 1987 HOOLEHUA

| WEEK                  | MON       | TUE       | WED       | THU       | FRI       | SAT       | SUN       | AVERAGE   |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <u>11/02-11/08/87</u> | <u>30</u> | <u>28</u> | <u>30</u> | <u>30</u> | <u>30</u> | <u>30</u> | <u>28</u> | <u>29</u> |
| <u>11/09-11/15/87</u> | <u>30</u> | <u>30</u> | <u>30</u> | <u>31</u> | <u>28</u> | <u>26</u> | <u>25</u> | <u>29</u> |
| <u>11/16-11/22/87</u> | <u>27</u> | <u>24</u> | <u>27</u> | <u>26</u> | <u>26</u> | <u>30</u> | <u>28</u> | <u>26</u> |
|                       |           |           |           |           |           |           |           |           |
|                       |           |           |           |           |           |           |           |           |
|                       |           |           |           |           |           |           |           |           |
|                       |           |           |           |           |           |           |           |           |

OTHER COMMENTS: *Nursery is now about 1' tall. Stands are good.*  
*B. O'Keefe*

WORK ORDER

Crop: CORN

NURSERY: 700 packets; 5-23 kernels per packet; \_\_\_\_\_ packets for Delay Planting

ESTIMATE  
NEEDING  
250 rows

GROWOUT: \_\_\_\_\_ packets at 60 kernels per packet

276  
to 15 ft each

PRODUCTION: \_\_\_\_\_ acres requested; \_\_\_\_\_ population requested

REQUESTED PLANTING DATE: ~~NOV 10-15~~ NOV 10-15

REQUESTED LABOR FOR: Planting 2 people for 2 days

Pollinating \_\_\_\_\_ people from \_\_\_\_\_ to \_\_\_\_\_

Harvesting 1-2 people from Mar 10 to 15 (~2 days)

ADDITIONAL COMMENTS OR INSTRUCTIONS:

INCLUDES WALBOT (STANFORD) + CHANDLER (OREGON)

ORDERED FOR WALBOT (STANFORD) AND  
CHANDLER (OREGON) WILL BE SENT  
SEPARATELY BUT PLEASE PLANT  
NEXT TO EACH OTHER

I have read the provisions of the agreement on the back of this work order and I hereby agree to all the terms and conditions of such agreement.

Address: Dept of Biological Sciences VIRGINIA WALBOT  
STANFORD, CA 94305 Customer

Telephone: (415) 723-2227

Date: Sept 23 1987

Acceptance of Work-Order/Agreement

HAWAIIAN RESEARCH, LTD.

By Peter H. Eichhorn  
PETER H. EICHHORN, General Manager

Date: 11-24-87

For the field season, we have evolved the following scheme:

***Dates***

***Duties of person in residence in Hawaii***

- |                   |  |
|-------------------|--|
| Dec. 25 - Jan. 1  | <ul style="list-style-type: none"><li>• Person there to count plants, supervise weeding check stakes, replace as necessary</li><li>• Number plants with MARKSALOT</li><li>• Check plant phenotypes against stake numbers</li><li>• Call cooperators and to report zero or low germination in time for replacement greenhouse plantings</li><li>• Score plant phenotypes at 6 weeks</li><li>• Realistically there are 2 - 3 days of work, unless there are some very early families</li></ul> |
| Jan. 1 - Jan. 14  | First shift field worker -- plants are 7-9 weeks old <ul style="list-style-type: none"><li>• shoot bagging</li><li>• early pollinations start about Jan. 5</li><li>• busy pollinating second week</li><li>• check individual pollinating instruction sheets against what will be possible (last minute chance for people to add to greenhouse plantings)</li></ul>   |
| Jan. 12 - Jan. 25 | Second shift field worker -- 8 - 10 week old plants <ul style="list-style-type: none"><li>• majority of pollinations</li><li>• late shoot bagging</li></ul>  |
| Jan. 25 - Feb. 10 | Third shift field worker -- >10 weeks <ul style="list-style-type: none"><li>• last pollinations</li><li>• scoring of late phenotypes: lesions, knots</li></ul>   |

Thus, someone is in residence for about 6 weeks during the season providing coverage of shoot bagging and pollination from about day 50 to day 100 post-planting. This should cover almost all corn stocks. Some materials are earlier and some are later in Hawaii than in California. Under optimal warm conditions, most materials will be a few days earlier in Hawaii than at home.

If several labs plant together, the expenses are pooled and costs calculated on a per row basis; thus, all living, transportation, and field expenses are added together, divided by the number of rows and then the monetary share of each lab calculated by adding/subtracting the calculated share from the actual expenses. This method assumes that each group donates an appropriate amount of time for the field work.

air fares: reserve early for times near Christmas holidays; search for bargain fares and for good inter-island fare from Oahu to Moloka

hotel: Molokai Shores or Wavecrest condominiums; currently \$1700/month for a two bedroom unit plus sleeping space in living room; easy for two groups to share for a few days. Swimming pool, small beach, various outdoor games are available. Molokai Shores is one mile from "town" where there is a library, post office, and a few stores. Wavecrest is 8 miles from town, further away from the corn.

car: essential; we usually use AVIS, about \$400/month for basic rental fee. Stanford has already insured all drivers so supplemental insurance is not required. The fields are usually about 10-18 miles from the condominium.

transport: several small airlines serve other islands from Honolulu; cheap one day round trip fares usually available from Molokai to other islands if you want to explore Maui, etc. Also fun to see the island from the air.

food: several restaurants ranging from raunchy to plush Sheraton resort. Food stores are the quality of 7-11 and have very high prices. Best fresh food from farmers' markets.

recreation: this is Hawaii after all.

Before harvest time, each person must specify whether ears are to be pooled and bagged with the stake or a paper tag designating the family, or whether individual ears are to be left in brown bags. The two methods are about the same price. The crop is treated for pests and then shipped directly to us from Hawaii. If the crop is large it can be cheaper to fly to Hawaii and sort through the harvest, i.e. scoring and discarding ears, and then returning with the treated corn as luggage.