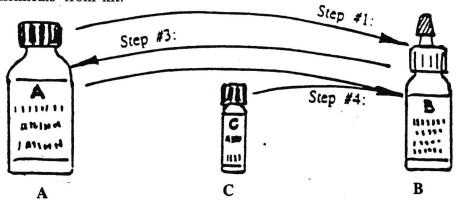
DAYLILY CONVERSION METHOD "A"- 2

Preparing chemicals from kit:



2 oz glass storage bottle for shipping 50 ml Kinetin, and storing Colchicine and Kinetin solutions.

1 dram glass bottle for storage of 1 ml of DMSO. 2 oz plastic mixing and dispensing bottle for shipping dry Colchicine, and for mixing with Kinetin and DMSO solutions.

Step #1: Pour contents (50 ml Kinetin solution) of Bottle "A" into Bottle "B" (dry Colchicine). [DO NOT REVERSE THIS STEP].

Step #2: Seal both caps and mix by shaking Bottle B.

CAUTION: Always wear rubber gloves and glasses when working with Colchicine.

Step #3: Return contents of Bottle "B"(Colchicine and Kinetin Solution) to Bottle "A"for storage.

Step #4: This step was necessary only when it was thought that DMSO would have a very short life expectancy after mixing into the other chemicals. Experiments indicate that if 1 ml of DMSO is added to 50 ml of Kinetin solution and is used within a few weeks, it works as well as the original Step #4 and is much easier.

Step #5: Tighten cap on Bottle "B" and mix by shaking.

Step #6: Remove red cap from Bottle "B" (dispensing) and apply approximately 10 drops per plant for each treatment. Recap bottle and refrigerate (not necessary for one day storage).

NOTE: These instructions are designed for under light systems at stable temperatures (60 to 78°F). Green houses may cause higher temperatures resulting in too much activity of the Colchicine.

DAYLILY CONVERSION METHOD "A"- 2

(Continued)

Sensitivity: The kit is based on treating 25 plants for 2 days (4 treatments) that are of high sensitivity (light yellow and melon). Low sensitive plants (purple, reds) should be treated 3 or 4 days. Pure yellows are usually treated for 3 days, and 2 days for all others. Also, if conservative methods are practiced (see sketch), 0.5 ml (approximately 10 drops or ½ eye dropper) is sufficient for each application per plant.

Treating reds and purple could reduce the number of plants treated to half (12). Not using evaporation control and being too generous could cut the number to half or less.

Evaporation Control: The plant must be flooded or moist during the time of treating. Low room humidity may call for repeated applications to accomplish this, and Colchicine will accumulate in the plant when the water evaporates. There will always be some variation in accumulation, but using the plastic cup will standardize applications therefore reducing another variable in the treatment.

Refer to attached sketches and notes on treating daylilies with Colchicine (clone method).

Note: Varieties will vary in sensitivity. Don't be afraid to increase or decrease treatment if repeated attempts to convert are killing too many (decrease treatment) or nothing is being converted (increase treatment).

Notes on Treating Daylilies with Colchicine (Clone Method)

- 1. **Selecting Plants:** Aside from variety, the plants should be well rooted, no severe division cuts, crowns of 3/4" to 1" diameter is ideal (very small or very large should be avoided), only single division. No small side plants (remove to crown), and use only vigorous growing plants; sick or runts are easy to kill with treatment.
- 2. **Timing of Treatment:** My rationale for the best time to treat is to pick an event when cell division is at a maximum and slightly before scapes will form. The reason for the maximum cell division is that the conversions only take place when a cell is in a phase of dividing. First scapes of mid-season plants usually form around early fall to early winter. Catching the scape cells when they are a microscopic few increases the chances of complete conversion and less chimeras.

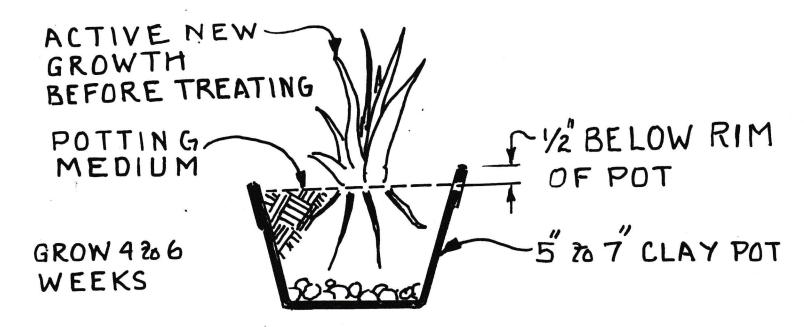
The time for both of these events is around September and October, about when plants start new fall growth. Other months can be used if the plant is actively growing.

- 3. **Chemical Application:** To thoroughly convert, you must go to the brink of killing and run the risk for some. Three factors affect the strength of the treatment:
 - (A) Solution strength. 0.2% Colchicine is most commonly used.
 - (B) Saturation of tissue around growing point. Usually four (4) applications over two (2) days under evaporation control (X cuts and plastic cup) of the carrier (water and wetting agent).
 - (C) The amount of deep-penetrating wetting agent (DMSO). 1 ml of agent to 50 ml of Colchicine solution is common.
- 4. **Ambient Temperature during Treatment and After-Care:** Consistent 75 to 80° is normally found in basements. Greenhouses, outside, and living quarters vary more which probably will work as well, but temperatures in mid to high 80s are more conducive to tissue rot, and near 32° reduces most activity.
- 5. **Lighting during Treatment and After-Care:** I believe low light intensity continuously is beneficial to survival of the treated plants. It seems to reduce rot and recovery time.
- 6. **Potting Plants:** I use 5" to 6" clay pots and substitute soil medium (Pro-Mix). Locate top of crown about ½" above top of medium. Pot approximately three weeks before treating if possible, and wait for new growth to start. Plant with the top of the crown at the top of the pot, however, after treating is complete, add medium to the pot to bring it up to the top of the crown. (This will encourage adventitious roots near the upper region of the crown.)

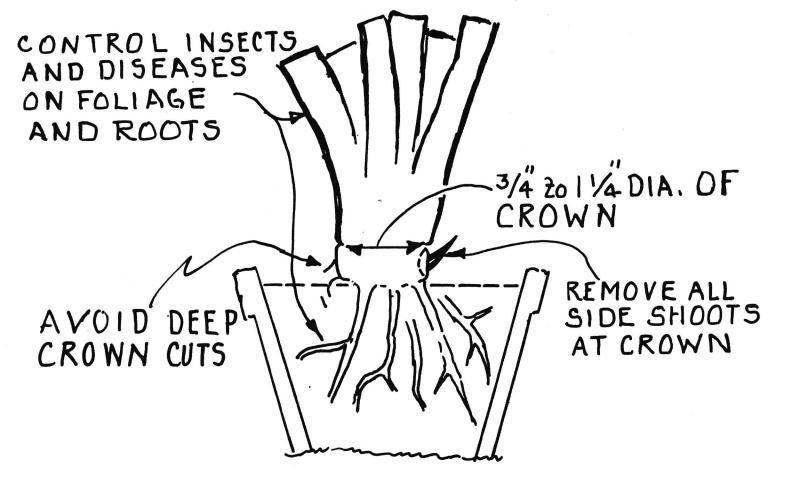
Notes on Treating Daylilies with Colchicine (Clone Method)

(Continued)

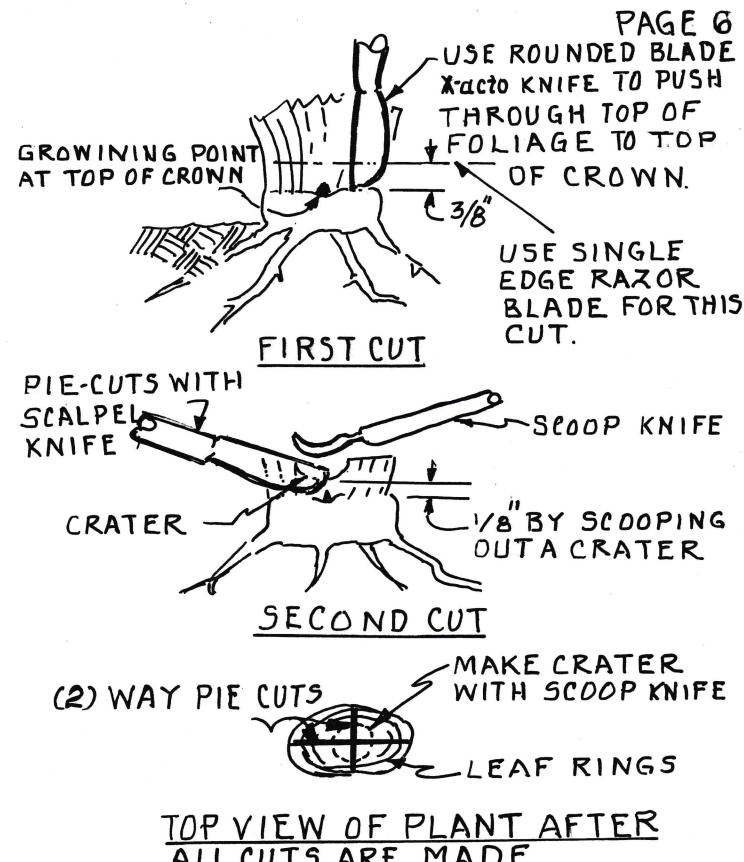
- 7. See illustrations for cutting plant and plastic cup location.
- 8. After about six weeks under continuous lights and after plants have grown out a few inches, move to more normal growing conditions, such as a cold frame or cool greenhouse. Slow growth at this stage seems to help produce healthier scapes in the spring.
- 9. **Identification:** Refer to articles in "Journal Summer 1993, Fall 1993 Vol. 48" and "Winter 1993, Spring 1994 Vol. 49." Note: Colchicine's effect on leaves, scapes, and flowers are common and can be misleading. You may choose to circumvent microscopic I.D. and go directly to hybridizing. If you do, be prepared to follow a lot of false trails.
- 10. **Chances of Success:** My experience, somewhat confirmed by other converters, is as follows:
 - (A) 40% will not convert even though most will show some signs.
 - (B) 40% will be converted chimeras which are destined to revert back to diploid (in a year or two). A few can be used before they revert.
 - (C) 10% will die or become useless, mostly due to rot and growth inhibition.
 - (D) 10% or less will be stable conversions, and, in time, most will be pollen-fertile.



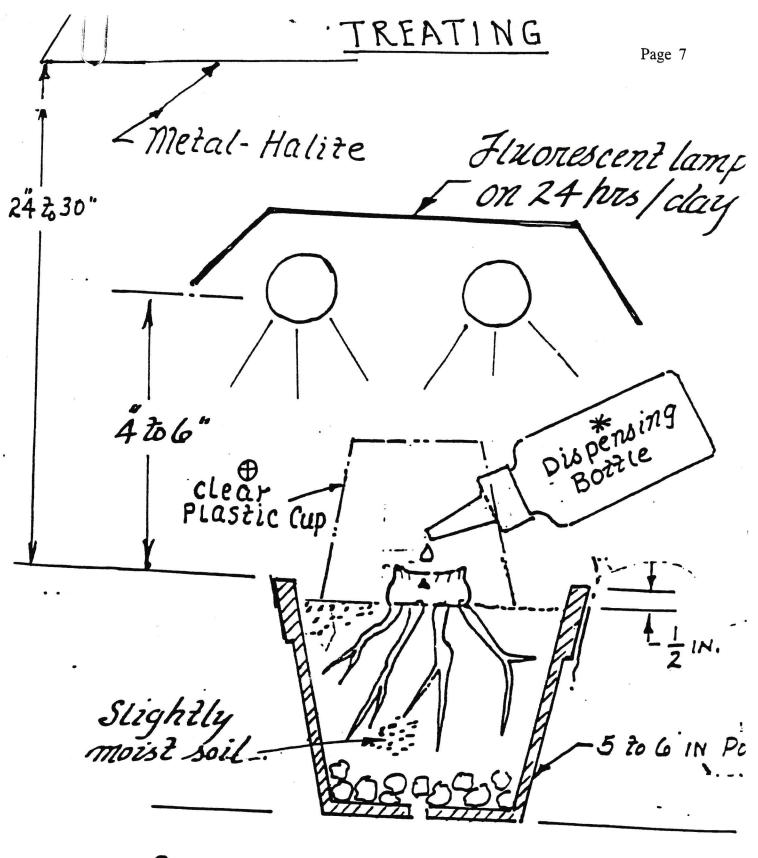
PREPARING THE POTTED PLANT



SELECTING THE PLANT



ALL CUTS ARE MADE



* 2% Colchicine Solution

with wetting agent

4 oz clear plastic cup. KEEP ON PLANTS
DURING AND 2 DAYS AFTER.