

Version 2

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Transplanting Tobacco Seedlings in the Greenhouse V.2

Lynn Doran¹¹Realizing Increased Photosynthetic Efficiency (RIPE)

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Works for me

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Ag SynBio Lab UIUC

Lynn Doran

Realizing Increased Photosynthetic Efficiency (RIPE)

SUBMIT TO PLOS ONE

ABSTRACT

Protocol for transplanting and growing tobacco seedlings in the UIUC RIPE greenhouse. Has been verified on Petite Havana and Samson cultivars.

For additional information on growing tobacco for research purposes refer to UIUC RIPE greenhouse guide "Growing Tobacco (*Nicotiana tabacum*) under Greenhouse Conditions, RIPE Project UIUC Field and Greenhouse, 2020"

PROTOCOL CITATION

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WHAT'S NEW

Included more details about Osmocote fertilizer incorporation in step 2. Added step 21 sub steps outlining re-potting procedures.

KEYWORDS

Tobacco, greenhouse, transplanting, planting, seedlings, *Nicotiana tabacu*, Petite Havana, Samsun

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IMAGE ATTRIBUTION

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GUIDELINES

Temperature

Tobacco's optimal temperature is 20-30°C with daytime temps between 23 - 26°C and nighttime temps between 20-23°C. During high humidity, high temperature days, greenhouse temperature cannot reach "ideal" growth temperatures due to limitations on evaporative coolers. Tobacco will still grow normally and produce seed outside of optimal temperature ranges. Watering frequency will need to be increased for plants to maintain vigor as temperatures increase.

Lighting

Natural lighting in a greenhouse can range anywhere from 0-2000 $\mu\text{molm}^{-2} \text{ s}^{-1}$. Supplemental lighting can increase the photosynthetic photon flux densities (PPFD) on cloudy days or to extend day length. Even the most advanced lighting systems only reach 500 $\mu\text{molm}^{-2} \text{ s}^{-1}$ unless mounted very close to the crop canopy. Tobacco grows best in high light but can still perform under shaded conditions.

Please Note The RIPE greenhouse is equipped with dual walled acrylic glazing and on clear summer days the PPFD is close to 2000 $\mu\text{molm}^{-2} \text{ s}^{-1}$. Supplemental lighting is provided in all bays with 1000W high pressure sodium fixtures which provide around 400 $\mu\text{molm}^{-2} \text{ s}^{-1}$ at the plant level. Daylength is set to 12 hours of supplemental light. If you want to grow a variety that is sensitive to daylength, we have a blackout tent with air circulation that can be used.

Day Length

Tobacco daylength requirements for flowering vary by variety. The experimental varieties Petite Havana NN and Samsun NN are day-neutral and will reach reproductive maturity regardless of the day/night cycle.

Humidity

Tobacco prefers high relative humidity between 60-80%.

Please Note Tobacco can grow in low humidity (30-40%). However, watering frequency is drastically increased under those conditions. In winter without a misting system and heat supplied by natural gas, the air dries very quickly.

Air Flow

Adequate air circulation between plants is required to reduce disease pressure. If your plants are all gently swaying in your growth space, you most likely have adequate airflow.

Please Note The RIPE greenhouse currently has two strong circulation fans in each bay, oriented at a downward angle providing circular airflow. For situations involving seedlings or close spacing we have installed a large oscillating fan in Bay 1 to increase airflow if necessary.

MATERIALS TEXT

- Pot, Classic 400: Used if keeping all transplants or after making selections for final growout. ([BFG Supply NS01-00300-0900](#))
- Saucer, 10": Used with Classic 400 pot ([Hummert International 65-840G0](#))
- Planting flat, 3" x 3", 18 well: Used if making selections for final growout, ([Grower's Solution, 1801 Deep Insert, 1801d](#))
- Planting flat, 1" x 1", 72 well: Used for preparing field plugs. ([GrowOrganic GP009](#))
- Propagation tray, 20"L x 10"W, no holes (Better Grow Hydro [5630](#))
- Humidity dome, 20"L x 10"W x 7" T, adjustable vented, used with planting flats only (Amazon, [YieldLab](#))
- Soil, Berger BM6 HP Myco Organic Fertilized Peat Moss Mix (Hummert International [10121000](#))
- Soil, [BUSH DOCTOR@COCO LOCO@POTTING MIX](#)
- Tweezers/Forceps (Fisher Scientific [10-270](#))
- Drench nozzle, Fogg-IT, Super Fine: 1/2 gallon per minute @ 40 PSI (Hummert International [19195000](#))
- Plastic plant label stakes (A.M. Leonard [VP-PS6](#))
- Labelling tape (Fisher Scientific [15-959](#)) and sharpie (Fisher Scientific [19-166-600](#)) or laser printable labels for identifying pots (Order information varies based on printer)
- Optional: Slow release fertilizer, [Osmocote® Plus 15-9-12 \(3-4 mo. Release\)](#)
- Optional: Liquid fertilizer, [JR Peter's 77860 Jack's Professional Peat Lite 20-10-20 Fertilizer, 25 lb](#)
- Optional: Total Dissolved Solids Meter, [HM Digital 716160 COM-100 WATERPROOF PROFESSIONAL SERIES Combo Meter, 7", White/Purple](#)

SAFETY WARNINGS

Annual Worker Protection Standard training required to work in greenhouse. Contact [UIUC ACES Plant Care](#)

[Facility](#) Coordinator to arrange training.

UIUC RIPE Greenhouse online training required to work in UIUC RIPE greenhouse. Contact [UIUC RIPE Greenhouse Manager](#) to arrange training.

BEFORE STARTING

Seedlings will be ready to transplant in approximately 10 to 14 days after planting when they've formed one to two true leaves. Do not wait too long to transplant. The root system begins to develop rapidly after the first true leaves. When the true leaves first appear, there is typically one branch in the root system making it easy to remove from the initial media if care is taken. Damaging roots during transplant will drastically affect development and result in non-uniform plants.

If planting transgenic plants, review all USDA-APHIS requirements for the RIPE APHIS permit for handling transgenic materials before beginning any experiment.

Initial Transplant



6w

1 Select the appropriate pot size for transplanting. Typical pot sizes:

- If keeping all transplants or after making selections for final growout: Classic 400 Pot
- If making selections for final growout: 3" x 3", 18 well planting flat
- If preparing plugs for transplanting into the field: 1" x 1", 72 well planting flat

Tobacco can reach maturity even in a small pot if the proper fertilizer solution is provided. Tobacco can be top-heavy and a larger soil volume will prevent the plants from falling over. Tobacco should not be allowed to dry out. Smaller pots will require more frequent watering. Classic 400 pot (1gal) is standard for tobacco. This size container allows enough soil to prevent fallen plants if watered properly and reduces soil waste after harvest

2

If transplanting into the Classic 400 pot, it is advisable to mix the slow release fertilizer into the soil while filling the pots. For a medium fertilization rate of a 1 gallon container, use  **17 g** of Osmocote® Plus 15-9-12. A tablespoon of Osmocote weighs about  **12-13 g** so if application does not need to be exact, 1.5_TBSP will suffice.

Fill the pot half full with soil. Add recommended rate of Osmocote. Mix in well with hands. Add remaining soil.

[More information on Osmocote recommended rates.](#)

For the smaller planting flats, it is too difficult to properly mix in the appropriate amount of Osmocote. Apply liquid fertilizer as needed in smaller planting flats.

3 Fill the appropriate sized pot completely full with soil.

- Use Berger BM6 HP Myco Organic Fertilized Peat Moss Mix soil if transplanting into Classic 400 pot or 3" x 3" 18 cell flat.
- Use Coco Loco if transplanting into 1" x 1" 72 cell flats for field propagation.



Coco Loco soil seems to help control the presence of fungus gnats.

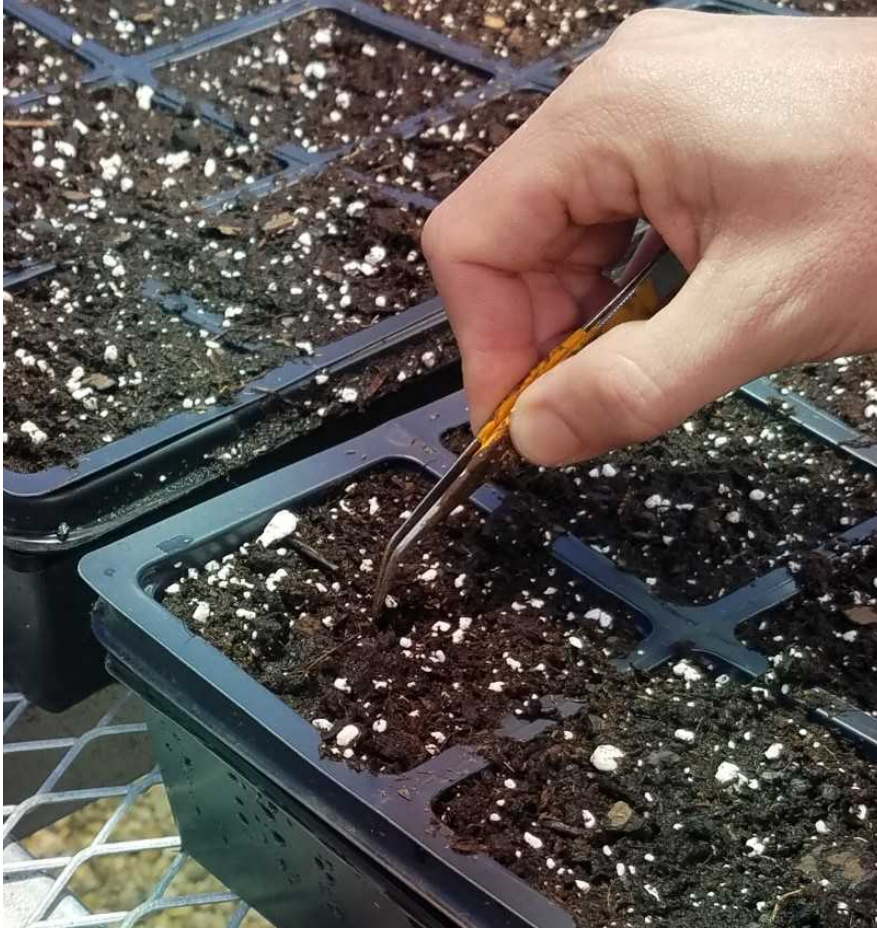
- 4 Tamp the soil down firmly with your hands. Trying to make as level of a surface as possible.
- 5 Using the super fine (0.5 GPM @ 40 PSI) fogg-IT drench nozzle, soak the soil until saturated to remove any air bubbles. If the soil settles more than about an inch, add additional soil and rewet. Do not overpack the soil.



- 6 Label pot twice. Once with sharpie on a plastic stake in the pot and once on the exterior of the pot using a sharpie and labelling tape or a laser printed label.

Laser printed labels are more robust. Handwritten sharpie labels tend to fade over the growing season.

- 7 Poke a small hole in the middle of the pot using tweezers.



- 8 Choose a healthy looking seedling with one to two true leaves but that isn't too big.



The root system begins to develop rapidly after the first true leaves. When the true leaves first appear, there is typically one branch in the root system making it easy to remove from the initial media if care is taken. Larger plants have larger root systems that can easily be damaged during transplanting. Damaging roots during transplant will drastically affect development and result in non-uniform plants.

- 9 Pinch the tweezers closed and insert them into the soil about a quarter inch deep about an inch away from the selected seedling.



- 10 Holding the tweezers closed, dig under and towards the selected seedling. Slowly bring the tweezers up to the surface to loosen the soil.
- 11 Once the soil has been loosened, use the tweezers to grab the plant by the stem as close to the roots as possible.



- 12 Gently pull up on the seedling until the roots are free from the soil.

If the roots or stem break, discard the seedling and select a different one. Additional seeds should have been sown at seeding to allow for breakage and provide more seedlings to select from.

- 13 With the tweezers lay the intact roots of the seedling that was freed from the soil over the hole in the new pot.



- 14 Use the tweezers to gently shove the roots of the seedling into the hole in the new pot.



15

Use the tweezers and finger to gently firm the soil around the seedling. Try to plant the seedling to the same depth on the stem that it was growing in the previous pot and the seedling leaves should not be touching the soil when transplant is finished.



Fungus gnat larva may be present in the soil. The larvae will eat the leaves if the leaves of the seedling touch the soil. The larvae cannot climb the stem.

- 16 Using the super fine (0.5 GPM @ 40 PSI) fogg-IT drench nozzle, soak the soil until saturated to remove any air bubbles and ensure good soil to root contact.



- 17 Place the planting flat in a non-perforated propagation tray, or place the Classic 400 pot in a 10" saucer. Fill tray with about 1" of water or fill saucer about half full with water.

18 

3d

If using a planting flat, cover with a humidity dome for at least the first three days after transplanting. Leave vents on dome half closed, half opened. There are no humidity domes available for the Classic 400 pot.



- 19 After at least 3 days, remove humidity dome and grow transplants at ambient greenhouse conditions: $\uparrow 23^{\circ}\text{C}$, $\uparrow 60\%$, 300-750PAR, and light cycle 12 hours on/12 hours off. Check daily and add water to keep tray filled with approximately 1" water or Classic 400 pot saucer half full of water.

Apply liquid fertilizer to planting flats as needed, if plants show nutrient stress such as yellowing. Most plants transplanted into planting flats will be transplanted into the field or transplanted again into Classic 400 pots with Osmocote before fertilizer is required. For plants in Classic 400 pots, slow release Osmocote fertilizer should be sufficient for remainder of the plant's life cycle.

Following transplant the plant will not use much water but it is important that the soil does not dry. Water from the bottom by filling the saucer at least half full or the tray 1" full for the entire life of the plant. Under less than ideal conditions, i.e. high temps/low humidity, it may be necessary to water the plants from the top, but this has been found to promote sore-shin, a fungal disease that will rot the stem at the base from which the plant cannot

recover. Frequent watering is essential and plants should be monitored daily to maintain the healthiest possible plants.

If plants in planting flats require liquid fertilizer, dilute 600 g of Peter's Profession 20-10-20 in a 9 liter container of water. Then measure 150 ml of the diluted fertilizer into a watering can with 3 gal of water. Use a TDS meter to test total dissolved solids and adjust to 150 ppm by adding more diluted fertilizer or more plain water.

- 20 Keep original seedlings watered and growing in the greenhouse for one to two weeks after transplanting. Replace any^{2w} transplants that die with a new transplant from the respective seedling pot.

Repotting 4w

- 21 If transplants were grown in a 3" x 3" planting flat, after 3 to 4 weeks and once selections have been completed,^{4w} transplant selected plants into Classic 400 pots. If transplants were planted directly into Classic 400s, maintain plants through necessary phenotyping or seed collection.

- 21.1 Plants should be repotted from 3" X 3" planting flats to Classic 400 pots when their leaf spread diameter is approximately 1-2 inches.



Signs of stress that indicate a plant has grown too large for it's pot and should be repotted immediately include:





Once plants show signs of stress, they will never grow or produce seed to their full potential even after repotting. Repotting too late will create added variability and negatively affect research measurements.

21.2 Prepare a Classic 400 Pot as described in [go to step #2 through step #6](#).

21.3 Make a 3" by 3" hole in the center of the pot.



21.4 Remove the plant with as much soil intact as possible from the 3" X 3" flat and place in the hole. Soil from the original pot and new soil should be level.



- 21.5 Gently firm the soil around the base of the plant with your hands.
- 21.6 Water thoroughly from the top down to ensure good root to soil contact.
- 21.7 Maintain plants through necessary phenotyping or seed collection.