Grafting Manual:
How to Produce Grafted Vegetable Plants
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Synopsis:
Watermelon grafting became a standard practice to manage disease as early as the 1930s. In addition, grafted plants are also used to manage salinity in soil and irrigation water.

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USDA United States Department of Agriculture National Institute of Food and Agriculture

Watermelon and Melon Grafting

The use of grafted watermelon (*Citrullus lanatus*) became a standard production practice in Japan in the 1930s as a means to manage Fusarium wilt, a soil-borne disease, and grafting was soon thereafter applied to melons (*Cucumis melo*). The benefits of using grafted plants have been increasingly recognized to address production challenges with the ban of the broad-spectrum soil fumigant methyl bromide (Davis et al., 2008; Lee et al., 2010). Today, commercial use of grafted watermelon plants in some regions of Asia and Europe accounts for up to 95% of total watermelon production, while about 30% of melons grown in Japan, 90% in Korea, and 5% in China are grafted (Lee et al., 2010). In addition to disease management, grafted plants are also used to manage salinity in soil and irrigation water. Beginning in the 1990s, commercial growers and home gardeners in the U.S. have gradually become more aware of the advantages of using grafted plants. Grafted watermelon and melon plants are used in the U.S. primarily to manage soil-borne diseases (e.g., Fusarium wilt, Verticillium wilt) by providing more vigorous, disease-resistant root system.

There are three methods commonly used to graft watermelon and melons: one cotyledon splice, hole insertion, and tongue-approach (Davis et al., 2008; Guan and Zhao, 2014; Zhao et al., 2016). The tongue-approach method was developed in the Netherlands, and is still popular in some European countries such as Spain and also in Japan. Some nurseries prefer to cut rootstock seedlings at the soil line (referred to as root excision) prior to grafting with the one cotyledon or hole insertion method, as this allows grafted plants to regenerate roots during the graft healing process (Guan and Zhao, 2015). Additionally, it is easier to handle the cuttings rather than whole plants, the work surface tends to be cleaner, and the seedlings are more suitable for automated grafting operations.

Some propagators believe that adventitious roots from the rootstock may develop a stronger root system than the original root system. We will provide a summary of the advantages and disadvantages of each method, but plant propagators often develop specific techniques for each method to optimize production of grafted transplants in their operation.

Grafting preparation

In general, watermelon plants are grafted when they are 14–21 days old. Each grafting method has specific requirements regarding seedling size (see below), but seedlings are usually ready to graft when the rootstock seedling has 1 fully emerged leaf and the scion seedling has 2.

In the one-cotyledon and tongue approach grafting methods, the scion and rootstock plants should have similar stem diameters at the time of grafting so their vascular bundles can be aligned and in complete contact with one another. In the hole-insertion grafting method, scion plants need to have a smaller stem size than rootstock plants. The scion and rootstock seedlings may not germinate or grow at the same rates, so it is important to conduct a preliminary test to determine their growth rates in your growing environment.

Figure 2. Supplies commonly used for grafting. (*Photo by Pinki Devi*)

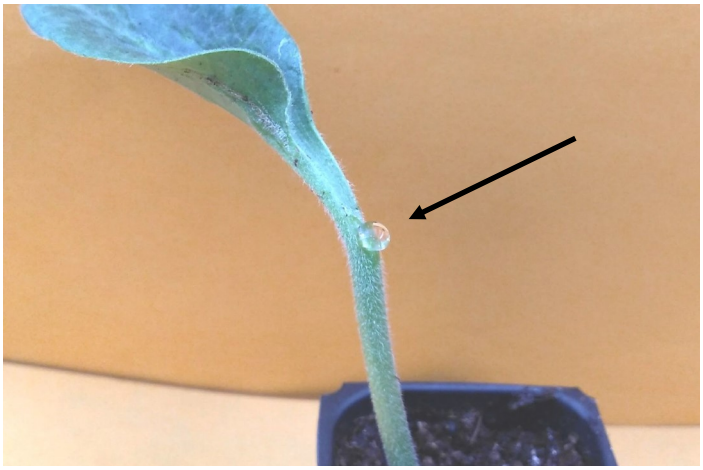

Figure 1. A water bubble will form from the cut plant stem if the seedling is too turgid at the time of grafting. (*Photo by Pinki Devi*)

To achieve uniformity in both germination and growth, planting dates must be optimized for both the scion and the rootstock. Seed more plants than necessary so you have a greater selection when matching stem diameters. Also, it is rare to get 100% graft survival for watermelon, so graft at least 20% more plants than needed. Adjust this rate as appropriate for graft survival in your production system.

Water both rootstock and scion plants 12–24 hours before grafting. Do not water plants immediately before grafting, unless they are wilted. If seedlings are too turgid, a water bubble will form when the plants are cut (Fig. 1), and this bubble will prevent a tight graft union from forming. Use only clean, sharp razor blades for grafting, and wash your hands with anti-bacterial soap or hand gel before working on the plants, or use latex-type surgical gloves.

While there are many tools that can be used for cutting vegetable plants for grafting, the double-edged razor blade snapped in half is most commonly used as it has the thinnest sharpest blade and is the least expensive (Fig. 2). If reusing grafting clips, wash them in warm, soapy water, sterilize them by soaking for 1 minute in a 10% bleach solution, and rinse them under tap water. Allow the clips to air-dry before reuse. Fill a spray

bottle with tap water in order to mist plants frequently during grafting.

A clean area such as work bench, with no direct sunlight is required for grafting. Grafting is commonly done in a greenhouse, but a shaded area may be needed so that temperature is 21-23 °C (70-73 °F). Do not graft near a fan to protect the plants from water loss and avoid disturbance to the graft union. If you are using a healing chamber, spray the inner surfaces of the chamber with water and add a thin layer of water to the chamber floor a few hours before grafting to raise the relative humidity to about 95% within the chamber. Do your grafting early in the morning or late in the day to avoid water stress and drying of the cut surfaces.

Grafting techniques

1. One-cotyledon grafting

The one cotyledon grafting method is by far is the most popular method used for watermelon and melons in Korea, Europe and North America. This method is also known as the splice graft, and was originally developed by Japanese engineers for use with automated grafting. Due to the procedure's simplicity and speed as well as the relatively low rate of rootstock regrowth (2-3%), it has become the most commonly used manual grafting method in Eu-

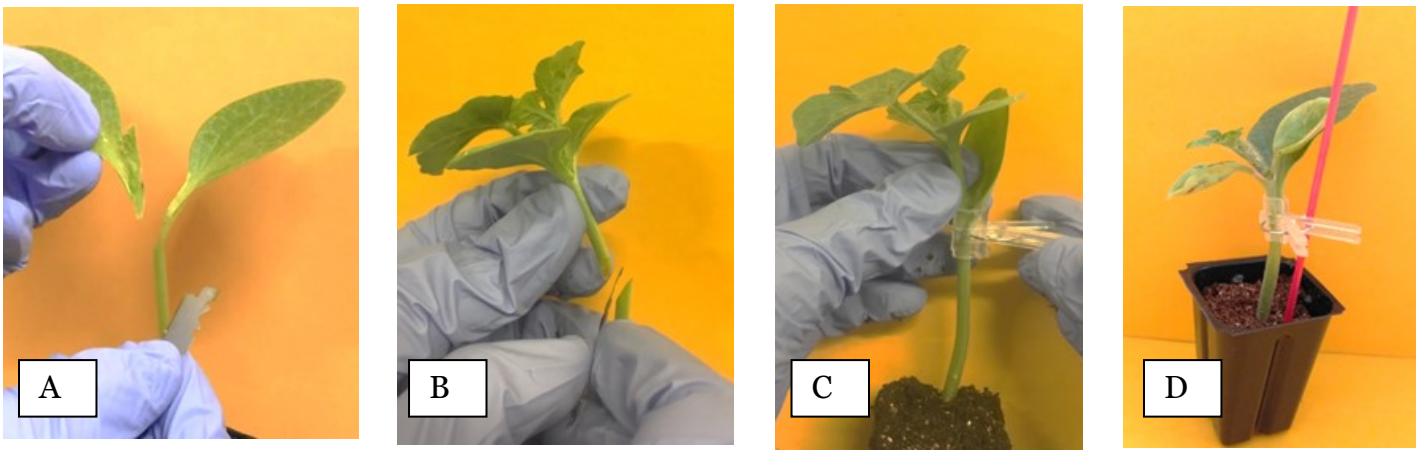

Figure 3. One cotyledon grafting: (A) cut the rootstock at a 60° angle with one cotyledon remaining on the plant; (B) cut the scion at a 60° angle below the cotyledons, where its diameter matches that of the rootstock; (C) join the two cut stems together; and (D) secure with a grafting clip. (*Photos by Pinki Devi*)

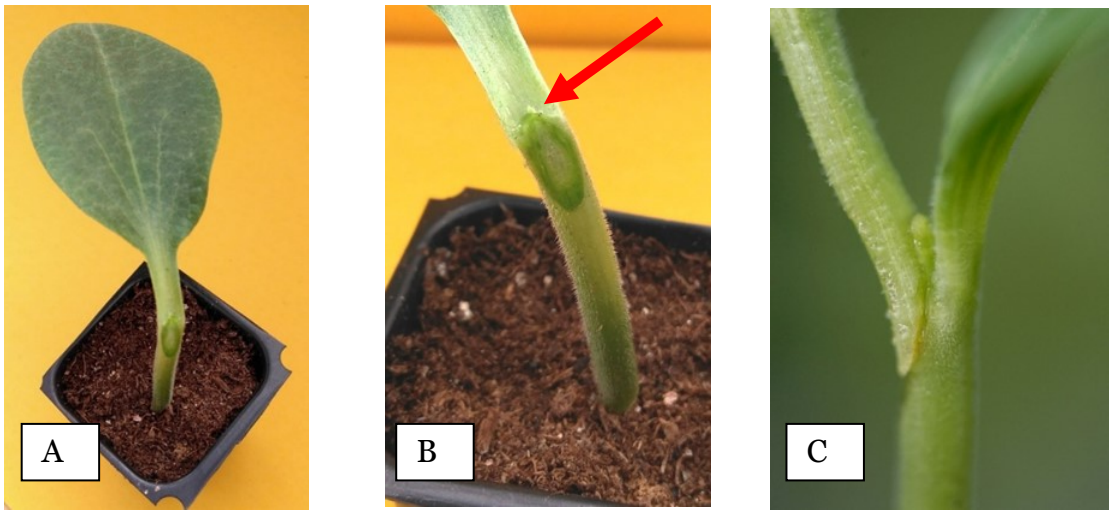

Figure 4. (A) & (B) The meristem tissue lies below the axillary bud at the base of the cotyledon; (C) if it is not completely removed, the rootstock will regrow. (*Photos by Pinki Devi*)

rope. Grafting machines can be used to graft watermelon and melons using this method, but high initial cost for equipment and strict requirement for uniformity of seedlings present obstacles for wider adoption of grafting automation of cucurbit crops.

Rootstock seedlings should have one true leaf, and scion seedlings should have two true leaves. Cut the rootstock at a 60° angle (Miles et al., 2013) so one cotyledon remains and one is removed (Fig. 3). Cut carefully so as to keep the remaining cotyledon firmly attached to the rootstock stem. The angled cut should also remove the apical meri-stem in the remaining cotyledon (these are undifferentiated cells at the base of the axillary bud) (Fig. 4).

It is important to remove all of the apical meristem to prevent future shoot growth of the rootstock. To better ensure that all of the axillary bud tissue has been removed, swipe the corner of the razor blade back and forth two times over the meristem area. Cut the scion at a 60° angle below the cotyledons, where its diameter matches that of the rootstock. Bring the two cut stem surfaces together, and hold them in place with a grafting clip. Place a small plastic straw through the clip to provide support as needed (Fig. 5); do not use wood/bamboo as it will mold in the healing chamber. Mist the grafted plants with water before placing them in the healing chamber.

Advantages:

- The most simple and rapid technique for grafting watermelon.
- Grafting automation can be conveniently accomplished.

Disadvantages:

- Requires careful control of humidity, light, and temperature after grafting.
- High losses and possible diseases or physiological disorders may occur if the healing environment is not optimal.
- Some meristem tissue may remain in the rootstock, requiring removal later in the production cycle.

2. Hole insertion grafting

The hole insertion method is the most widely used method for watermelon and melon grafting in China and Japan because it tends to have a high success rate with relatively minimal management during the healing period. Rootstock seedlings should have one small true leaf, and scion seedlings should have just the cotyledons or the first true leaf just emerging. The diameter of the scion stem must be smaller than

Figure 5. Place a small plastic straw through the clip to provide support. *(Photo by Pinki Devi)*

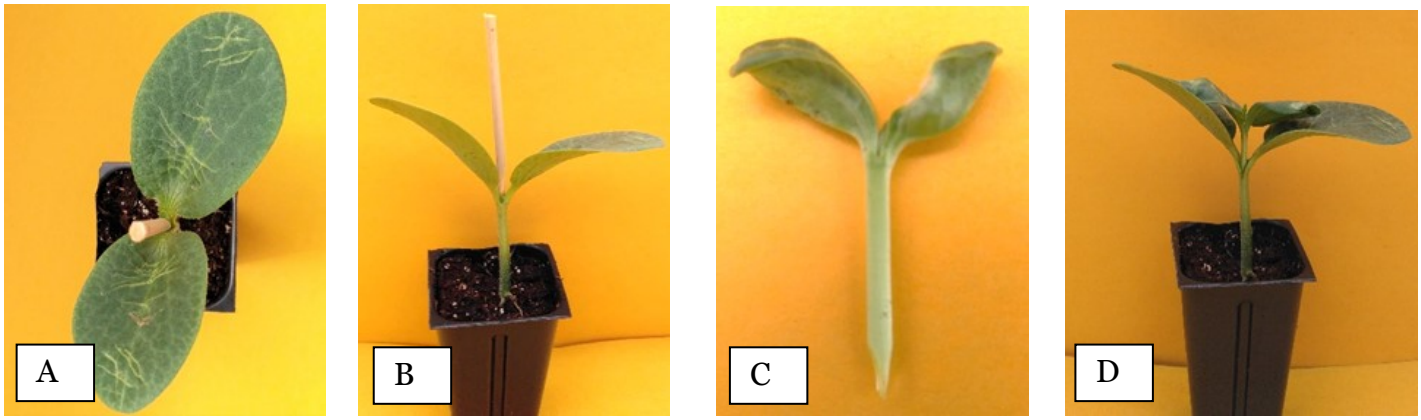

Figure 6. (A) & (B) Create a hole in the rootstock that removes the cotyledons and damages the meristem tissue; (C) cut the scion at a 45° angle on two sides to form a wedge; (D) insert the scion into the rootstock. (*Photos by Pinki Devi*)

the diameter of the rootstock stem so that the scion can be inserted into a hole made between the two cotyledons of the rootstock. Because seedlings can grow quickly in a day or two at this young growth stage, this grafting method has a short grafting window and timing of planting rootstock and scion seeds is critical.

With a pointed probe, remove the true leaf, the apical meristem, and the axillary buds from the topmost growing point of the rootstock plant (Fig. 6). It is important to remove all of the apical meristem and the axillary buds to prevent future shoot growth of the rootstock. Japanese grafters commonly use a bamboo probe as it is more effective at creating the hole and removing the apical meristem (Fig. 7). Use the probe to create a hole in the top of the rootstock where the tissue was removed; leave the probe inserted in the growing point while cutting the scion.

Figure 7. Wooden probe commonly used by Japanese farmers for hole insertion grafting. (*Photo by P. Devi*)

Cut the scion below the cotyledons at a 45° angle on two sides to form a wedge and insert it into the rootstock as the probe is removed. Oftentimes, the scion cotyledons are positioned to avoid overlap with the rootstock cotyledons, however, this is not necessary as long as the cut surfaces are oriented so that they are in good contact. It is not necessary to use a grafting clip to hold the two plants together. Mist the grafted plants with water and place in healing chamber.

Advantages:

- A grafting clip is not essential, which saves time and labor involved in collecting grafting clips after healing.
- Tends to have a high success rate.
- Maximizes the contacting surface area between rootstock and scion which helps create a strong graft union.

Disadvantages:

- Requires slightly more skill than most other grafting techniques.
- It may require more time to graft than some of the other grafting techniques depending on the grafter's skill and the grafting operation.
- Regrowth of the rootstock will occur if not all the meristem tissue has been removed.
- In some cases, internal rooting of the scion through the pith of rootstock may occur, especially when the scion stem is not oriented in the rootstock stem properly.

3. Approach (tongue) grafting

The approach grafting method is popular for small-scale grafters who are grafting up to a few hundred plants. It is slower than other methods but it has a high success rate. However, when grafting melon plants using melon (*C. melo*) rootstocks, it may be challenging to use the approach grafting method because melon rootstocks tend to have thin stems; therefore, it is easier to use squash rootstocks that have thicker stems for melon grafting using this method.

Both rootstock and scion should have one or two true leaves and similar stem diameter. Remove the rootstock and scion seedlings from the seedling trays and cut a 45° downward slit

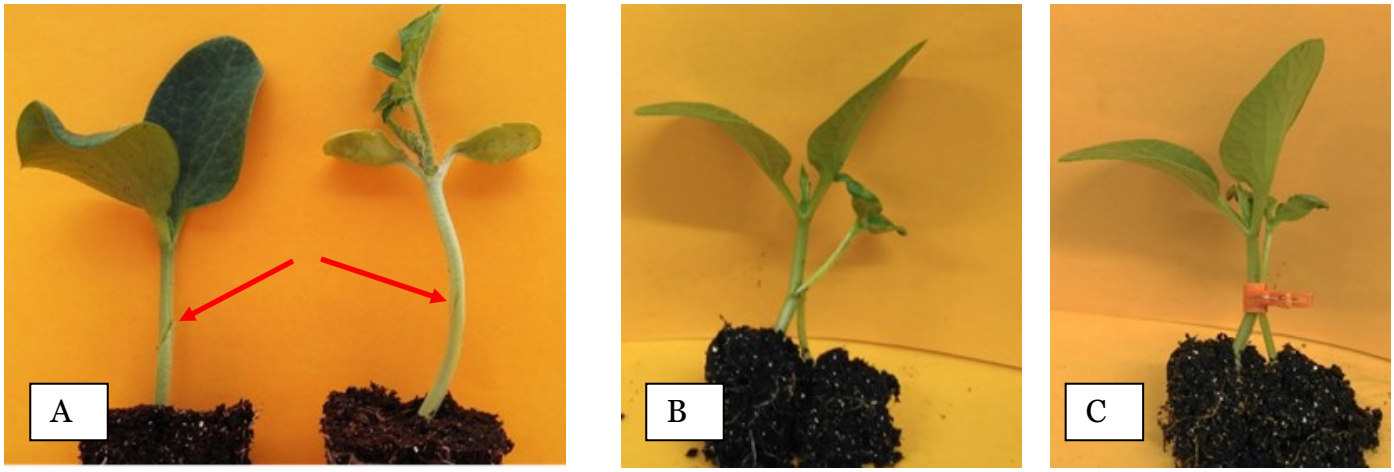

Figure 8. (A) Cut a 45° downward slit halfway through the rootstock stem below the cotyledons, and cut an identically angled upward slit in the scion stem; (B) bring the two cut stems together so the plants are joined; (C) attach a clip to secure the plants. (*Photos by Pinki Devi*)

Figure 9. A larger (left) and smaller (right) chamber used to heal grafted vegetables. (*Photos by Carol Miles*)

halfway through the rootstock stem below the cotyledons, and cut an identi-cally angled upward slit in the scion stem (Fig. 8). The angle and location of the cuts must be relatively precise so the scion can be placed on top of the rootstock. Join the two cut stems together and attach a clip or securely wrap the joined stems in plastic wrap or parafilm. Place the joined plant in a transplant tray or small pot that is just large enough to fit both rootballs. Mist the plant with water and place it on a greenhouse bench. Water the plant as needed. Cut off the top of the rootstock 5 days after grafting. Wait 7 days (12 days after grafting), then cut off the bot-tom portion of the scion.

Advantages:

- A relatively simple technique.
- A high humidity and low light environment is not required for successful healing of the graft union; a shaded greenhouse environment is sufficient.
- There is no shoot regrowth from the rootstock.

Disadvantages:

- Relatively slow grafting process.
- Requires more space for grafted transplants on the greenhouse bench.
- Not suitable for rootstock with hollow stems.

Figure 10. Healed watermelon plant with regenerated roots. (*Photos by Yuan Liu*)

Figure 11. Rootstock regrowth (squash-like leaves) from grafted watermelon (left) and melon (right) plants. (*Photos by Carol Miles and Xin Zhao*)

• It can be challenging when rootstock and scion plants look similar, making it difficult to distinguish between rootstock and scion seedling after grafting.

Healing chamber regime

Following grafting, it is critical to follow a successful graft healing regime (Johnson and Miles, 2011). Whatever healing chamber structure you use, it is necessary to control the humidity and temperature during the healing period, especially during the first 48-72 hours after grafting. After the critical 48-72 hours, gradual reduction of humidity and an increase in the amount of light is important for graft healing and disease prevention.

The following healing schedule is based on the greenhouse grafting environment at Washington State University Northwestern Washington Research and Extension Center in Mount Vernon (Johnson et al., 2011), where the average greenhouse temperature is 78/70 oF day/night (26/21 oC), and the relative humidity is 45%-60% in the late spring, when grafting usually occurs. Your green-house or grafting environment may be different (higher or lower temperature and humidity), and you may need to adjust the exposure times for grafted plants so they are not stressed when introducing them back into the greenhouse environment. The key is to slowly acclimatize the grafted plants without causing permanent wilting, which will lead to plant death.

Place grafted plants in a pre-misted humidity chamber (Fig. 9). In the following schedule, Day 1 is the day of grafting, and all chamber openings are during the day.

• Day 1. Close plastic of healing chamber; cover chamber with black fabric.
• Day 2. Keep chamber closed and covered with black fabric.
• Day 3. Open the chamber add water to chamber floor if needed (should be wet) close the chamber, and fold the black fabric up and away from the front of the chamber.
• Day 4. Open chamber for 15 minutes, wet floor of chamber if needed, reclose chamber, fold black fabric half way up all sides of the chamber.
• Day 5. Open chamber for 30 minutes, wet floor of chamber if needed, reclose chamber, remove black fabric from sides but keep top covered.
• Day 6. Open chamber for 1 hour, wet floor of chamber if needed, reclose chamber, remove black fabric entirely.
• Day 7. Open the chamber for 3 hours, wet floor of chamber if needed, reclose the chamber.
• Day 8. Open chamber for 6 hours, wet floor of chamber if needed, reclose chamber
• Day 9. Remove plants from the chamber and water them.

If greenhouse conditions are very bright and hot (greater than 90°F / 32 °C), place plants in a shaded area in the greenhouse for a few days to transition them before fully exposing them to regular greenhouse conditions.

If the root excision method was used for rootstock seedlings, the grafted plants should have regenerated roots by the time they are removed from the healing chamber (Fig. 10).

Remove rootstock regrowth

For plants grafted with the one cotyledon or the hole insertion methods, scout plants in the greenhouse during and after the healing process, and remove rootstock regrowth as soon as it appears. It is more time efficient and effective to remove rootstock regrowth when grafted plants are very young, before they are placed in the field. As plants get older, rootstock regrowth can cause graft failure. Shoot regrowth from the rootstock competes with the scion for water and nutrients, and reduces yield.

Preparation for transplanting into the field

Although vascular connection is established between scion and rootstock at approximately 7 days after grafting, it takes at least 14 days after grafting for the graft union to fully heal. After

removing plants from the healing cham-ber, allow them to rest in the greenhouse for 5 days, and then move them outside for 3 days, so they can harden off before transplanting. Adjust this schedule as needed if plants appear stressed when they are introduced into each new environment.

Leave the clips on after transplanting to provide extra support, especially in windy conditions. Do not place grafted transplants into the field under very windy conditions. Grafting clips generally do not fall off the stem as the stem increases in diameter, so be sure to remove them 1-2 weeks after transplanting. Clean and sterilize grafting clips before you use them again (see Grafting Preparation above).
When transplanting, make sure the graft union remains above the soil line. If the graft union is buried, the scion will root into the soil and any advantages provided by the rootstock, such as resistance to soil-borne diseases, will be lost.

Field maintenance of grafted plants
Check the plants once a week the first month after transplanting to see if the rootstock has regrown (Fig. 11), and remove rootstock shoots immediately. It is very challenging later in the season to go through the field and remove rootstock suckers as the vines will be covering the field. However, many commercial rootstocks are extremely vigorous and will quickly overtake the scion variety if allowed to grow, so continued diligence for rootstock regrowth removal is recommended.

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