

How to cross wheat

The following section aims to illustrate how to perform crosses in wheat. There are many different techniques but we will only show our method here. A description of each part of the wheat plant can be found in the [Introduction to wheat](#), along with a [Glossary](#), to help with the specific vocabulary. Further directions developed by the John Innes Centre Germplasm Resources Unit can be found at <https://www.youtube.com/watch?v=XrrqLrxLXak&feature=youtu.be>; <https://www.youtube.com/watch?v=D7vF3hsh5Mg> and in the WISP course materials.

a) Materials:

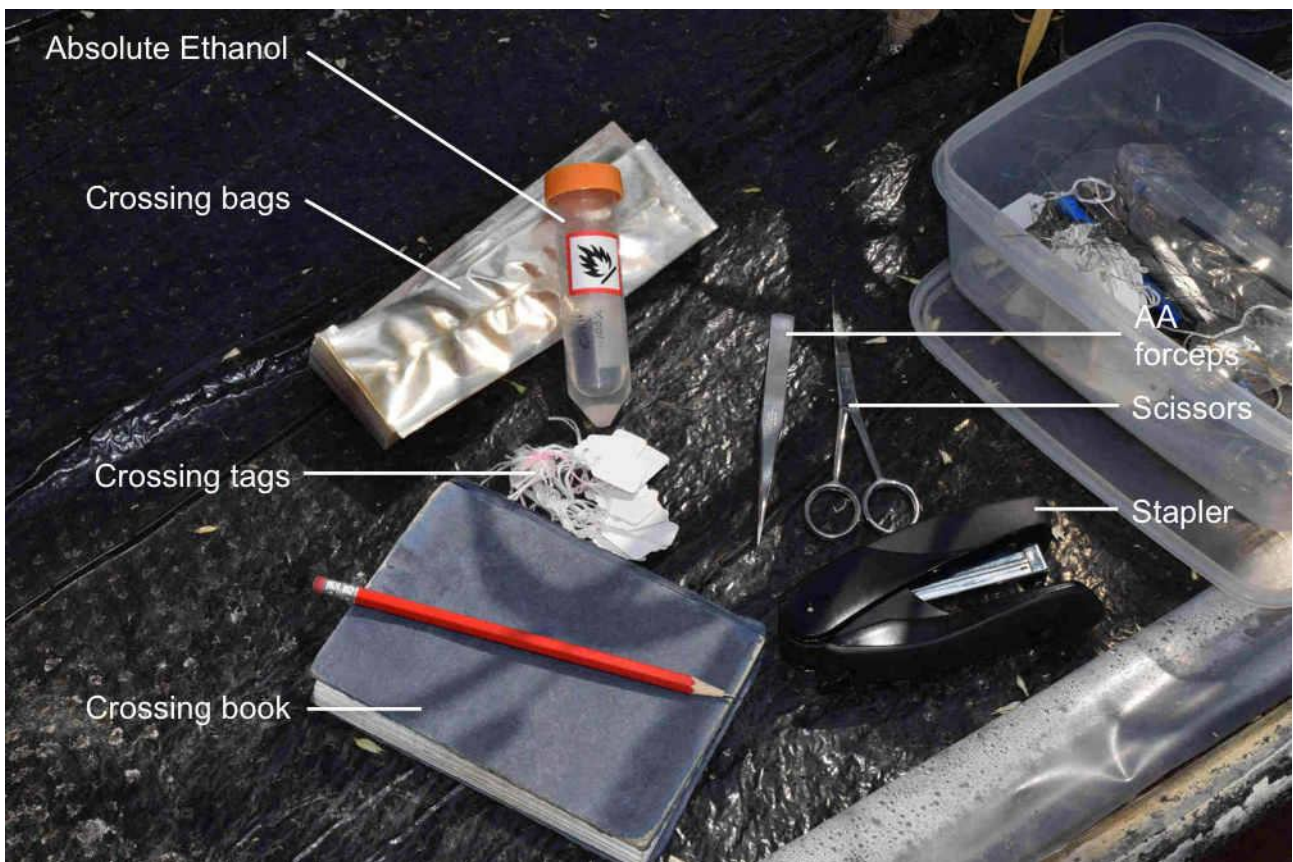


Figure 1. Wheat crossing kit

Figure 1 shows the basic toolkit for wheat crossing. The forceps need to be sharp enough to efficiently remove florets and anthers. Scissors are used to cut the florets during emasculation. Absolute ethanol sterilization of the forceps and scissors between each plant helps to avoid cross-pollination. After the emasculation as well as after making the cross itself the spike needs to be bagged to avoid any cross-pollination. The plants are labelled with crossing tags and crossing information is recorded in a small notebook that should be brought along to the glasshouse.

Knowing the heading stages of the cultivars to be crossed is essential to synchronise emasculating and pollination. Sowing sequential batches of the plant material ensures having a sufficient number of individuals concomitantly mature for these steps.

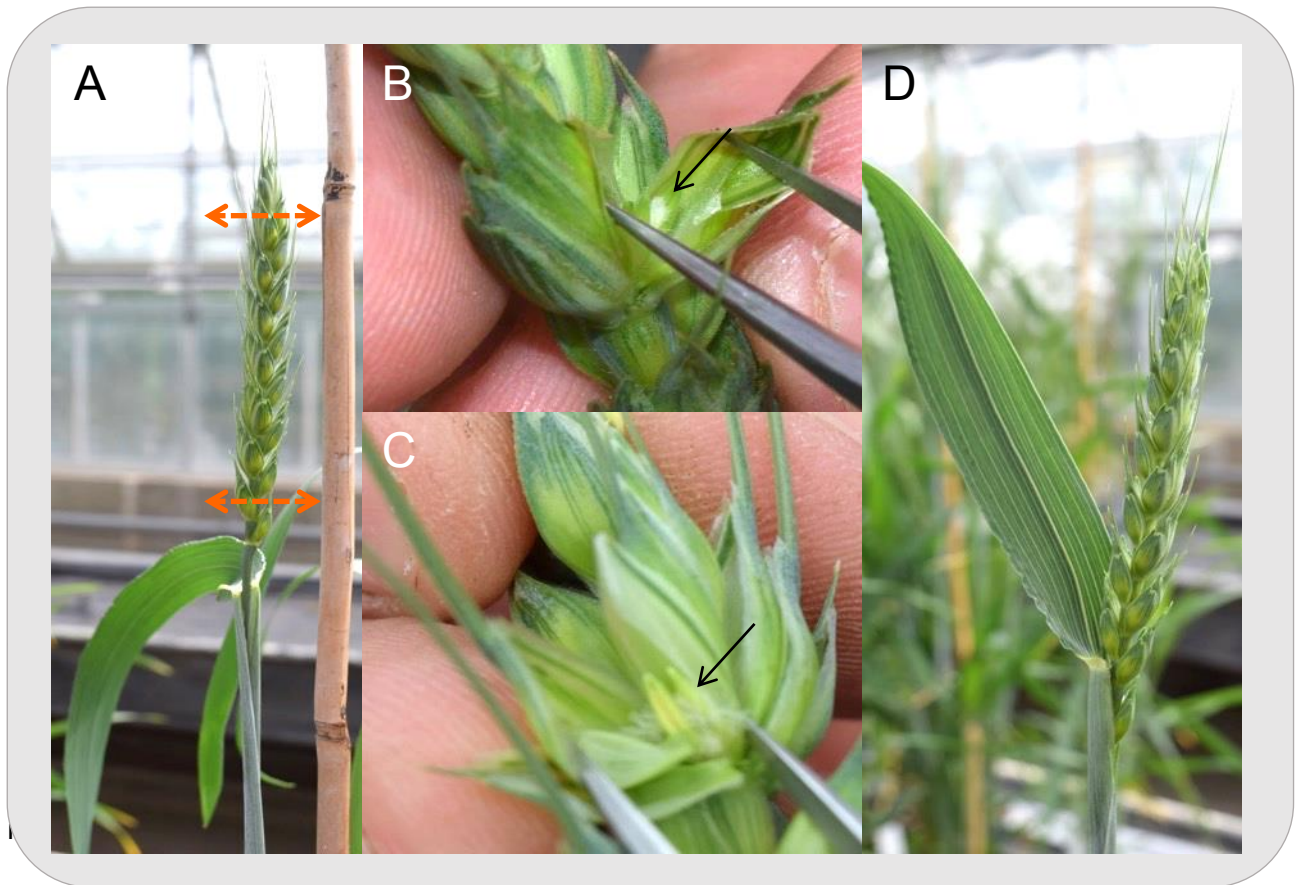


Figure 2. When to perform spike emasculating.

(A) The spike is fully emerged from the flag leaf and would thus be ready for emasculating. Red arrows indicate spikelets that will be removed before proceeding. (B) Black arrows point out a green anther and the un-developed stigma versus (C) slightly yellow anthers and feathered stigma, which is a too advanced stage for emasculating. (D) Depending on the cultivar (here Paragon), flowering may already occur before the spike is fully emerged from the flag leaf. It is thus important to verify the anthers' developmental stage.

Spikes should be ready to be emasculated at GS59 (see [Wheat development](#)), which corresponds to the complete emergence of the spike from the flag leaf (**Figure 2A**). However, depending on the cultivar, flowering could happen while the spike is not yet fully emerged from the flag leaf (**Figure 2D**). It is thus important to check the anthers' development: If the anthers are small and green with a tight stigma, emasculating can be performed; but if the anthers start to become slightly yellow, the hand heat could speed up their maturation and pollen could start spilling by the time the whole spike is being emasculated (**Figure 2B** and **2C**). Consequently, self-pollination may occur.

For emasculating, first remove spikelets located at the tip and base of the spike using forceps or scissors; these spikelets are quite asynchronous to the rest of the spike and also are frequently sterile (**Figure 2A**). Then remove the florets in the centre of each spikelet, which develop asynchronously to the outer florets (**Figure 3**). Cut across each spikelet leaving roughly two-third of it to make both anther removal and pollination easier (**Figure 4**).

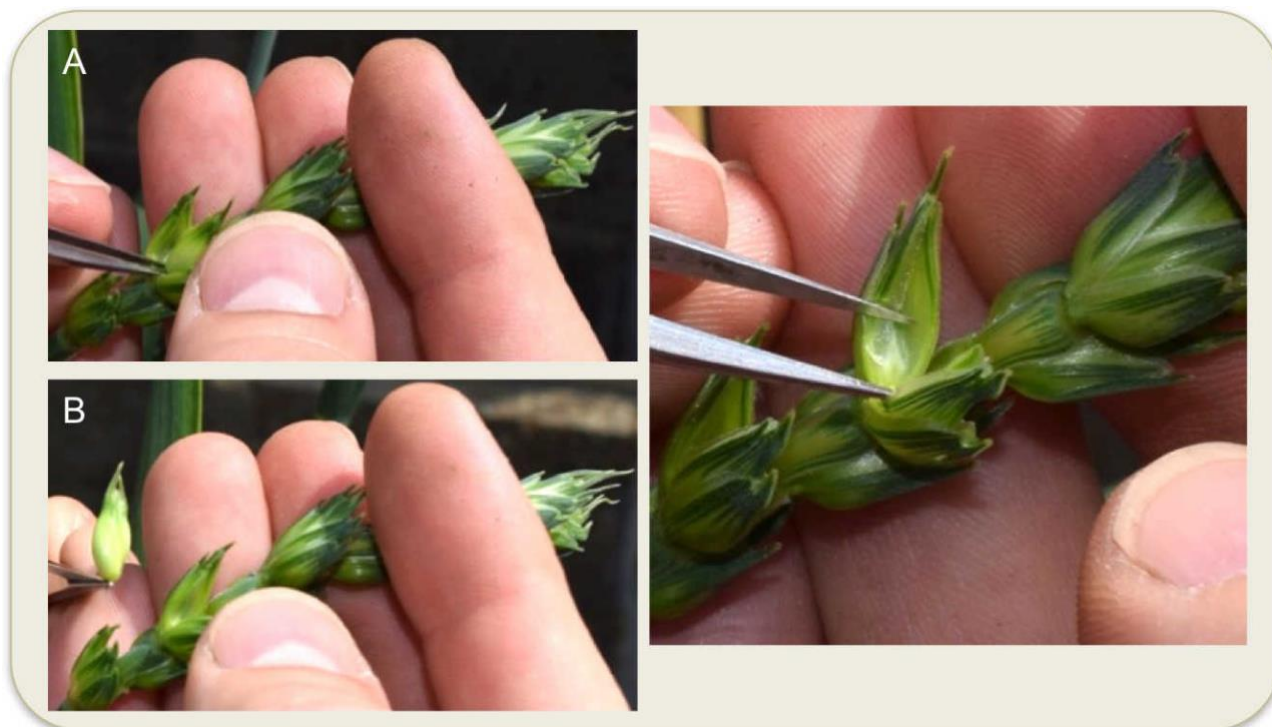


Figure 3 Central floret removal.

(A) Place the forceps at the base of the floret and (B) pull it out in one go. (C) Ensure that no anthers have been left in the centre of the spikelet.

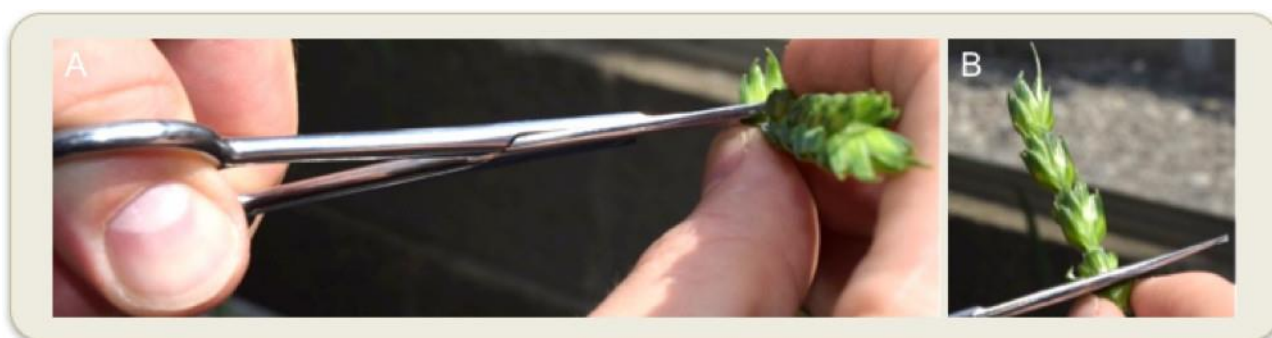


Figure 4. Cutting across each spikelet with fine pointed scissors will help anther removal and pollination.

Then remove the three anthers of each floret using the forceps. **There must be three anthers per floret** so count them carefully in order not to miss any of them as it could lead to self-pollination. Sometimes an anther might not develop properly; look for a small, white anther-like structure at the base of the ovary. The positions of the anthers are predetermined, so after a bit of training you should be able to know the anther locations automatically. In addition, make sure that the removed anthers are still green and that no pollen has come out. Remove the anthers by holding them by their tip and try to pull out the three at once but make sure to not damage or extract the stigma (**Figure 5**). Search for any remaining anthers when the whole spike has been emasculated (**Figure 6**). Anthers can slip off your forceps and fall back onto the spike without you realizing it!

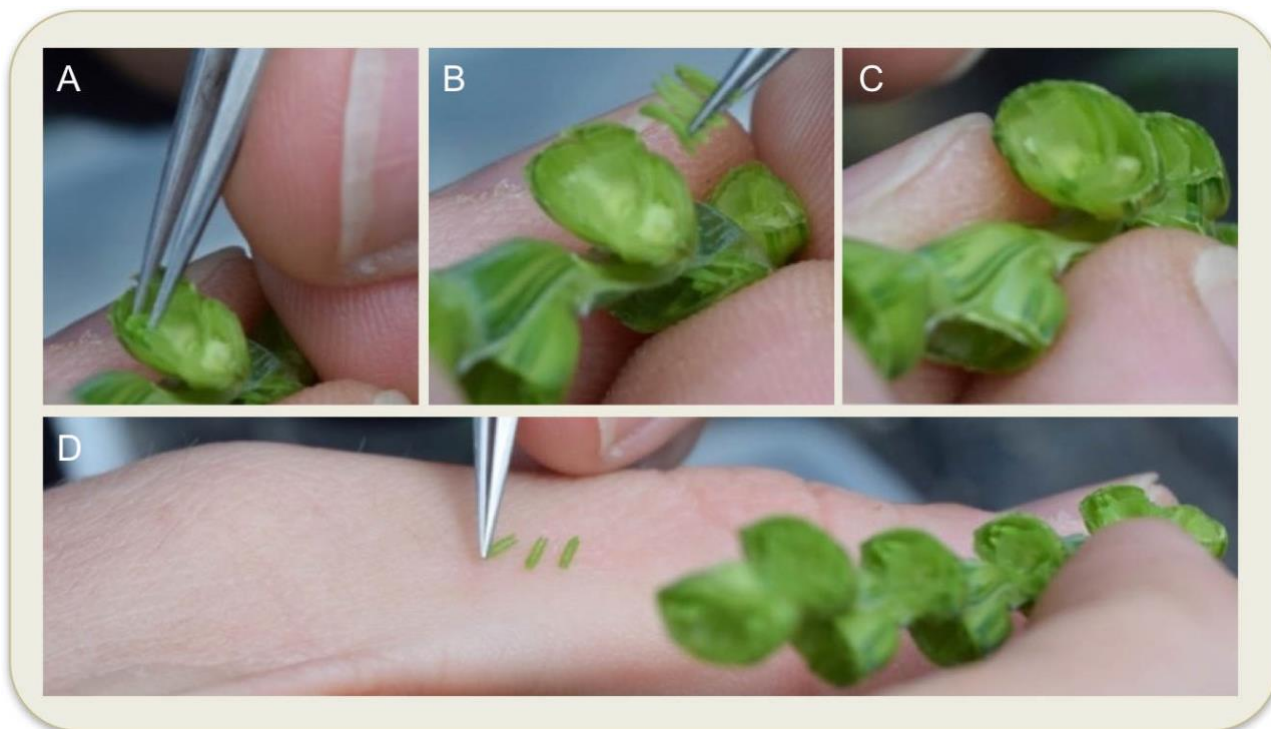


Figure 5. Anther removal.

(A) Hold all the three anthers by the tip to not damage the stigma and (B) pull them out in one go. (C) Ensure that no anther is left in the spikelet and (D) count the removed anthers. There must be three of them in each floret.



**Figure 6.
Emasculated ear**

Use a crossing tag to label the receptor plant (genotype, date, etc.) and record the information in the crossing notebook as well. Cover the emasculated spike with a crossing bag to avoid any cross-pollination and secure it with a tie; be careful to not damage the emasculated spike. To differentiate non-pollinated and pollinated donor, the crossing tag can be left hanging for the first and put into the bag with the spike for the latter.

c) Pollination:

Both pollen and stigma have to reach the proper developmental stage for an efficient pollination. Stigma receptiveness can be checked daily after emasculation; it is usually mature two or three days after emasculation. A mature stigma is 'fluffy'/feathered compared to an immature one which is tightly curled up (Figure 7).

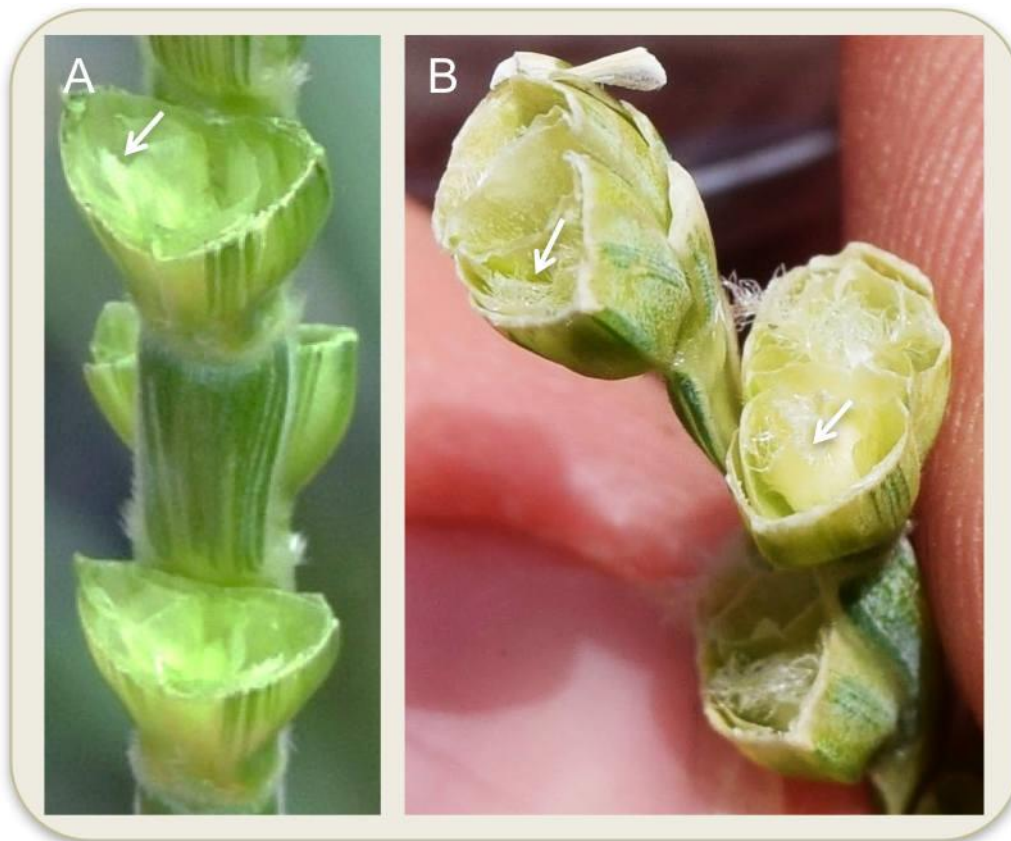


Figure 7. Immature stigma versus mature stigma.

(A) Immature stigma is tightly curled up and very small compared to (B) the mature one, which is feathery and fanned.

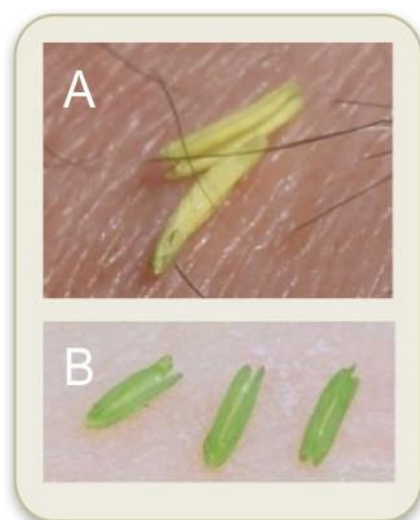


Figure 8. Extreme late and early developmental stages of the anthers.

(A) Anthers are clearly yellow and fat, they already have released their pollen. A little might remain but this pollen would be too old for a good pollination. (B) The anthers are green; they are too young so the hand heat would not be sufficient to advance them to the mature stage.

Pollen maturity is reached when the anthers start to turn yellow and be slightly rounded. Figure 8 illustrates anthers that have already released the pollen (A) and others that have not reached the right developmental stage yet (B). Pollen viability is measured in **minutes**; only fresh pollen will result in a successful cross!

Carefully remove the anthers from the floret and place them on the base of your thumb (Figure 9). The warmth of your hand will trigger the anthers to reach maturity; the anthers will start to inflate and

then split open at the top, potentially releasing some of the pollen on the hand. Having different batches of pollen donors increase the chance of having mature pollen when the stigma is receptive.

These anthers can thus be used to pollinate the receptive stigmas of the receptor plant. Carefully hold one anther by the tip and tap it inside the floret to drop the pollen. Usually the pollen from one mature anther can be used to pollinate two to three florets (**Figure 10**). If a lot of donor pollen is available, it might be useful to pollinate again the day after to ensure that pollen will be in contact with the stigma. If not, try to aim at the receptive part of the stigma to save as much pollen as possible.

Once the whole spike has been pollinated, record the date of the pollination on the crossing tag with the name of the donor (write it down in the notebook as well) and bag the ear with the tag inside this time. Use a tie to seal the bag (**Figure 11**).

After approximately seven days you should be see the ovary enlarging; this means that your cross has worked.

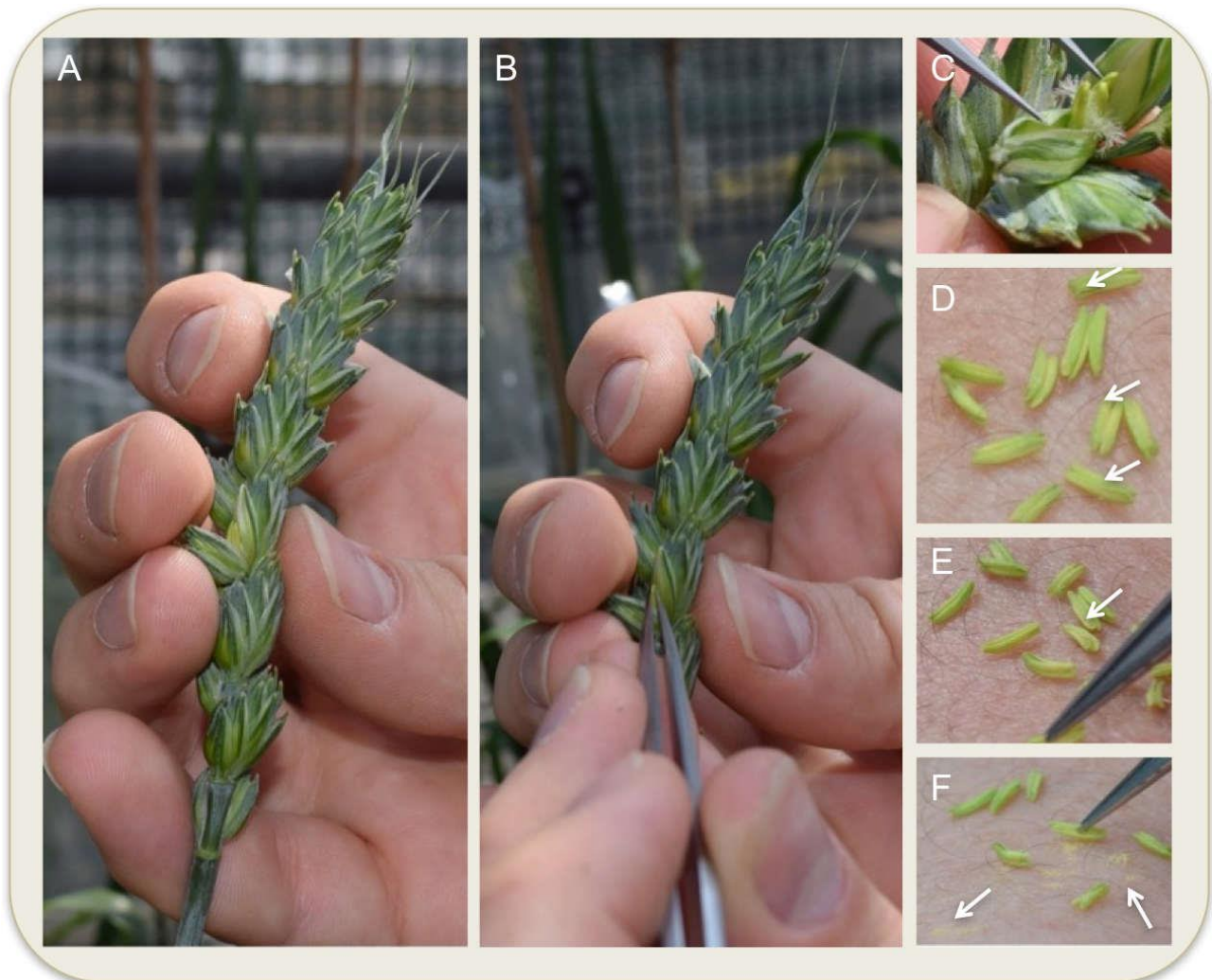


Figure 9. Mature anthers.

(A, B and C) look for mature anthers inside the florets, they usually started to turn yellow and are slightly inflated. Remove them from the floret with the forceps and place them at the base of the thumb so that the warmth of your hand will trigger their maturation: (D) The two sections of the

anthers start to separate from each other, (E) then they split open and the pollen is visible inside. (F) Finally they start spilling the pollen onto the hand and are ready to be used to pollinate the receptor plant.

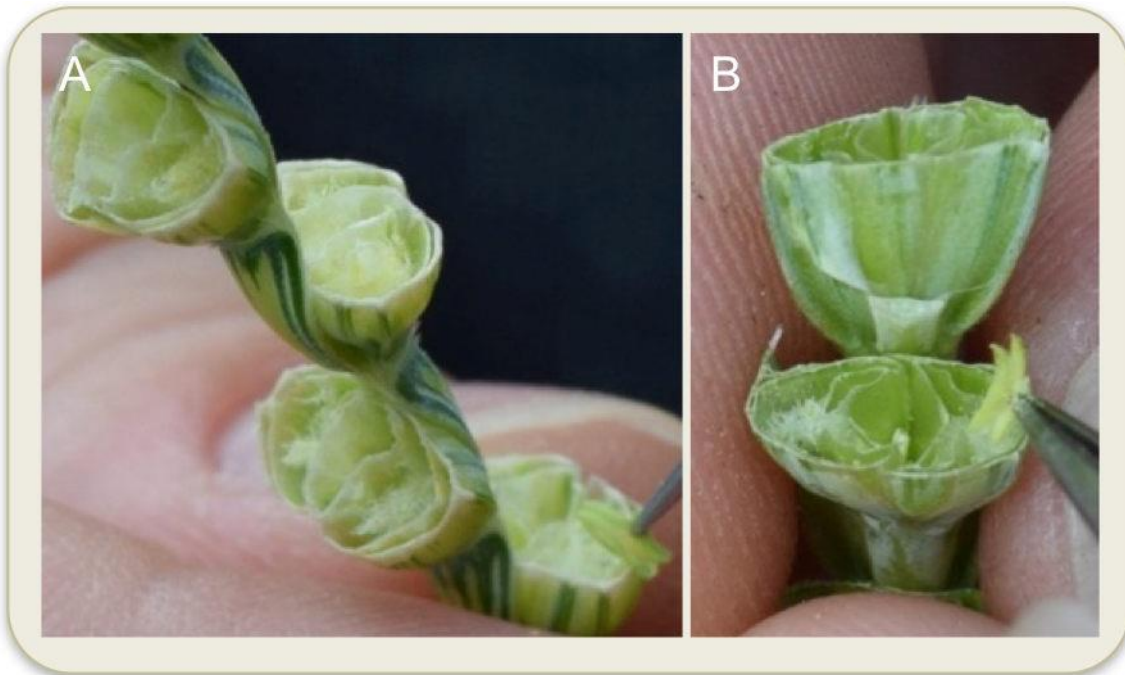


Figure 10. Pollination.

(A and B) Hold the mature anther with the forceps and tap it inside the floret to drop the pollen. Carefully use the anther to mix the pollen into the stigma.



Figure 11. Pollinated receptor plant bagged with the crossing tag inside

d) Alternative Pollination

It is possible to accelerate and enhance your crossing output by altering the traditional pollination process. This can be done using one of two following methods, the 'detached spike' pollination or the 'attached spike' pollination. Both methods will undoubtedly save time as there is no requirement to search for individual anthers at the correct stage. They can also achieve high seed set levels on a par with traditional pollination methods.

Detached Spike Pollination

Select a donor parent spike where anther extrusion has occurred on the spikelets in the middle of the spike (**Figure 12 A**). This indicates that the spike has begun anthesis and pollen will be available. Snip the spike from the plant leaving a 5-10cm section of peduncle and trim the spikelets at a 45° angle, exposing the anthers to the warmth and light (**Figure 12 B**). Leave the spike in a 15ml falcon tube with water in full sun or elevated to the lights if they are on. After a few minutes additional anthers should have extruded from the spikelets and these will be full of fresh pollen (**Figure 12 C**).



Figure 12. Preparing donor spike for detached spike pollination.

(A) The central spikelets of the spike to be used for donor pollen should show signs of dehiscence in the form of extruded anthers.

(B) Cut the spikelets at a 45° angle to expose the anthers to the warmth and light.

(D) After a few minutes additional anthers showing dehiscence will be visible (white arrows), these are ready to use to pollinate your emasculated spike.

Cut the top off the crossing bag and gently insert the inverted spike being careful not to disturb the spike (**Figure 13A**). Once the spike is in the bag twizzle it around the emasculated spike and the pollen will fall. Look for pollen on the side of the crossing bag as a sign that the emasculated spike has been pollinated (**Figure 13B**). The spike can be removed and left in the falcon tube again if

required. It is also possible to just snip one side of the ear at a time so that it can be used twice. In cold or cloudy conditions, the anthers do not ripen as quickly and therefore this method may not be as beneficial.

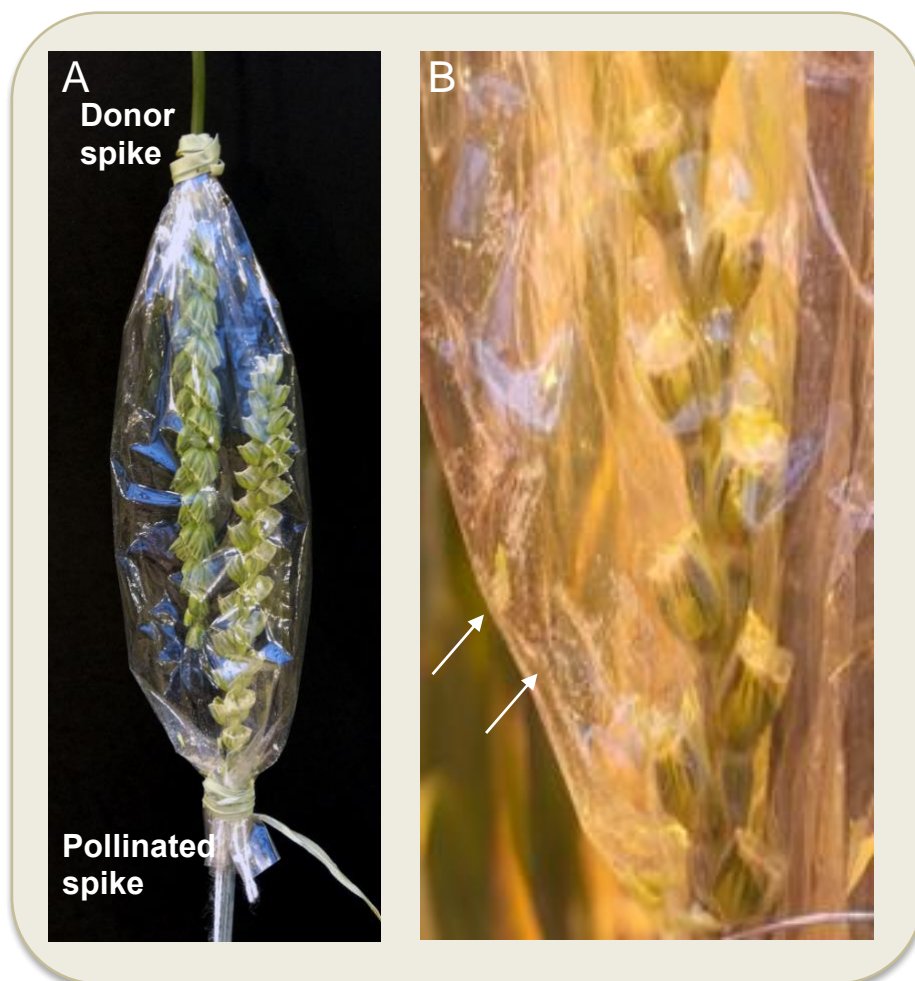


Figure 13. Detached spike pollination.

(A) When additional anthers are visible in the donor spike (Figure 13C), carefully invert the spike and insert it in to the top of the crossing bag, disturbing the spike as little as possible. Twizzle the spike to enable pollen to fall on to the emasculated spike.

(B) Pollen should be clearly visible on the inside of the crossing bag (white arrows).

Attached Spike Pollination

Alternatively, it is possible to pollinate without cutting the spike from the plant. This is perhaps more challenging as it requires the spikes to be of equivalent height, with the pollen donor slightly higher to allow pollination of the terminal spikelets. However, as the spike is still attached to the plant the emasculated spike will be subject to multiple waves of pollen. This method can be especially useful if you will be absent for 2-3 days and pollination will need to occur in that time.

Select a spike at a similar stage to the spike requiring pollination. As with the previous method trim the spikelets at a 45° angle and simply insert the pollen donor in the bag with the emasculated spike. As the donor spike progresses through anthesis pollen will fall on to the emasculated spike (**Figure 14**). Slight agitation of the plants by a gentle tap or breeze may assist this procedure. Again, visible pollen on the inside of the bag will suggest that pollination has taken place.



Figure 14. Attached spike pollination.

Insert the trimmed pollen donor spike in the bag with the emasculated spike. As anthesis progresses pollen will fall on to the stigmas of the emasculated spike.