



# **PHYTOTOXKIT**

## **Test procedure**



1

**RAPID METHOD FOR DETERMINATION OF  
THE WATER HOLDING CAPACITY (WHC) OF  
TEST SOILS**



2

SIEVE AIR-DRIED SOIL THROUGH A SIEVE WITH A 2mm MESH  
TO ELIMINATE ALL COARSE MATERIAL





### 3

- FILL A GRADUATED 50 ml CYLINDER TO THE MARK WITH PURE (distilled or deionized) WATER
- FILL A 100 ml BEAKER WITH 90 ml SIEVED SOIL





4

POUR THE 50 ml WATER IN THE BEAKER WITH THE SOIL



**5**

MIX THE WATER THOROUGHLY WITH THE SOIL TILL THE SOIL IS  
COMPLETELY WATER SATURATED





6

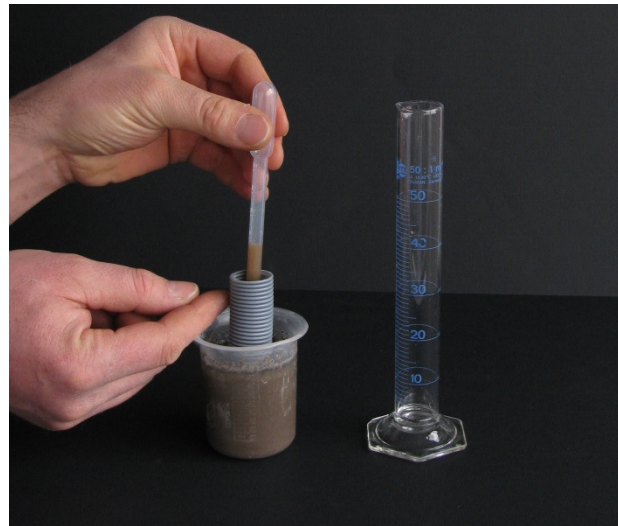
WAIT FOR THE SOIL/WATER MIXTURE  
TO REACH EQUILIBRIUM, LEADING TO  
A WATER SATURATED SOIL PHASE  
AND A LAYER OF WATER ON TOP





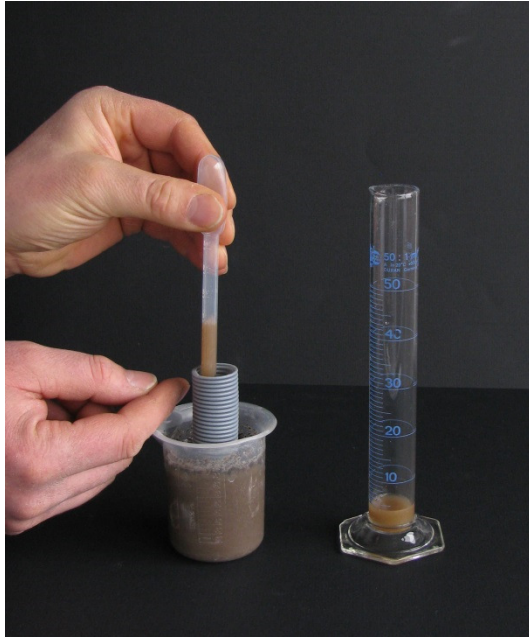
7

- TAKE THE MICROSIEVE CYLINDER AND BRING IT DOWN VERTICALLY IN THE BEAKER WITH SOIL AND WATER, TILL THE BOTTOM OF THE SIEVE TOUCHES THE SUPERNATANT
- GENTLY LOWER THE MICROSIEVE CYLINDER A LITTLE FURTHER DOWN, SO THAT IT GRADUALLY FILLS WITH SUPERNATANT



8

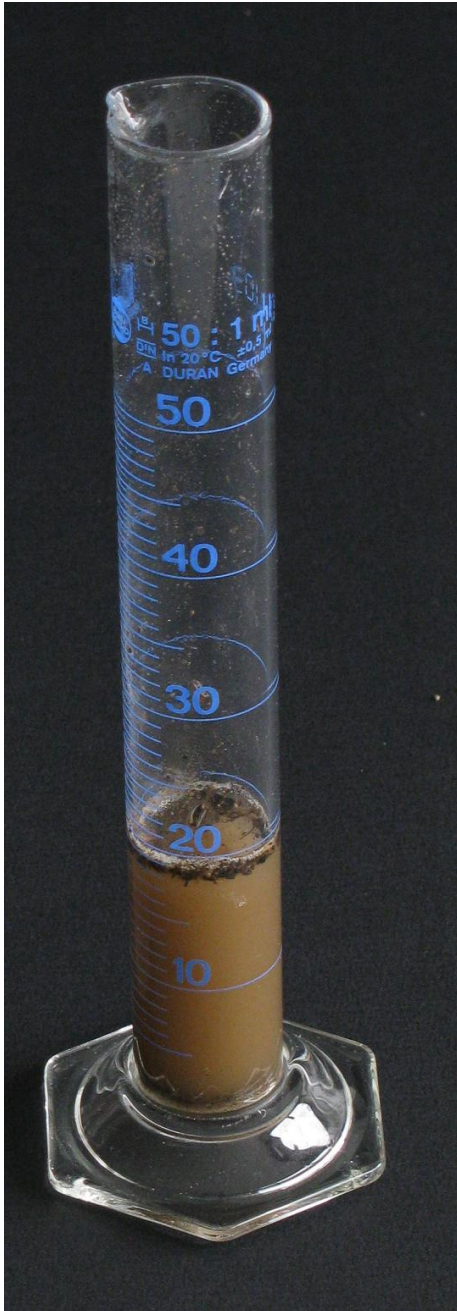
SUCK UP THE SUPERNATANT FROM THE MICROSIEVE WITH THE WIDE MOUTH MICROPIPETTE AND TRANSFER IT INTO THE GRADUATED CYLINDER



9

REPEAT THE FORMER OPERATIONS, PUSHING EACH TIME THE MICROSIEVE CYLINDER A LITTLE FURTHER DOWN INTO THE WATER/SOIL MIXTURE, UNTIL NO SUPERNATANT PENETRATES ANYMORE INTO THE MICROSIEVE





# 10

CALCULATE THE VOLUME OF WATER ( $V_{\text{sat}}$ ) NEEDED FOR COMPLETE HYDRATION OF THE TEST SOIL.

THIS VOLUME IS EQUIVALENT TO THE VOLUME OF WATER THAT HAS BEEN ADDED TO THE SOIL (= 50 ml) MINUS THE VOLUME OF SUPERNATANT WATER (S) RECOVERED IN THE GRADUATED CYLINDER

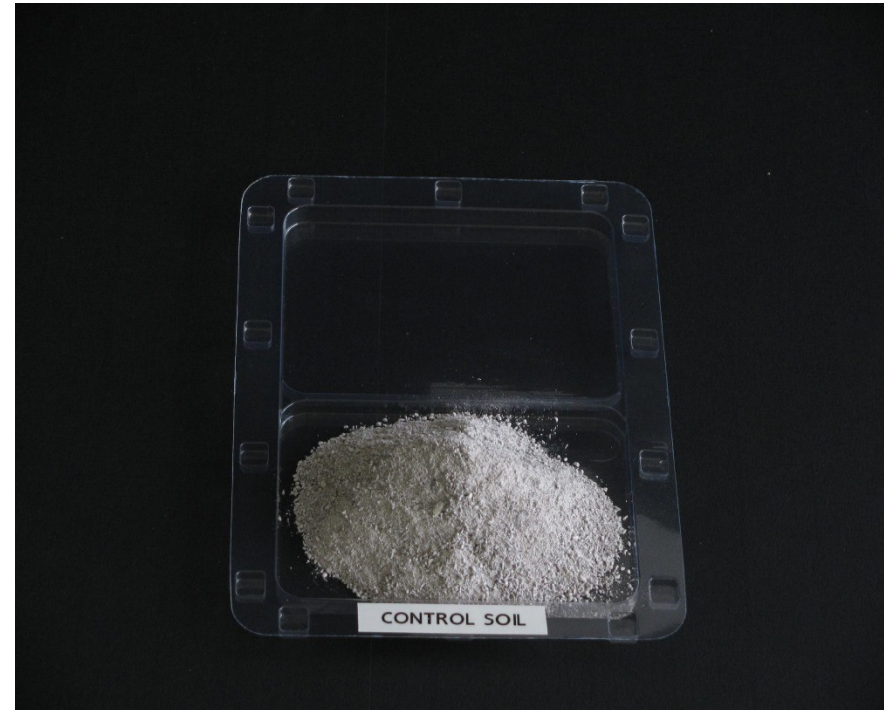
$$V_{\text{sat}} (\text{ml}) = 50 - S$$



# 11

**ADDITION OF REFERENCE SOIL AND TEST SOIL TO THE TEST PLATES  
AND HYDRATION OF THE SOILS**

**1. CONTROL SOIL**



# 12

POUR THE CONTENTS OF ONE BAG WITH REFERENCE SOIL (90 ml) IN THE LOWER COMPARTMENT OF A TEST PLATE





# 13

- TAKE A **50 ml** SYRINGE AND FILL IT TO THE **35 ml** MARK WITH PURE WATER  
(35 ml is the volume of water giving 100% water saturation of 90 ml reference soil)
- EMPTY THE CONTENTS OF THE SYRINGE BY DROPPING THE WATER SLOWLY OVER THE WHOLE SURFACE OF THE SOIL IN THE TEST PLATE



**14**

- WAIT A FEW MOMENTS FOR THE WATER TO HYDRATE THE SOIL COMPLETELY
- WITH THE AID OF A SPATULA, SPREAD THE WET SOIL EVENLY OVER THE TOTAL SURFACE OF THE BOTTOM COMPARTMENT OF THE TEST PLATE



**15**

FLATTEN THE SURFACE OF THE SOIL TO OBTAIN A LAYER OF UNIFORM DEPTH

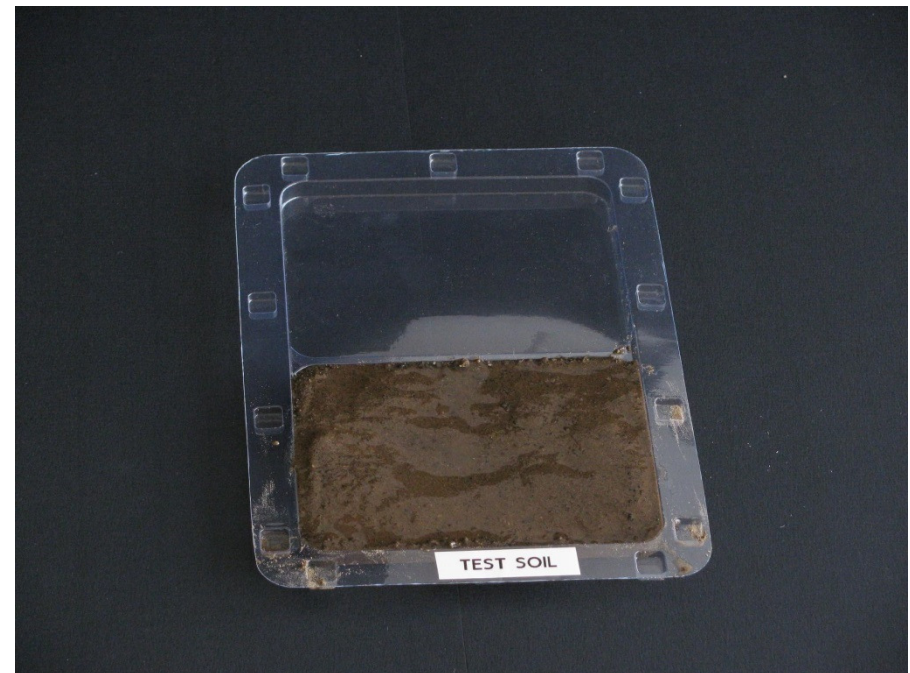




# 16

## 2. TEST SOIL

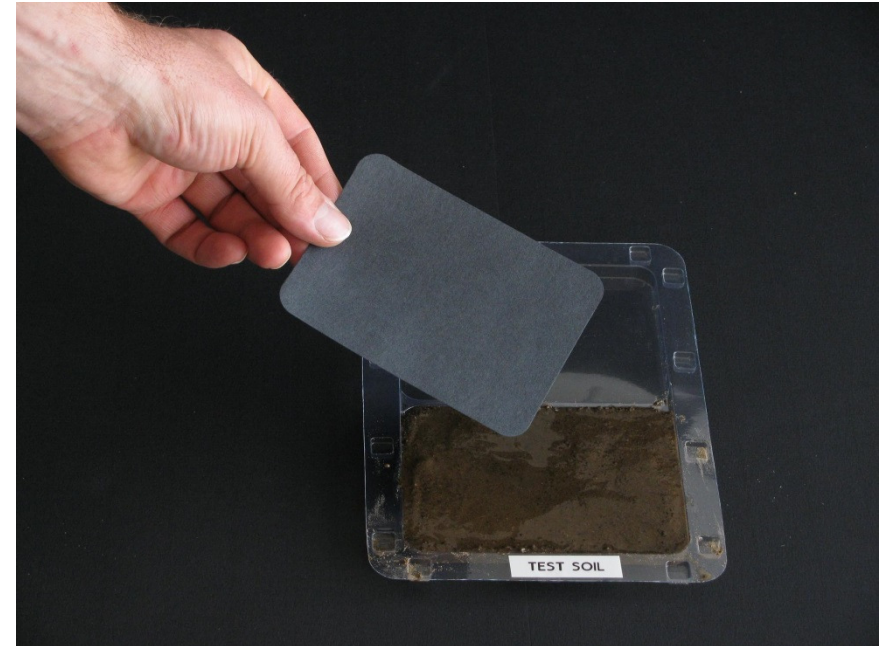
- FILL A **100 ml** BEAKER TO THE **90 ml** MARK WITH THE SIEVED TEST SOIL
- TRANSFER THIS VOLUME OF SOIL TO THE BOTTOM COMPARTMENT OF A TEST PLATE



# 17

- HYDRATE THE TEST SOIL WITH A VOLUME OF WATER EQUAL TO  $V_{sat}$  (see PICTURE 10)
- PROCEED FURTHER AS INDICATED IN PICTURES 14 AND 15 TO FLATTEN THE SURFACE OF THE SOIL



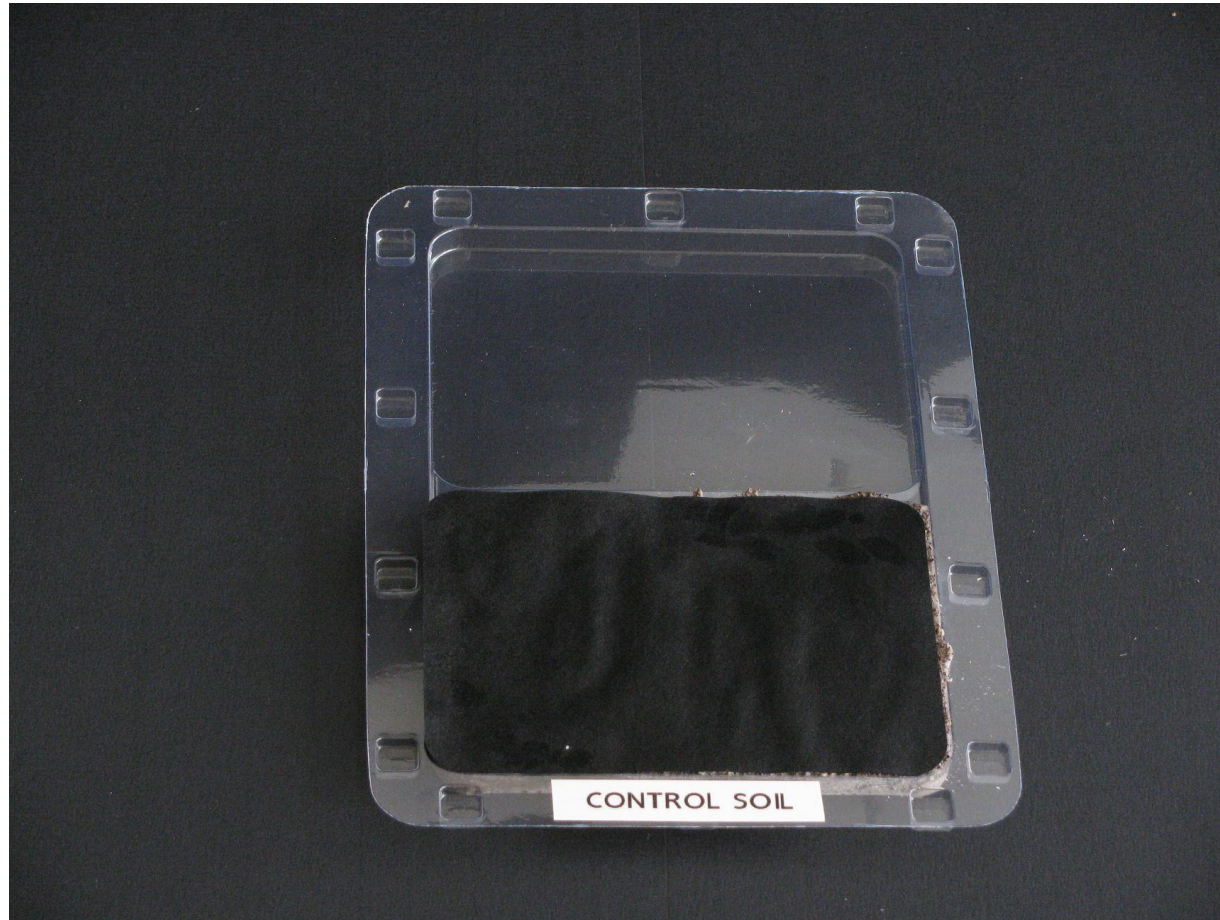


# 18

**PLACING OF THE FILTER PAPER AND SEEDS IN THE TEST PLATE**

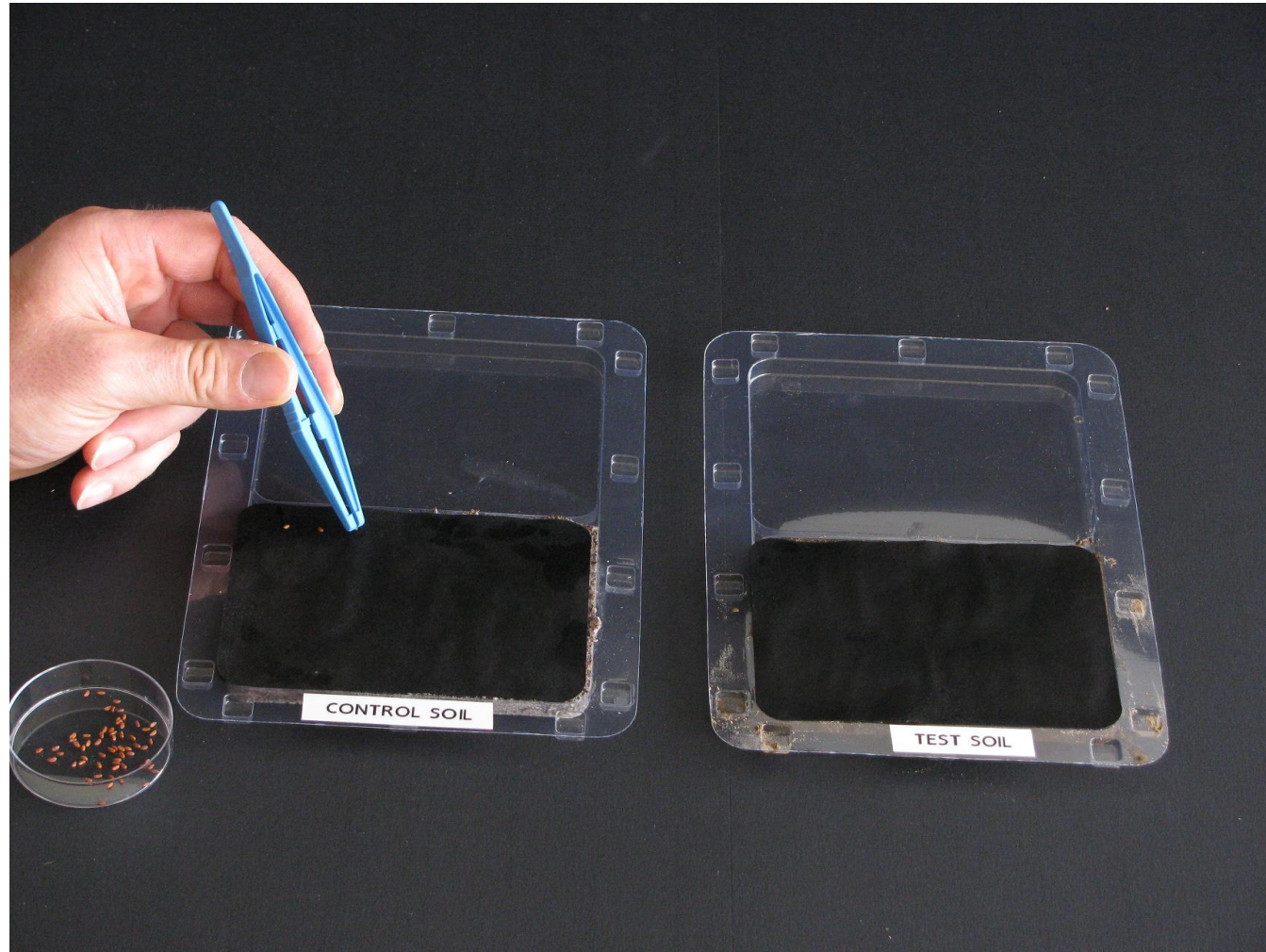
**1. PLACING OF THE FILTER PAPER**





**19**

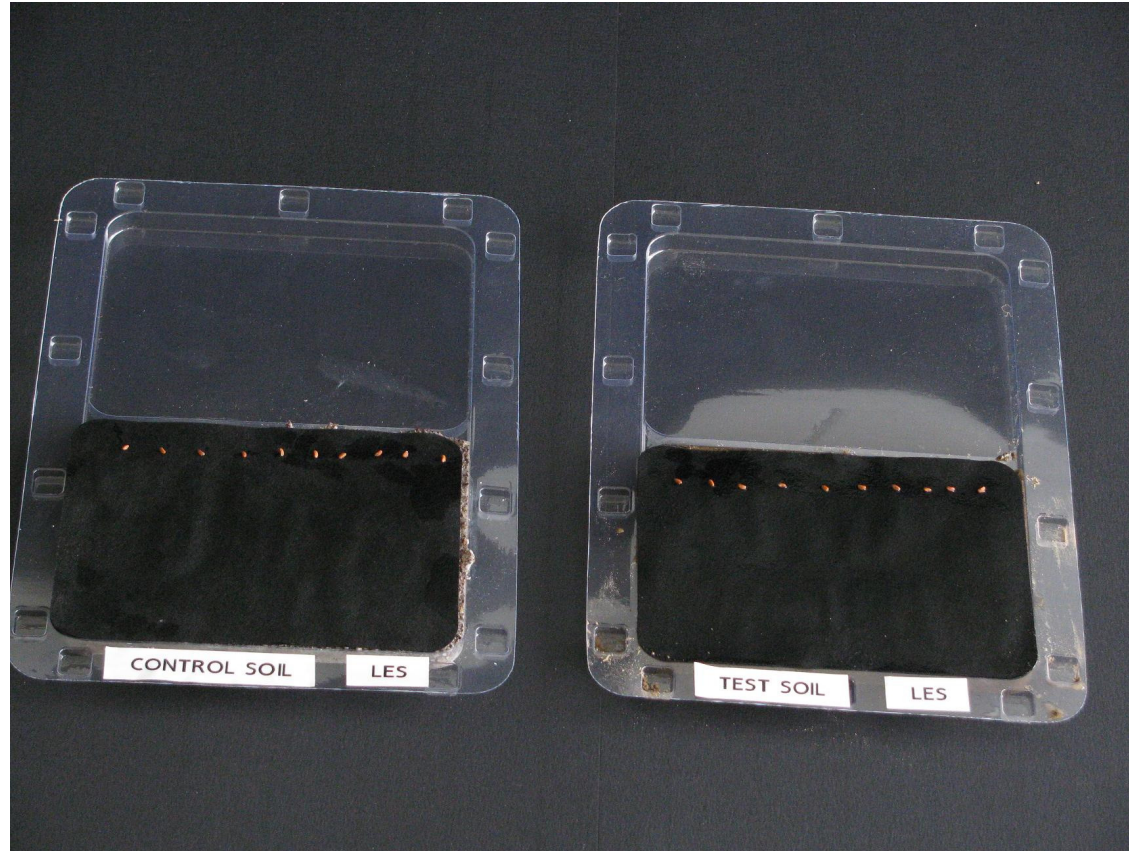
PUT A BLACK FILTER ON TOP OF THE HYDRATED (CONTROL AND TEST) SOILS IN ALL THE TEST PLATES AND WAIT UNTIL THE FILTER IS COMPLETELY WET



**20**

**2. PLACING OF THE SEEDS**

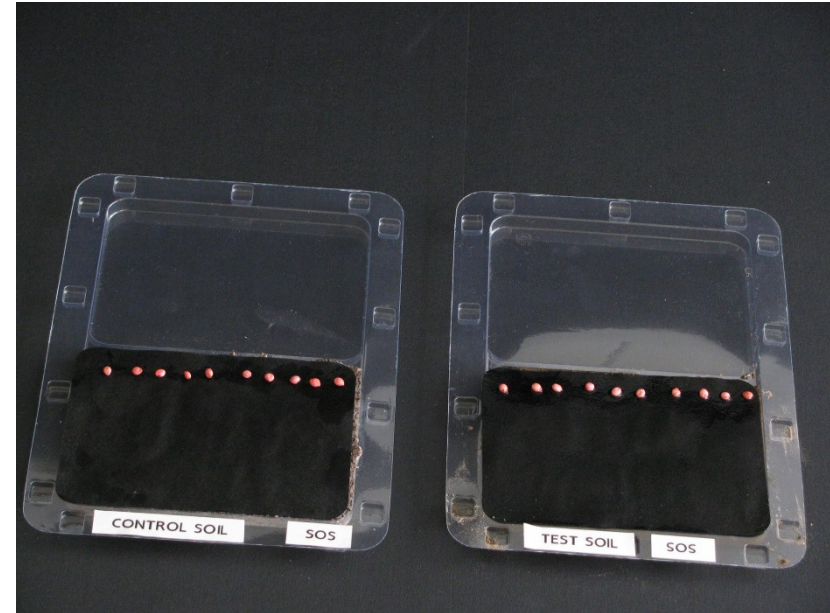
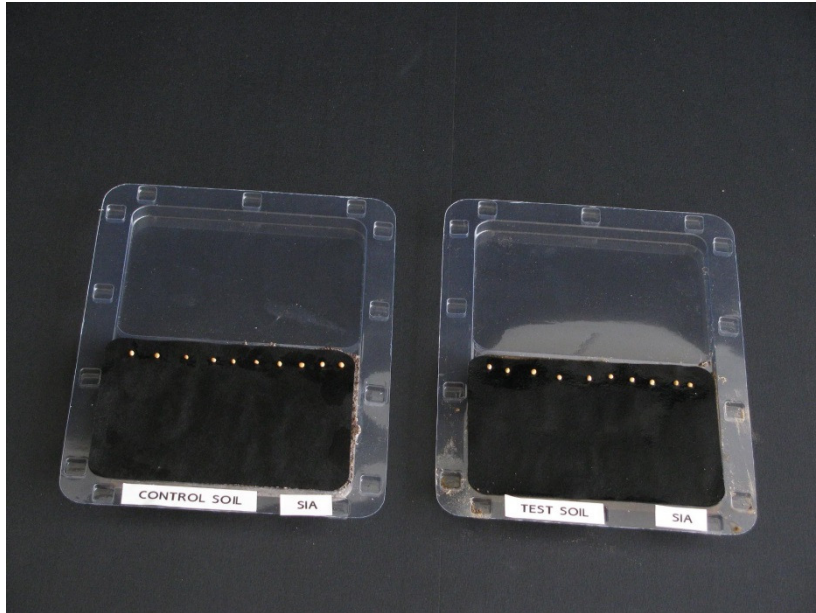




## 21

- PLACE **10 SEEDS OF THE SAME TEST PLANT** ON TOP OF THE FILTER PAPER IN ONE ROW AND AT EQUAL DISTANCE OF EACH OTHER

*(The test plates are provided with marks on the middle ridge and on the lateral sides to facilitate the correct positioning of the seeds)*

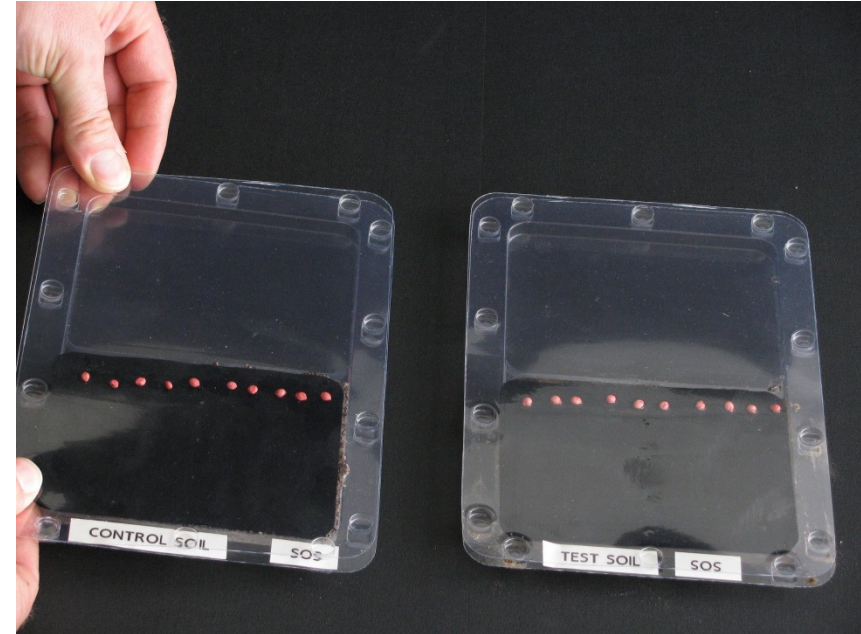
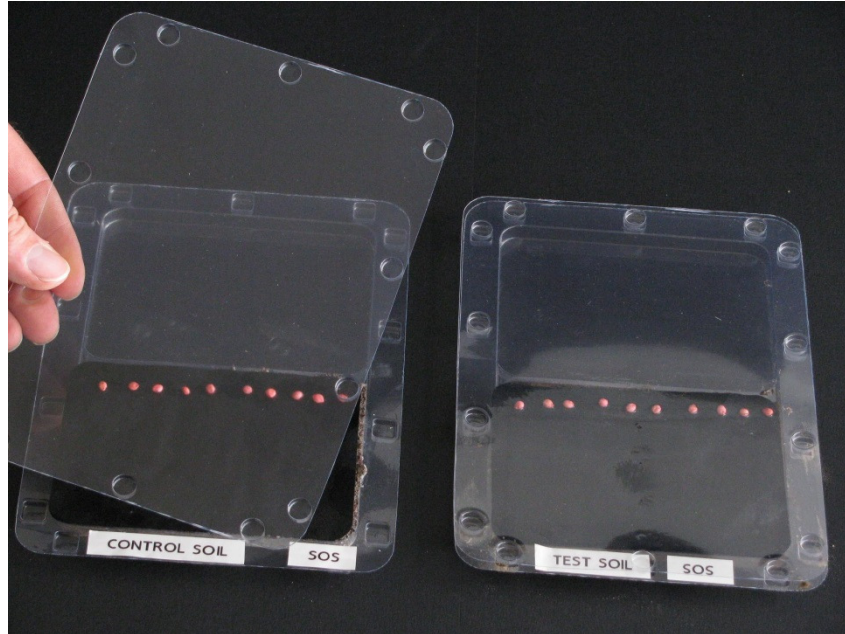


## 22

PHYTOTOXKIT TESTS ARE NORMALLY CARRIED OUT IN 3 REPLICATES  
WITH 3 DIFFERENT SEEDS :

- \* THE MONOCOTYL SORGHO (***Sorghum saccharatum***) (SOS)
- \* THE DICOTYL GARDEN CRESS (***Lepidium sativum***) (LES)
- \* THE DICOTYL MUSTARD (***Sinapis alba***) (SIA)





# 23

### 3. CLOSING OF THE TEST PLATES

CAREFULLY PLACE THE COVER ON THE TEST PLATE AND CLICK THE PROTRUDING PARTS INTO THE CORRESPONDING HOLES OF THE BOTTOM PART

# 24

## INCUBATION OF THE TEST PLATES

PUT THE TEST PLATES VERTICALLY IN THE  
CARDBOARD HOLDERS (6 plates per holder)

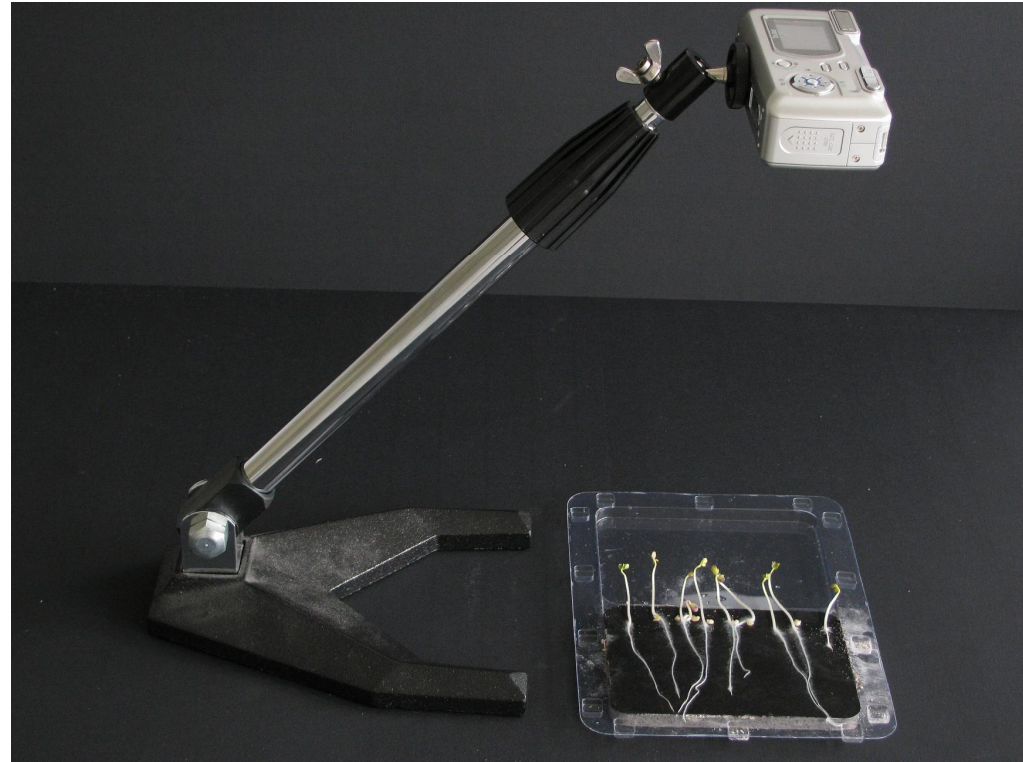






# 25

PUT THE CARDBOARD HOLDERS WITH  
THE TEST PLATES IN THE INCUBATOR  
AND INCUBATE **AT 25° C, FOR 3 DAYS,**  
**IN DARKNESS**

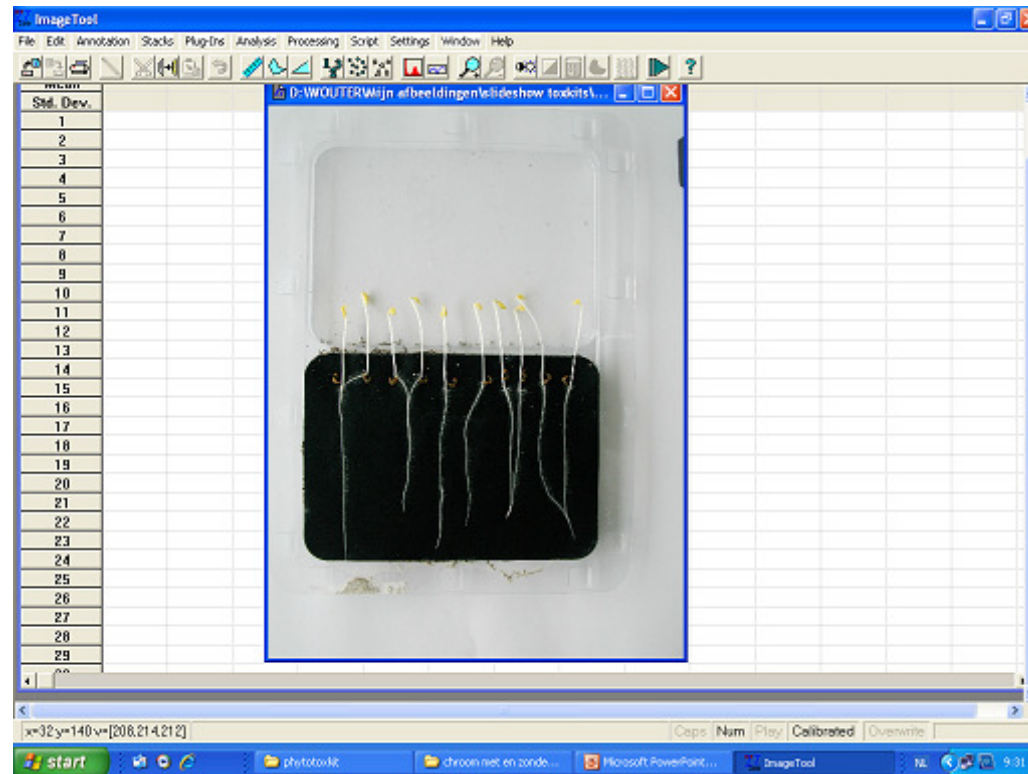


# 26

## IMAGE RECORDING AT THE END OF THE EXPOSURE PERIOD

- TAKE THE TEST PLATES OUT OF THEIR HOLDER
- PUT THE PLATES ON A HORIZONTAL SURFACE
- TAKE A PHOTO OF EACH PLATE WITH A DIGITAL CAMERA
- TRANSFER THE PHOTOS TO A COMPUTER FILE





# 27

## ANALYSIS AND MEASUREMENTS

- MEASURE THE LENGTHS OF THE ROOTS (AND THE SHOOTS) WITH AN APPROPRIATE IMAGE ANALYSIS PROGRAM
- PERFORM THE PRESCRIBED DATA TREATMENT TO CALCULATE THE PERCENTAGE INHIBITION OF SEED GERMINATION AND ROOT GROWTH OF THE PLANTS IN THE TEST SOIL VERSUS THE REFERENCE SOIL