**A Summary of the Study on the Effect of Karrikin on the Germination of Thermodormant Lettuce (*Lactuca sativa*) Seeds**

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 Karrikins are compounds found in smoke released from the burning of plants. These compounds have been linked to growth regulation, particularly seed germination. The goal of this project was to examine the effect of karrikin on inducing germination of thermodormant lettuce (*Lactuca sativa*) seeds. Over the course of the spring 2016 semester, trials were run to determine the germination rates of lettuce seeds treated with different concentrations of the smoke-derived compound karrikin at 33 ̊C, an inhibiting temperature. Our data indicated that karrikin concentrations between 0.2 µM to 0.8 µM increased germination significantly at 33 ̊C, and that 0.4 µM karrikin solution produced the highest germination rate.

In the fall 2016 semester, an object-based image analysis program\* was developed to quickly quantify seed germination. By using an image station, clear and consistent pictures were taken which maximized accuracy of the image analysis program (Figure 1). The automated counts were compared to manual counts (Figure 2). It was found that, on average, the percent germination estimated using the automated program was off by 5.16%. This allows for clear distinction between treated and control sets, however, further improvements of the automated program will be necessary. Aided with a more high-throughput approach, it is expected future studies could quickly explore the effects of karrikin on other important food crops, such as rice or corn. Eventually, we hope this research will to the development of effective treatments in agriculture for higher yields with lower seed waste.

\*Python script is attached.



*Figure 1.* Table-top imaging set-up. The light from two desks lamps was dispersed using a clear plastic container and a red background added contrast to the seeds.

*Figure 2:* Comparison of manual and automated germination counts.

# A program to estimate germination rates of lettuce seeds.

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# by: Kari Miller

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# Import necessary packages

import cv2

import numpy as np

import pylab

import scipy

from skimage import color

from skimage import measure

# Function to threshold for total seeds

def thres\_seed(image):

 blur = cv2.GaussianBlur(image,(5,5),0)

 retval, thres = cv2.threshold(blur, 43, 255,cv2.THRESH\_BINARY)

 grayscaled = cv2.cvtColor(thres,cv2.COLOR\_BGR2GRAY)

 ret3,thres\_img = cv2.threshold(grayscaled,0,255,cv2.THRESH\_BINARY+cv2.THRESH\_OTSU)

 #thres\_img = np.invert(thres\_img) #maybe remove later

 return thres\_img

# Function to threshold for germinated roots

def thres\_root(image):

 blur = cv2.GaussianBlur(image,(5,5),0)

 retval, thres = cv2.threshold(blur, 120, 160,cv2.THRESH\_BINARY)

 grayscaled = cv2.cvtColor(thres,cv2.COLOR\_BGR2GRAY)

 ret3,thres\_img = cv2.threshold(grayscaled,0,255,cv2.THRESH\_BINARY+cv2.THRESH\_OTSU)

 thres\_img = np.invert(thres\_img)

 return thres\_img

# Function to determine total seed count

def count\_seed(image):

 params = cv2.SimpleBlobDetector\_Params()

 params.blobColor = 0

 params.filterByArea = False

 params.minArea = 50

 params.filterByCircularity = False

 params.filterByInertia = False

 params.minInertiaRatio = 0

 params.filterByConvexity = False

 detector = cv2.SimpleBlobDetector\_create(params)

 keypoints = detector.detect(image)

 return len(keypoints)

# Function to determine germinated count

def count\_root(image):

 params = cv2.SimpleBlobDetector\_Params()

 params.blobColor = 0

 params.filterByArea = True

 params.minArea = 50

 params.filterByCircularity = False

 params.filterByInertia = True

 params.minInertiaRatio = 0

 params.filterByConvexity = False

 detector = cv2.SimpleBlobDetector\_create(params)

 keypoints = detector.detect(image)

 return len(keypoints)

# Main program

def main():

 # Import image

 date = input("What date did you perform the trial currently in the test folder? ")

 imgs = ["0.0\_", "0.2K\_", "0.4K\_", "0.6K\_", "0.8K\_", "1.0K\_", "0.2C\_", "0.4C\_", "0.6C\_", "0.8C\_", "1.0C\_"]

 img\_names = []

 for name in imgs:

 name = name + date + ".jpg"

 img\_names.append(name)

 for image in img\_names:

 img1 = cv2.imread(image)

 img2 = cv2.imread(image)

 #threshold for seed count

 seed\_img = thres\_seed(img1)

 #determine number of seeds

 seeds = count\_seed(seed\_img)

 #threshold for root count

 root\_img = thres\_root(img2)

 #determine number of roots

 roots = count\_root(root\_img)

 #determine germination percentage

 percent = roots/seeds \* 100

 print("The percent germinated for ", image[:4], " is: ", roots, "/", seeds, " = ", round(percent, 4))

main()