Exercise 3

Crop Loss Prediction and Modeling for Management Decisions
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1. Objectives
    To explore the relationships of plant growth and
    nematode multiplication with preplant population
    densities, and to develop critical point models of these
    relationships.

2. Length of time required
    2 x 3 hr laboratory sessions, 2 months apart.

    Preparation of material: Sow tomato seeds in
    vermiculite on 2 dates: 7 weeks before exercise and 4
    weeks before exercise.   Two weeks after sowing,
    transplant seedlings into sand in small pots or
    styrofoam cups.   Ensure large quantities of _Meloidogyne_
    inoculum by starting culture pots in the greenhouse
    three months before the experiment.   Two days before the
    experiment, extract juveniles from soil in the culture
    pots.   Extract eggs from the roots in the culture pots
    by cutting the roots into short lengths and stirring
    them for 3 min in 4% commercial bleach.   Using a 100
    mesh over a 500 mesh screen, sieve eggs from bleach
    solution and root suspension immediately to prevent
    mortality.

3. Procedure
    Set up procedure
    1 a) Work in 4 groups.   From the eggs and second-stage
           juveniles of _Meloidogyne_ sp. provided, each group
           will prepare 4 replications of dilutions of 0, N,
           2N, 4N, 8N, 16N, 32N, 64N where N=1000.   Prepare
           4 extra replications of inoculum level N for use
           in 1c.   Prepare 31 extra replications of inoculum
           level 16N for use in Part 2.

       b) Inoculate 5" pots of sand by washing the inoculum
           into a planting depression.   Label the pots
           appropriately.

       c) Inoculate 4 extra pots with inoculum density N.
           After 12 days, wash the roots from these pots.
           Stain the roots by boiling carefully in acid
           fuchsin-lactic acid (see Method A) (a slight
           modification of the acid fuchsin staining
           technique is given in Exercise 33).   Rinse roots
           and place the stained roots in a few drops of
           glycerin between glass plates.   Count the

juveniles in the roots.

d) Devise and set up a check in your experiment which will demonstrate that the symptoms seen on the plant are caused by the nematodes and not by anything else you may have introduced.

e) The two groups will plant 5-week-old tomato seedlings in the pots and 2 groups will plant 2-week-old seedlings (7 and 4 weeks after sowing, respectively).

Takedown procedure

Soil and root systems will be examined and plant growth assessed after 2 months.

f) Cut tops off tomato plants at soil level. Obtain fresh weight for each top.

g) Using the cork borers provided, take a five-core sample from each pot. Bulk samples for the four replications of the same treatment in one 600-ml beaker, i.e., this will result in 8 beakers each containing 20 soil cores. Extract nematodes using a Cobb's sieving (40 mesh over a 325 mesh screen) or an elutriation technique. Rinse gently to avoid dislodging egg masses adhering to root fragments in the sample. (Root fragments retained on 40 mesh screen should be replaced in rinsed beakers containing 200 ml water and processed in Section 1h).

Soil juveniles will be retained on the 325 mesh screens. Collect these in a beaker, carefully reduce the volume of water by pouring sample through 325 mesh screen and rinsing nematodes back into beaker. Place samples on Baermann funnels and determine number of juveniles after 3 days. Calculate number of juveniles per pot.

h) Extract eggs from the egg masses attached to the root sample obtained in 1g above. Add 20-ml sodium hypochlorite solution (bleach), stir for 5 min. Take a 10 ml sample from the suspension, dilute out in 100 ml water in a 150 ml beaker. Extract the eggs from this sample on a 500 mesh sieve. Stain with acid fuchsin in lactic acid solution (3 drops) and bring to boil. Count eggs in the sample and calculate number of eggs per pot. The total number of eggs (1h) and second-stage (1g) per pot represents the final nematode population (P_f) for this experiment.

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i) Wash root systems gently from soil, place each root system in water and score it using the gall index chart provided. Calculate the average index value for each treatment. (See Method B)

PART I:

(i) What evidence do you have that the symptoms were caused by the nematodes you inoculated?

(ii) What are the symptoms caused by _Meloidogyne_ sp. on tomato?

(iii) What was the percent infectivity of your inoculum? Were you able to accurately determine the infectivity of your initial inoculum from the stained roots (1c)? If not, why not?

Adjust actual inoculum rates on the basis of the % infectivity of your inoculum.

(iv) What was the effect of increasing nematode density on plant growth and disease symptoms?

(v) Compare the symptom severity and effects on growth when seedlings of different ages are inoculated (check with other groups).

(vi) Plot P_f against P_i (log scale) and draw the reproduction curve. (see Ferris, 1985a)

(vii) Plot plant weight against log P_i.

(viii) Plot plant weight against root gall index.

PART II:

The following will require some background reading. A discussion of the concepts and calculations may be addressed in a written report or in class.

(i) Based on Seinhorst's articles and on the data collected, can you determine values for the maximum rate of reproduction (a) and the equilibrium density (E)? Discuss the relationship between a, E, P_i and P_f for the conditions under which the experiment was conducted.

Background for question (i):

Seinhorst has derived a model to describe the relation between the rate of increase in a nematode population and

the density of that population.

This model depends on 2 constants which indicate the host status of a plant to a plant parasitic nematode:

1. "a" describes the maximum rate of reproduction in a given system, and

2. "E" describes the equilibrium density, which is that population density at which the available food is just sufficient to maintain the existing population (Seinhorst, 1966).

Thus, a and E are specific to both the nematode species and the host under consideration. They are also dependent on external conditions, such as soil type and water, and will change over time, since plants grow and change during an experiment. According to Seinhorst, changes in the values of a and E during the experiment do not affect the shape of the curve for the relation between initial and final densities of the population, but only its position relative to its coordinates (Seinhorst, 1966).

The rate of increase of a nematode population depends on the population density (P), such that at low P, the rate of increase is high, while at high P, the rate of increase is low. Where P = E, the rate of increase is O.

The multiplication of the nematode population may be expressed as:

$$P_f(P_i)^{-1} = aE[(a-1)P_i + E]^{-1}$$

When P_i is very low, or approaches 0, the multiplication rate is high and approaches "a". When $P_f = P_i$, population is not increasing, and the population density equals the equilibrium density (P = E as stated above).

(ii) Based on Ferris (1985a), determine the multiplication curve (P_f/P_i vs. ln P_i) and fit the model $P_f/P_i = a P_i^{-b}$, based on the data collected.

(iii) Regress plant weight with log initial population.

(iv) Determine parameter values for equation
$y = m + (1-m)Z^{P-T}$ for P>T, y = 1 for P<T. Graph the function and include the data points. Assuming this damage function to be field applicable, determine the economic threshold for a management practice costing 25% of the potential crop value (see Ferris, 1985b).

(v)   Determine whether method (iii) or (iv) resulted
        in better fit.   Determine predicted and actual
        plant weight.   Calculate sums of squares of
        deviations of observed data from model
        predictors.

(vi)   Regression of plant weight with root gall index.

(vii)   Do these values and relationships change when
        seedlings of different sizes (ages) are used?
        Comment.

2. Plant Density Experiment:

    Agricultural crops are spaced in the field so as to
    make optimum use of resources (light, nutrients,
    water, etc.).   If the effect of nematodes is to
    reduce the plant growth, available resources are no
    longer optimally utilized.   Consequently, it would
    seem that adjusting plant spacing might be a method
    of compensating for nematode damage in some crops.
    Verify this theory for above ground growth of
    tomatoes parasitized by root-knot nematodes.   Each
    group is provided with 10 trays (approximately 35 cm x
    25 cm) of nematode-free soil.   Determine optimal plant
    spacing to maximize fresh weight per tray in the
    presence and absence of nematodes as follows:

    a) Plant separate trays of nematode-free soil with
        1, 2, 4, 8, and 16 tomato seedlings.   Plant an
        equivalent series with 16N inoculum level
        prepared in Part 1a.   Determine maximum foliar
        growth per plant during the takedown lab.

    b) Take down the plant spacing experiment after 2
        months.   Cut plants off at soil level and weigh
        total fresh top growth in each tray.   The
        nematode population density on each plant in the
        infested soil is equivalent to the 16N treatment
        in Part 1 of this lab.   Determine expected growth
        reduction at this nematode density and test the
        theory that knowledge of the population density
        of nematodes would allow appropriate adjustment
        of plant spacing to maximize production.

        EXAMPLE: If maximum production in non-infested
        trays occurs at 4 plants/tray and nematode
        density 16N reduces plant growth by 50%, then
        maximum productivity in the nematode infested
        soils should occur at 8 plants/tray.

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A. ROOT STAINING WITH ACID FUCHSIN-LACTIC ACID

1. Wash roots from soil under running water.

2. Drain the excess water on a paper towel.

3. Heat root system in beaker of acid fuchsin-lactic acid solution until it boils.

4. Moderate, galls numerous, mostly discrete

5. Roots may be stored in lactic acid solution if you are unable to examine them immediately.

6. Cut roots into small pieces. Spread the roots between two glass plates in a few drops of glycerin and view under dissecting microscope.

7. Be sure to press the roots firmly between the glass plates to facilitate views of the stained nematodes.

1% acid fuchsin	
acid fuchsin powder	1 gm
water	100 ml

lactic acid solution	
lactic acid	1750 ml
glycerin	126 ml
water	124 ml

0.01% acid fuchsin-lactic acid	
1% acid fuchsin	1 ml
lactic acid solution	99 ml

B. ASSESSMENT OF DEGREE OF GALLING BY Meloidogyne spp.-
   (Daulton and Nusbaum, 1961)

Infection Class	Index Value	Description of Index Value
0	0	Free from galls
1	1	Trace, less than 5 galls
2	5	Very slight, trace to 25 galls
3	10	Slight, 26 to 100 galls
4	25	Moderate, galls numerous mostly discrete
5	50	Moderately heavy, galls numerous, many coalesced
6	75	Heavy, galls very numerous,

		mostly coalesced, root growth slightly retarded
7	90	Very heavy, mass invasion, slight root growth
8	100	Extremely heavy, mass invasion, no root development

REFERENCES AND SUGGESTED READING

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