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Determination of Leaf Necrosis Caused by *Cercospora arachidicola* Hori in Peanut as Measured by Loss in Total Chlorophyll [1]

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ABSTRACT

Percent leaf necrosis can be used to assess severity of early leafspot caused by *Cercospora arachidicola* Hori on peanut *(Arachis hypogaea* L.). Measurements of leaf necrosis (%), as determined by area, and the corresponding loss (%) in total chlorophyll content were made. Correlations were calculated using linear regression, and logarithmic curve fit analyses. High positive correlation values were obtained between leaf necrosis and loss in total chlorophyll. Therefore, this method is suggested for a reasonably accurate and fast assessment of leaf necrosis of peanut caused by *C. arachidicola.*

Keywords: Leafspot, disease assessment, insoluble polyvinylpyrrolidone, polyphenols.

Early leafspot disease of peanut *(Arachis hypogaea* L.) caused by *Cercospora arachidicola* Hori is a major problem in peanut growing areas of the world. This disease causes leaf necrosis and defoliation. One parameter in assessing disease severity is a measurement of leaf necrosis (%), determined by the formula, $(\frac{A-B}{A}) \times 100$, where A = total leaf area, and B = leaf area after excising leafspots.

This measurement is simple and fast when the number of leafspots per leaf is small, but it is laborious and time consuming when a large number of leafspots per leaf occur.

Loss in total chlorophyll has been used to determine injury to pinto bean leaves caused by air pollutants (4). The purpose of this investigation was to test the feasibility of utilizing the loss in total chlorophyll to assess the severity of leaf necrosis in peanut caused by *C. arachidicola.*

Materials and Methods

The *C. arachidicola* culture used for inoculations was isolated from a leafspot-susceptible peanut cultivar 'Comet' grown in Caddo County, Oklahoma. The fungus was grown for production of conidia on a peanut-oatmeal-agar medium (3). Conidial suspensions were obtained by flooding 15-20 day old cultures in petri-plates with sterile distilled water, followed by filtration through four layers of cheese-cloth to remove most of the mycelial fragments.

A leafspot susceptible peanut cultivar, 'Spanhoma', was used in these experiments. Six-week-old plants were inoculated by misting them with a conidial suspension (2×10^4 conidia/ml) of *C. arachidicola* using a DeVilbiss atomizer (No. 152). Inoculated plants were then placed in a clear plastic chamber on a greenhouse bench. Temperature in the chamber was maintained at 27-30 C and relative humidity was maintained at 80-90% by hanging wicks of cheesecloth on both sides of the chamber. Ten to 12 days after inoculation, necrotic spots began to appear on peanut leaves.

Leaf necrosis (%) determinations were calculated using the formula described earlier. Leaf area (cm^2) was measured with a Lambda area meter.

Total chlorophyll was extracted by grinding 0.5g (fresh wt.) of leaf tissue, and 0.5g of water-insoluble polyvinlypyrrolidone (PVP) in 25 ml of 80% aqueous acetone for 90 sec. in a Virtis homogenizer at 40,000 rpm. The mixture was then filtered through Whatman #1 filter paper, and the total chlorophyll content in the fltrate was measured spectrophotometrically at 652 nm (1). Total chlorophyll content in the leaf tissue was calculated on two bases: a) weight to unit of leaf area (i.e. mg of total chlorophyll/cm^2 leaf area), and b) weight to weight (i.e. mg of total chlorophyll/g of fresh leaf tissue). Loss (%) in total chlorophyll was calculated using the formula, $(\frac{C-D}{C}) \times 100$, where C = total chlorophyll content in control normal leaves, and D = total chlorophyll content in necrotic leaves.

Results and Discussion

In earlier experiments, insoluble PVP was not used in the process of extracting total chlorophyll from leaf tissue. This resulted in low correlation values between leaf necrosis (%) caused by *C arachidicola* in peanut and loss (%) in total chlorophyll, because polyphenols released from the necrotic leaf tissue interferred with chlorophyll determinations. Therefore, to preclude interference with chlorophyll determinations, insoluble PVP subsequently was used in all extractions to bind polyphenols released from necrotic tissue (2).

Twenty-five determinations were made of leaf necrosis (%) and the corresponding loss (%) in total chlorophyll. Correlations between leaf necrosis (%) and loss (%) in total chlorophyll were calculated by linear regression and logarithmic curve fit analyses; in all cases, high positive correlation values were obtained. In linear regression analyses, correlation values of (r=0.83) and (r=0.79) were obtained (Fig. la, lb), depending on the method used to calculate total chlorophyll content in leaf tissue (i.e. mg total chlorophyll/cm^2 leaf area or mg total chlorophyll/g fresh wt. tissue). In logarithmic curve fit analyses, correlation values of (r = 0.85) and (r = 0.79) were obtained (Fig. 2a, 2b) respectively, by the two methods used in calculating total chlorophyll content in leaf tissue. These data suggest that measuring the loss (%) in total chlorophyll can be used for a reasonably accurate and fast method of assessing severity of leaf necrosis of peanut caused by *C. arachidicola.*

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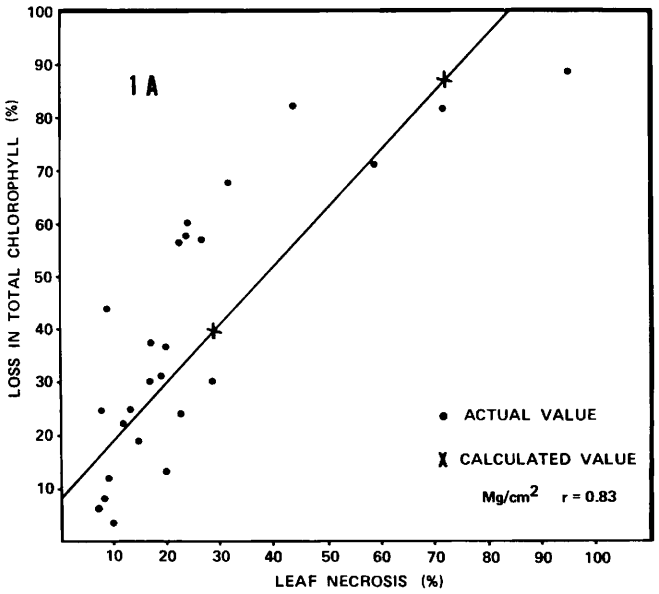


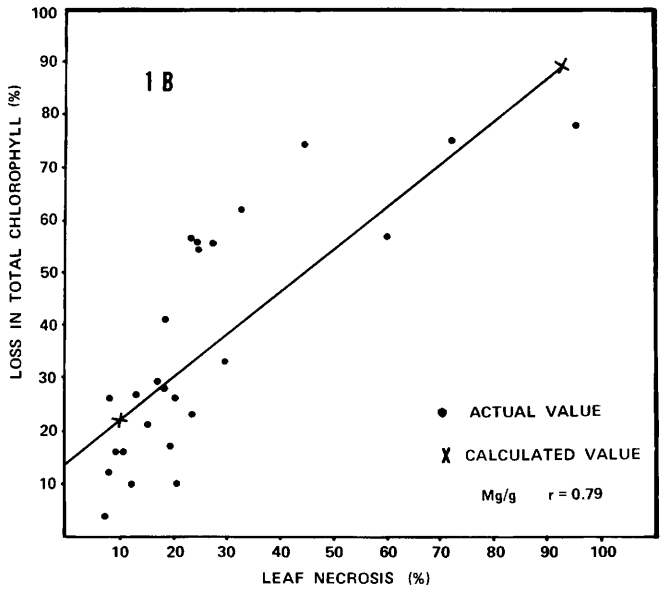
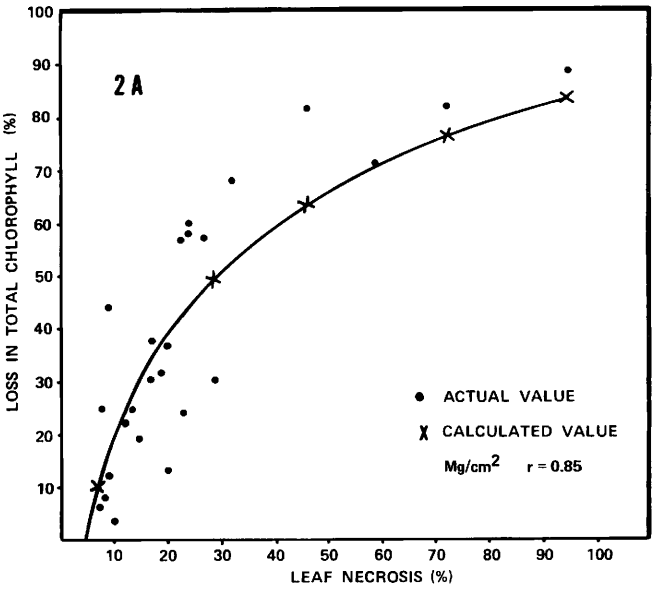


Fig. 1. Linear regression between loss (%) in total chlorophyll and leaf necrosis (%) in peanut caused by *Cercospora arachidicola*.
A. Total chlorophyll content was expressed as mg total chlorophyll/cm^2 of leaf area.
B. Total chlorophyll content was expressed as mg total chlorophyll/g of fresh wt. tissue.



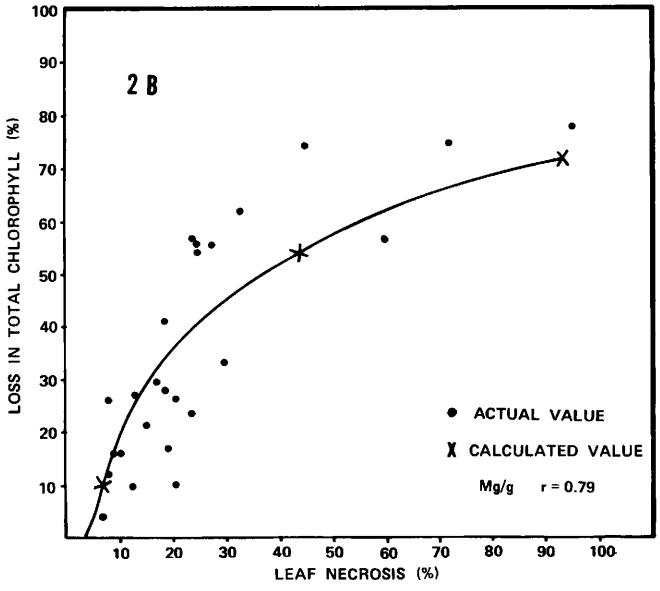


Fig. 2. Logarithmic curve fit between loss (%) in total chlorophyll and leaf necrosis (%) in peanut caused by *Cercospora arachidicola*.
A. Total chlorophyll content was expressed as mg total chlorophyll/cm^2 of leaf area.
B. Total chlorophyll content was expressed as mg total chlorophyll/g of fresh wt. tissue.

Literature Cited

1. Aron, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta Vulgaris*. Plant Physiol. 24: 1-15.

2. Loomis, W.D. and J. Battaile 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochem. 5: 423-438.

3. Smith, D.H., 1971. A simple method for producing *Cercospora arachidicola* conidial inoculum. Phytopath. 61: 1414.

4. Todd, G.W. and W.N. Arnold, 1961. An evaluation of methods used to determine injury to plant leaves by air pollutants. Bot. Gaz. 123: 151-154.