

Effect of Time of Inoculation with *Cladosporium caryigenum* on Pecan Scab Development and Nut Quality

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ABSTRACT

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Pecan nut clusters were inoculated with conidia of *Cladosporium caryigenum* on 10 dates ranging from 15 May to 11 September. Comparison of disease increase models based on a logarithmic curve-fitting regression analysis indicated that the 10 epidemics resulting from inoculations could be classified into three groups: early, mid-, or late-season. The midseason group had the greatest rate of disease increase, which may correspond to

greater midseason nut susceptibility. Early season disease initiation decreased nut size, weight, percent oil, moisture, and protein content. Mid- and late-season disease initiation did not adversely affect any of these quality parameters. This indicated that the major effect of pecan scab on nut quality occurred prior to mid-June, the time of the initiation of shell (endocarp) differentiation.

Additional key words: disease rating scale, *Fusicladium effusum*.

The most destructive and widespread disease of pecan, *Carya illinoensis* Koch, is pecan scab (4). The effect of pecan scab, which is caused by *Cladosporium caryigenum* (Ell. et Lang) Gottwald [= *Fusicladium effusum* (Wint.)] (9), on pecan production has been difficult to measure due to the influence of weather, which varies widely throughout the pecan belt, and also to differing cultivar susceptibilities, which have changed considerably over time (3,5).

Pecan scab and other fruit and foliar diseases have been implicated in reducing both yield and kernel quality, although most disease assessments have been made only at the end of the season (8,18-20). Pecan nuts infected by the pathogen early in the season usually drop before they are half grown (15).

Measurements of pecan scab disease increase over time have been made previously (10); however, we are aware of only one attempt to correlate disease severity with yield losses (8). Problems associated with infection from controlled inoculations, lack of acreage for adequate experimental replication (mature orchards often accommodate only 9-12 trees per acre), site variability, cultivar uniformity, planting patterns, and the tendency to bear less in alternate years, have made the collection of yield data associated with pecan scab both difficult to obtain and of questionable significance. Therefore, rather than approach the effect of scab on pecan production directly by measuring yields, we found it useful to approach the problem by measuring various nut quality parameters.

Measurements of oil and free fatty acid content have traditionally been used as indicators of pecan kernel quality (2,7,14,16). Kernel rancidity is associated with oxidation of the unsaturated fatty acids that predominate among the lipids of pecan oils (17). The average oil and protein contents are known to be 70 and 10%, respectively (6). Kernel moisture content relates to crispness, texture, and storage stability, and is optimum at 3.5-4.0% (7,17). In addition, size of unshelled nuts and kernel weights are used by the pecan industry to compare crop and cultivar quality (1).

The purpose of this study was to investigate the effect of pecan scab epidemics of various duration and intensity on disease development, nut drop, and nut quality in an attempt to determine under what conditions pecan scab is commercially damaging to the crop.

MATERIALS AND METHODS

The study was conducted in a 10-yr-old pecan orchard of the cultivar Cherokee planted on ~9 × 9 m spacing. The experimental design consisted of 11 treatments representing epidemics of differing duration. The duration of each epidemic was controlled by the inoculation date (15 May, 29 May, 12 June, 26 June, 10 July, 24 July, 7 August, 21 August, 4 September, 11 September, and an uninoculated control). Each treatment was replicated eight times, in a completely randomized design. Each replicate was an individual tree on which 25 nut clusters were tagged and observed throughout the season and harvested individually.

Each nut cluster was kept free of disease by compressed-air hand sprayer application of Super-Tin 4-L (triphenyltinhydroxide, 0.79 g/L) at 14-day intervals. Control measures for each treatment ceased 14 days prior to the inoculation of the trees in that treatment group. Inoculations were made by atomizing suspensions of $2.5-4.0 \times 10^5$ conidia per milliliter to run off on nut clusters and the surrounding foliage. Conidia were collected from 42-day-old sporulating oatmeal agar cultures of *C. caryigenum* isolates 773b or 81-1f, both virulent isolates from cultivar Cherokee pecan. Each tree received a single inoculation to establish the pathogen in that treatment group. Following each inoculation, the sprinkler irrigation system was run overnight to ensure high relative humidity and adequate phylloplane moisture for infection.

Determinations of the effect of pecan scab on nut size and kernel weight were made at harvest by measuring in-shell nut diameter and shelled kernel weight of all nuts from each nut cluster (~500-750 fruits per treatment). Chemical quality parameters (ie, the percentages of kernel oil, kernel protein, kernel free fatty acids, kernel moisture, and the relative saturations of kernel oils) were assayed by using standard techniques (13). Disease ratings made throughout the season were based on percent shuck surface area diseased: 1 = no disease, 2 = trace-6%, 3 = 7-25%, 4 = 26-50%, 5 = 51-75%, 6 = 76-94%, 7 = 95-99%, and 8 = 100%. This grading system is similar to one proposed by Horsfall and Barratt (11) and

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differs from that proposed for pecan scab by Hunter (12) in having three additional grades that allow greater accuracy of grading in the higher disease ranges.

Subsamples of 10 clusters per treatment, observed on at least four of the eight trees per treatment, were utilized as the basis for calculating both percent disease and percent fruit drop over time throughout the duration of the test.

RESULTS

A high correlation coefficient ($r = 0.975$) was obtained when disease intensity was plotted against epidemic initiation date as determined by least squares curve-fitting analysis (Fig. 1). There was a direct relationship between disease duration and end-of-season disease intensity. The later in the season the disease was initiated on the fruit clusters, the lower was the disease intensity achieved at the end of the season.

In addition to end-of-season disease intensity, individual disease progress curves were generated for each treatment to study postinoculation disease increase (Fig. 2). The major increase in disease for most treatments occurred during mid-season (July through August). Least squares curve-fitting regression analysis demonstrated that logarithmic curve approximations gave the best overall fit for the 11 disease progress curves (Fig. 1). These logarithmic curve approximations demonstrated a relationship between disease increase and the time of onset of each epidemic (the latter estimated by the point at which the model line crossed the X-axis). An uncontrolled epidemic (treatment 1) and an early season inoculation (treatment 2) had similar epidemic initiation dates (15 May and 29 May, respectively) and gave rise to epidemics with similar logarithmic disease progress curves. Early season disease control followed by midseason inoculations (treatments

3-5) demonstrated the expected sanitation effect by causing a delay in epidemic initiation date. Disease development from midseason inoculations with *C. caryigenum* resulted in three similar disease progress curves, which had steeper initial ascents than progress curves for the uncontrolled epidemic and early season epidemics (Fig. 1). Early and midseason disease control followed by late-season inoculations (treatments 6-10) and the uninoculated control (treatment 11) delayed epidemic initiation date. In several cases, the rate of disease increase was reduced in conjunction with late-season disease initiation. This reduced rate of disease increase was expressed as less-steep model line ascents (treatments 6, 8, 10, and 11) (Fig. 1).

Major fruit drop occurred during two general time periods: mid- to late June and late July to late August (Fig. 1). Following the second drop period, very few additional nuts dropped during the remainder of the season. Comparison of nut-drop curves and total nut drop for all inoculation dates demonstrated less than 12% difference among the treatments except for treatment 5 (Fig. 3).

Inoculations in mid- or late May significantly reduced nut diameter and kernel weight compared to the control (Fig. 4B and C); however, inoculation after mid-June had no significant effect on nut diameter or kernel weight (Fig. 4C). Scab had a significant effect on percent oil and percent moisture (Fig. 4D and E), but not on quantity of free fatty acids nor on iodine number (an indicator of polyunsaturation of oils) (Fig. 4F). A logarithmic curve gave the best fit to the data of percent protein versus inoculation date, with a correlation coefficient of $r = 0.69$ (Fig. 4F). Although not a high correlation, the logarithmic curve suggested that the greatest change in percent kernel protein occurred during the first two post-nut-set treatments (ie, between May and mid-June) (Fig. 4F). The puzzling effect of decreasing kernel moisture and protein with decreasing epidemic duration stimulated further analysis involving these quality parameters. Initial nut cluster values of percent

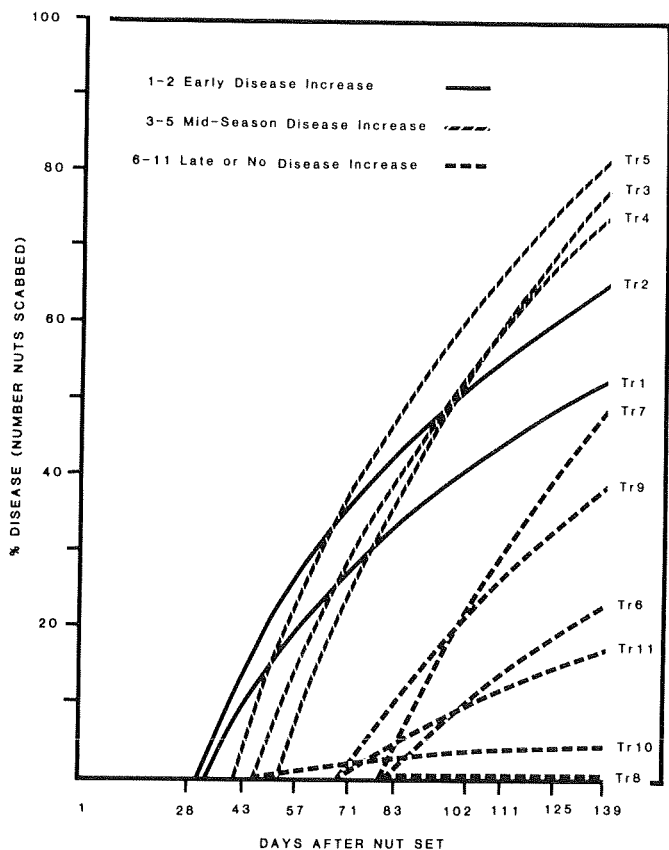


Fig. 1. Logarithmic approximation models of disease progress curves for each of the 10 inoculation dates and one uninoculated control (Tr 11), grouped by epidemic initiation date and disease increase model lines. Mid-season disease increase model lines have a steeper ascent while early and late-season disease increase model lines are less steep in their ascent. This may be indicative of increased tissue susceptibility during this time.

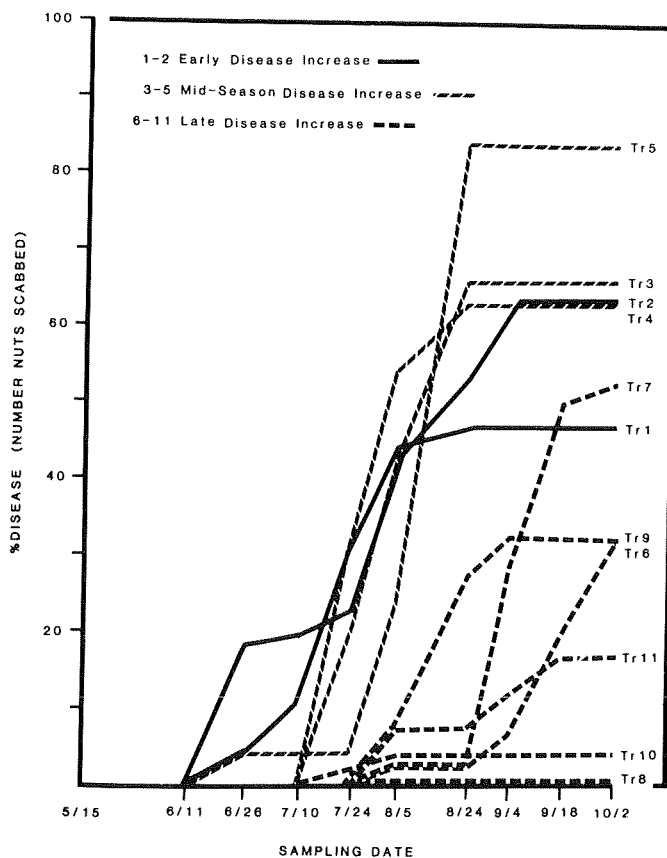


Fig. 2. Disease progress curves for each of the 10 inoculation dates and one uninoculated control (Tr 11), grouped by acceleration stage. Notice that the major increase in disease occurred during mid-season (July through August) followed by a leveling off for the remainder of the season.

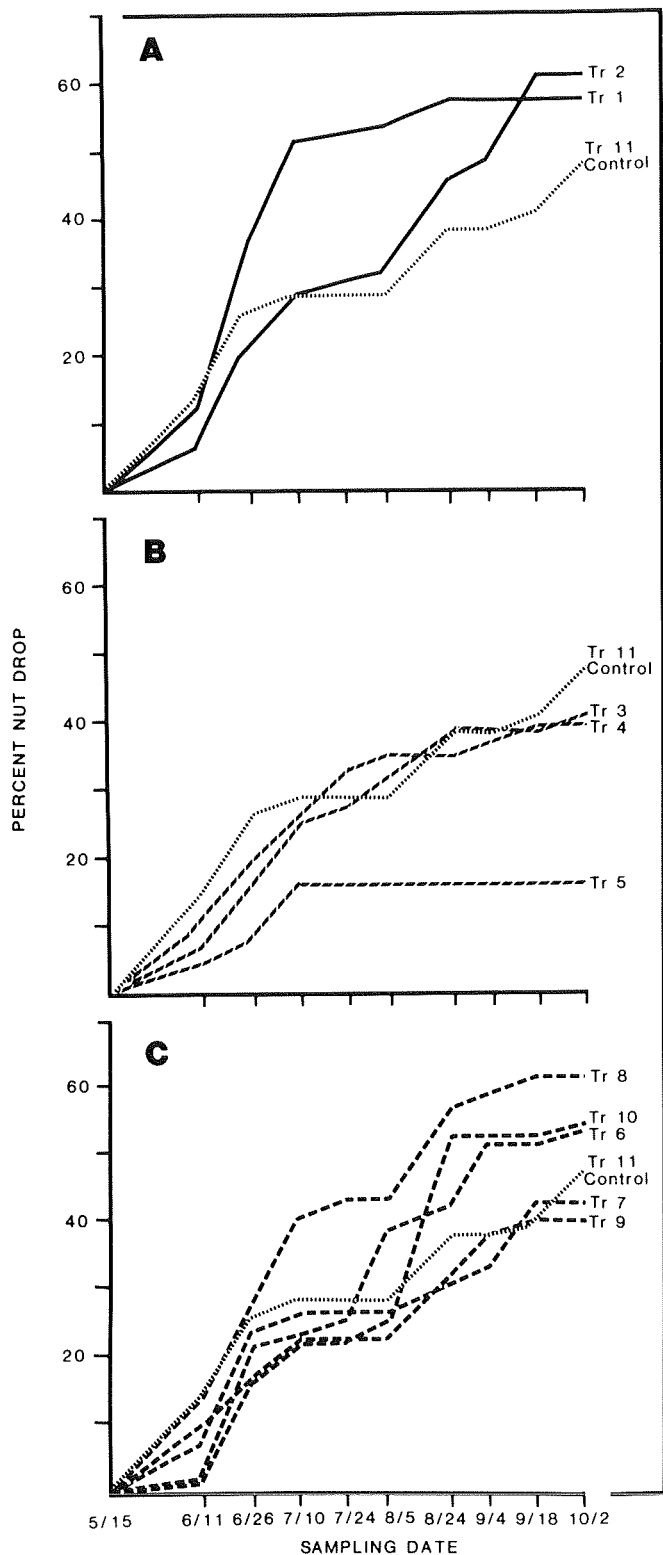


Fig. 3. Nut-drop curves for each of the 10 inoculation dates and one uninoculated control (Tr 11). Most end of season nut-drop totals differed less than 12% from the season-long control. A, The early season nut drop associated with high early season disease levels (Tr 1 and Tr 2) is offset by a decrease in late-season nut drop for that treatment. B and C, Mid- (Tr 3, Tr 4, and Tr 5) and late-season (Tr 6 through Tr 10) disease initiation resulted in no significant differences in overall nut drop from the season-long control (Tr 11).

protein per gram of kernel weight and percent moisture per gram of kernel weight were multiplied by the average kernel weight per cluster to remove the effect of nut weight from the data. The data were then reanalyzed by multiple comparison of treatment means. Results of this second analysis indicated that both percent protein and percent moisture actually increased as disease duration decreased when the effect of kernel weight was removed. Least squares curve-fitting analysis also demonstrated a greater correlation coefficient ($r = 0.906$) between percent protein and inoculation date for the transformed data (Fig. 5).

DISCUSSION

The data and analysis of epidemics indicated a large effect of early season scab on nut and kernel quality and relatively little effect of mid- and late-season scab. Both nut size and kernel weight were strongly affected by scab infections before mid-June and only mildly affected afterwards. Oil content was also reduced by scab infection, but only following inoculation prior to mid-June. Interestingly, nuts scabbed early in the season had a greater percent moisture and percent protein per gram of kernel weight. Further analysis on a per kernel basis, however, demonstrated less protein and moisture in these kernels associated with epidemics of longer duration. The restriction in nut size and restriction in incorporation of oils into the kernels of nut clusters associated with early season scab infections caused both protein and moisture to appear to make up a larger percentage of the kernel, while in fact both protein and moisture were also reduced in these kernels.

Disease progress curves were classified in three groups: A—Those resulting from early season inoculations, which showed disease increases from mid-June to late July; B—midseason inoculations corresponded with disease increases from mid-July to early August; and C—those resulting from late-season inoculations and the uninoculated control, which corresponded to disease increase from early August thru mid-September. The last group of disease progress curves (group C), those resulting from late season inoculations, also corresponded with lower end-of-season disease levels when compared to disease levels resulting from early and midseason inoculations. The last inoculation date and the uninoculated control gave rise to very low disease levels just prior to harvest and very minor disease increases in late July.

The effect of early season scab can be seen by examining year-end disease levels corresponding to early inoculation dates. The disease progress curves associated with these inoculation dates can be separated from the other curves by their early initiation and different phases of disease increase. Apparently, it was only during this time period (ie, nut set to mid-June) that cultivar Cherokee nuts were appreciably affected by scab infection. After this time period, the endocarp (shell) begins to differentiate. The relatively steep ascent of midseason disease progress curves may be indicative of greater disease susceptibility of nut shucks (exocarp tissue) during the corresponding rapid nut-expansion-stage (Fig. 1).

No appreciable differences were found in overall nut drop among treatments, thus indicating little or no effect of scab epidemic initiation date or disease intensity on total nut drop compared to the control (Fig. 3). This was especially surprising since treatment 5, which had the highest percent number nuts infected (Fig. 2), had the least nut drop (Fig. 3B). Early season scab apparently caused an average of less than 10% deviation in nut drop from the control even though scab in the plots was severe at times (Fig. 3A). Mid- and late-season scab also caused less than 12% variation in nut drop from the control (Fig. 3B,C). One explanation might be that although early season scab caused an increased early season nut drop it might act as a thinning mechanism causing fruit to drop prior to the time of natural drop. Thus, the tree may actually shed nearly an equivalent number of its nuts earlier in the season, which it would have aborted later without having to support their growth and development. Therefore, net drain of the tree's energy reserves may have been less from early season nut abortion due to scab than from its later natural thinning tendencies.

This study represents a preliminary investigation into areas of scab research that have previously received little attention. The

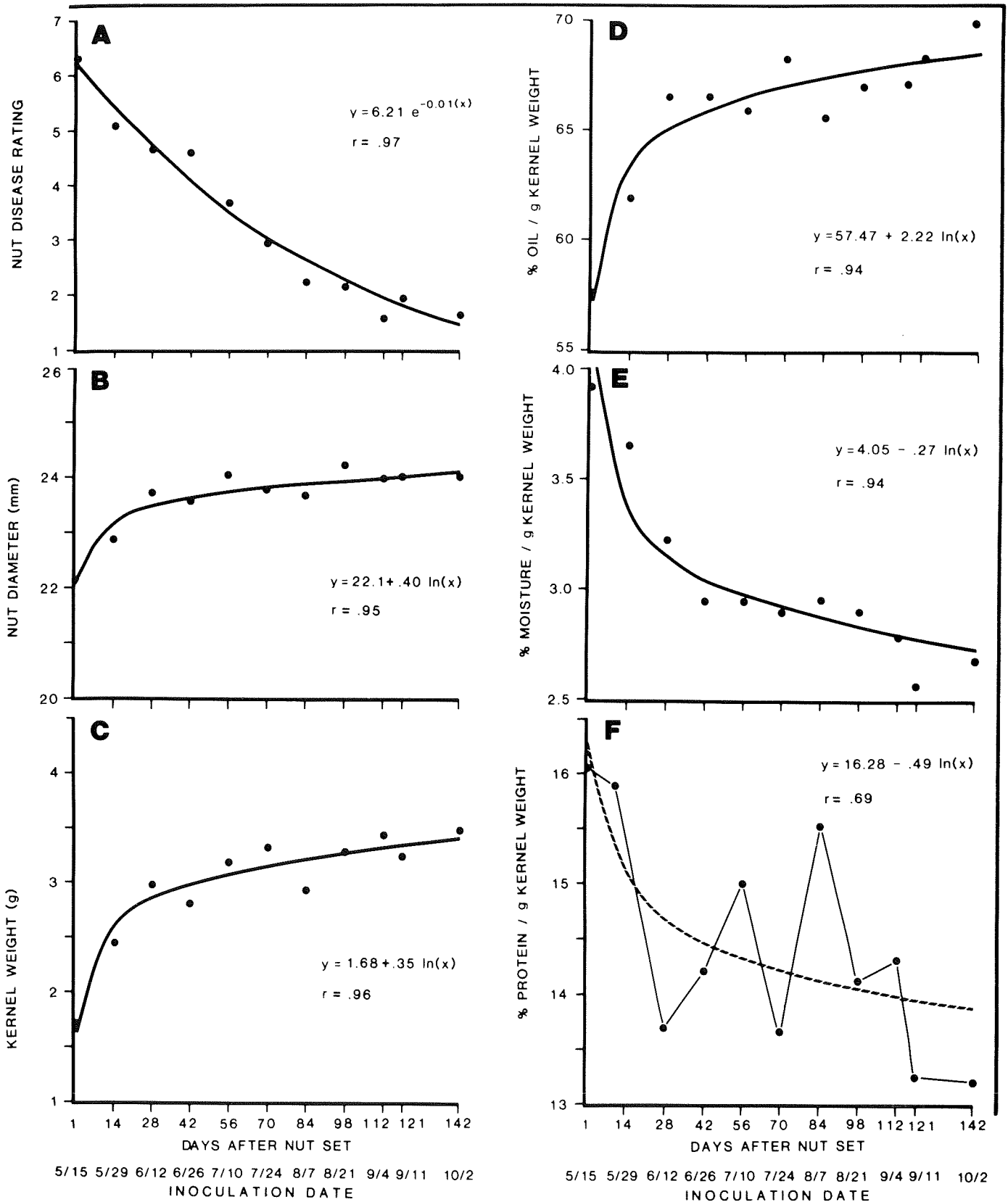


Fig. 4. The effect of pecan scab on nut quality. **A**, Season end disease rating for each of the eleven treatments. **B-F**, The effect of infection date on five parameters of nut quality. Individual points are overall averages for each treatment (10 inoculation dates and one uninoculated control which is plotted at 10/2 in each graph). Notice how the incidence angle of the predicted lines changes rapidly through the first two or three inoculation dates then levels off indicating that the major effect of pecan scab on nut quality occurs early in the season.

LITERATURE CITED

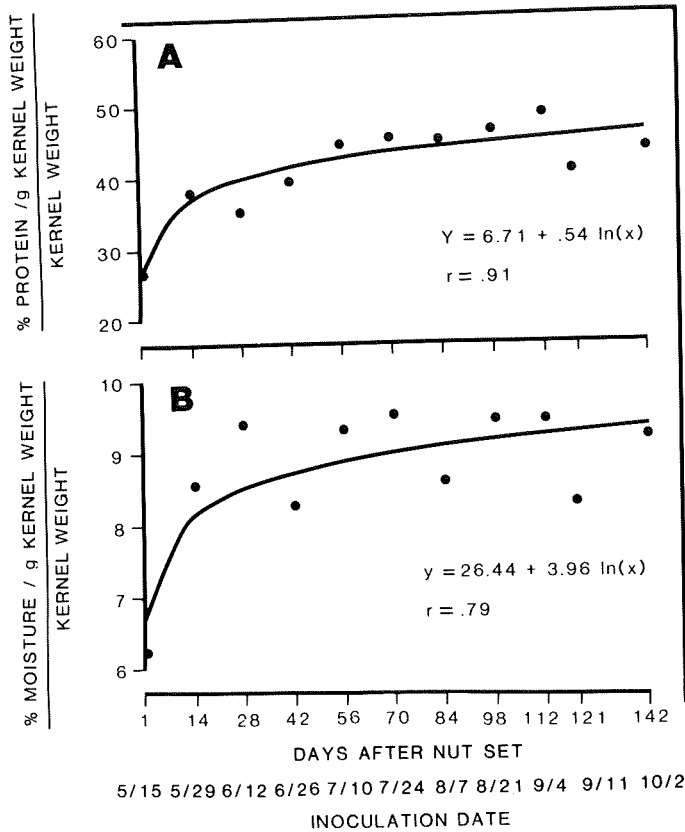


Fig. 5. The effect of pecan scab inoculation date on percent kernel protein and percent kernel moisture; effect of kernel weight removed. Notice that the major reductions in these quality parameters correspond to disease initiated from early inoculation dates. No appreciable loss in quality was found from disease initiated after late June. The point plotted at 10/2 represents the uninoculated control.

results of this study have raised many questions relating to the present scab disease control programs. Although the data imply that mid- and late-season scab may not be of sufficient economic importance to warrant the intense control it now receives, much additional research is required to determine the value of late-season fungicide sprays, especially for the maintenance of healthy foliage to reduce the tendency to bear less in alternate years, to reduce overwintering inoculum, and to maintain manageable levels of so called minor foliar diseases of pecan such as Zonate leafspot, powdery mildew, vein spot, downy spot, etc.

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