

# Crop Loss Assessment for Flue-Cured Tobacco Cultivars Infected with Tobacco Mosaic Virus

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## ABSTRACT

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Effects of inoculation date and incidence (percent plants infected) of tobacco mosaic virus (TMV) on yield, quality, and value of flue-cured tobacco were studied at two locations in North Carolina for 2 yr. Inoculation of seven susceptible cultivars with TMV in 1980 resulted in significant losses, which averaged 13, 16, and 16% in yield, grade index, and value, respectively. There were no differences in effects of inoculation at 7, 35, 49, or 63 days after transplanting either year of the investigation. Inoculation of 15, 30, 60, or 100% of the plants per plot with TMV in 1981 produced reductions of 7, 10, 17, and 30% in yield and 7, 14, 21, and 36% in value. No differences in leaf area and grade index of the tobacco were associated with the different incidences of the virus. Regression models were developed for estimating losses in flue-cured tobacco yield and value from TMV incidence data obtained through surveys conducted across North Carolina.

Tobacco mosaic virus (TMV) was estimated to cause losses of more than \$1 million annually in flue-cured tobacco production in North Carolina during the period 1960-1965 (3). Estimates of annual losses in value caused by TMV from 1966 to the present range from a low of 0.03% (\$1,583,055) in 1967 to a high of 0.88% (\$9,460,000) in 1980 (13). These estimates were made, however, by a subjective comparison method and their accuracy is unknown. Gooding (4) suggested a loss assessment method specifically for tobacco in North Carolina, but this method has never been used.

Valleau and Johnson (15) were among the first investigators to attempt to characterize the effects of TMV on tobacco yield and value. In 1927, they reported as much as 60% reduction in crop value caused by early mosaic infection. McMurtrey (10) conducted similar studies on Maryland tobacco and noted an average yield reduction of 30-35% and more than a 50% reduction in gross value. He also reported a relationship between time of infection with respect to plant growth and development and the subsequent severity of effects. Wolf and Moss (17) reported

similar findings in a study of the mosaic disease in flue-cured tobacco in North Carolina. They observed yield losses of 31, 30, and 17% when plants were inoculated at transplanting, 1 mo later, or at the topping stage, respectively. Losses in value were reported to be 55, 42, and 24% when plants were inoculated at transplanting, 1 mo later, or at topping. Chaplin (2) reported yield losses of 20% in yield and 24% in value when plants were inoculated at transplanting. Losses of 17% in yield and 29% in value were observed when plants were inoculated at the one-half grown stage. TMV inoculation at topping time had no apparent effect on yield or value. More recently, R. G. Paddick (*personal communication*) in Australia has related losses in tobacco yield and quality to TMV inoculation time.

This study was initiated with the following objectives: 1) to determine the effects and relative importance of date of TMV infection and percent TMV infection in North Carolina, 2) to study the effects of the interactions of these variables with cultivar and location on yield, quality, and value of flue-cured tobacco, and 3) to develop a TMV yield loss model to estimate regional yield losses from tobacco disease incidence survey data.

## MATERIALS AND METHODS

Experiments were conducted at the Border Belt Tobacco Research Station near Whiteville, NC, and the Upper Piedmont Research Station near Reidsville, NC, in 1980 and 1981.

Tobacco plants were transplanted from seed beds to the field with plant spacings of 60.96 cm and row spacings of 1.07 m. Inoculum was prepared and applied by methods similar to those

reported by Gooding (3). About 24 hr before inoculations, fresh tobacco leaf tissue systemically infected with the ATCC-1 isolate of TMV was homogenized in 0.005 M NaHPO<sub>4</sub>-KPO<sub>4</sub> buffer (1 g tissue/2 ml buffer) at pH 7.2 and pressed through cheesecloth. This filtrate was further diluted with buffer to give a tissue-buffer ratio of 1:10, then 1.5 g of 600-mesh Carborundum was added to the inoculum for each 100 ml of solution and the filtrate was stored until used.

Field inoculations of TMV-susceptible plants were made with an artist's airbrush (Thayer and Chandler, Inc., Chicago, IL 60610). Inoculum was propelled by carbon dioxide at 87.8 kg/cm<sup>2</sup> (30 psi) pressure. Plants were inoculated by spraying an area about 1.5 cm in diameter on the abaxial surface of a leaf about 5 cm long and near the top of the plant until the area was water-soaked. This procedure was then repeated on the next oldest leaf. All plants in each treated row were inoculated. TMV-resistant plants were not inoculated.

Border rows were placed between blocks inoculated at different dates as well as at each end of the test area in order to minimize spread of the virus among treatments. All field operations after inoculation were performed on control plots first and subsequently in reverse order to the sequence of inoculations to further minimize spread among treatments. In addition, all equipment was sprayed with a detergent solution immediately after passing through inoculated rows.

A split-plot experimental design with whole plots arranged according to a randomized complete-block design was used in 1980. Three replicates were used at Whiteville and four replicates were used at Reidsville. Whole plots consisted of 20-plant rows inoculated with TMV at 7, 35, 49, or 63 days after transplanting or left untreated to serve as controls. Subplots were rows of individual flue-cured tobacco cultivars. Seven cultivars susceptible to TMV (NC-2512, NC-2326, NC-744, McNair-944, NC-95, NC-82, and Speight-G28) and three resistant to the virus (NC-628, Coker-86, and VA-770) were used.

Leaf area and number of leaves per plant were assessed weekly in 1980 beginning 3 wk after transplanting. Four plants in each row were randomly selected and marked at the first observation. Leaf area was assessed by

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measuring the length and width of one leaf at the top, middle, and bottom layer of each plant in the sample. Leaf dimensions were then multiplied by 0.6345 to estimate the area of each leaf (12). Mean estimated leaf area for each plant sampled was multiplied by the number of leaves of each plant to obtain leaf area per plant. The four values of this variable obtained in each subsample were then averaged to provide a single estimate of leaf area per plant for each row.

In 1981, a split-split-plot design was used with seven replicates at each location. Whole plots consisted of flue-cured tobacco cultivars McNair-944 or NC-2326 arranged according to a randomized complete-block design. Subplots were blocks of rows inoculated 7 or 35 days after transplanting or not inoculated to serve as controls. Sub-subplots consisted of different TMV incidences, ie, different numbers of randomly assigned and inoculated plants per 20-plant row, which corresponded to 15, 30, 60, or 100% infection at Whiteville and 5, 15, 30, 60, or 100% infection at Reidsville. Weekly stand counts and visual assessments of the number of TMV-infected plants per row were made throughout the 1981 growing season.

After final harvest, all primings of all plots were cured, weighed, and graded by federal tobacco inspectors. A 0-99 tobacco grade index that groups federal tobacco grades according to equivalent value (16) was used to estimate the quality of each harvest or priming. This index estimates quality by measuring three characteristics of flue-cured tobacco leaves (leaf type, body, and color) that affect their market value. Flue-cured tobacco quality, however, includes other characteristics not reflected in the grade index. Average market prices for all flue-cured tobacco grades were obtained from

the Crop Science Department of North Carolina State University. The value for each harvest of each plot was calculated by multiplying the priming weight by the average market price of the priming grade. Yields and values were each summed and grade index values averaged over all harvests for each plot to obtain the total yield, total value, and average grade index of each plot. These variables were analyzed by analysis of variance. The Waller-Duncan test ( $k$ -ratio = 100) was used to identify significant differences among means for the different inoculation dates even though using a multiple-comparison procedure to analyze quantitative treatments is not considered appropriate from a statistical point of view (11). Use of quantitative treatments generally implies that the relationship between individual treatments is not as important to the experimenter as the overall relationship between the set of treatments imposed and the response variable(s) being measured. We considered the Waller-Duncan procedure appropriate in this case because our objective was to identify critical infection dates within the growing season rather than to characterize a general relationship between TMV inoculation date and tobacco yield, quality, and value.

Total yield, total value, and average grade index were also regressed upon date of TMV inoculation and/or percent TMV infection. Percent differences in total yield and value as well as in average grade index between the virus treatments were calculated by subtracting mean values of these variables for the different virus treatments from similar mean values for each susceptible cultivar control. These estimates of loss caused by TMV were also subjected to an analysis of variance and were regressed upon date of TMV inoculation and/or percent

TMV infection. Differences among treatment means were tested for significance by the Waller-Duncan procedure ( $k$ -ratio = 100). Residual plots for each regression were obtained and evaluated subjectively.

A survey of growers' fields was conducted in 1981 to study the pattern of TMV infection. Ten fields in which TMV was a problem were located in the county surrounding each test site and bimonthly observations of each field were made throughout the growing season. Survey sampling procedures were adapted from those of Main and Proctor (9). Each field was divided into four quadrants of equal size. Within each quadrant, two sample blocks were selected by a random procedure and marked. Each block consisted of five adjacent plants in four adjacent rows. Each sample block was observed for the number of TMV-infected plants until all plants were topped. At the final observation, the percent TMV incidence in each field was also assessed by the technique of Gooding et al (5).

## RESULTS

Typical symptoms (8) were observed on all inoculated plants within 14 days after inoculation. In 1980, TMV inoculation 7, 35, or 49 days after transplanting caused highly significant losses in yield, value, and quality (Table 1). Maximum losses at Whiteville were caused by inoculations 49 days after transplanting, whereas those performed 35 days after transplanting had the most severe effect at Reidsville. Maximum yield losses observed were 18% at Whiteville and 24% at Reidsville. Maximum differences in grade index and value were 23 and 19%, respectively, at Whiteville and 31 and 28%, respectively, at Reidsville.

Virus treatment effects were generally greater at Reidsville than at Whiteville, but the relative effects of the different inoculation dates were similar at both locations. TMV inoculation 7 or 35 days after transplanting reduced the average grade index of the treated plots in 1980 but not in 1981. TMV incidence did not significantly reduce average grade index in 1981. Reductions in grade index could not be attributed solely to any single character of the cured leaf but appeared to result from deviations in color, leaf body, and leaf type from the desired standards. Losses in grade index were often larger than yield losses but their effect on value seemed to be less than that exerted by losses in yield.

In 1981, inoculation 7 days after transplanting had the most severe effect on yield, grade index, and value at Whiteville but not different from the other inoculation date of 35 days after transplanting (Table 1). Maximum losses of 32% in yield, 37% in value, and 20% in grade index were observed in plots 100%

**Table 1.** Effect of time of inoculation with tobacco mosaic virus (TMV) on yield, grade index, and value of 10 TMV-susceptible cultivars of flue-cured tobacco

| Inoculation date (days after transplanting) | Yield (kg/ha) | Yield loss (%) | Value (\$/ha) | Loss in value (%) | Grade index <sup>x</sup> |
|---|---------------|----------------|---------------|-------------------|--------------------------|
| <b>1980<sup>y</sup></b>                     |               |                |               |                   |                          |
| Control                                     | 3,033 a       | ...            | 1,650 a       | ...               | 41.17 a                  |
| 7   | 2,632 b       | 13.14          | 1,373 bc      | 16.75             | 30.49 b                  |
| 35  | 2,421 c       | 20.20          | 1,259 c       | 23.69             | 32.07 b                  |
| 49  | 2,605 bc      | 14.03          | 1,388 bc      | 15.85             | 38.67 a                  |
| 63  | 2,865 ab      | 5.58           | 1,551 ab      | 6.02              | 41.94 a                  |
| Waller Duncan LSD                           |               |                |               |                   |                          |
| $k$ -ratio = 100                            | 384           | NS             | 202           | NS                | 4.74                     |
| <b>1981<sup>z</sup></b>                     |               |                |               |                   |                          |
| Control                                     | 3,533 a       | ...            | 1,940         | ...               | 11.96                    |
| 7   | 3,165 b       | 32.25 a        | 1,620         | 26.59             | 10.94                    |
| 35  | 3,058 b       | 27.07 a        | 1,730         | 36.31             | 10.86                    |
| Waller Duncan LSD                           |               |                |               |                   |                          |
| $k$ -ratio = 100                            | 132           | 7.35           | 90            | 9.53              | NS                       |

<sup>x</sup> Average quality was measured by a 0-99 grade index of federal flue-cured tobacco grades (16).

<sup>y</sup> Means summarize results from two locations with three replicates at one site and four replicates at the second site; means within a column followed by the same letter are not significantly different. NS = not significant.

<sup>z</sup> Means summarize results from two locations with seven replicates at each location; means within a column followed by the same letter are not significantly different. NS = not significant.

infected with TMV at this location. The data from Reidsville in 1981 were not included in the analysis because control plots were extensively contaminated with TMV about 35 days after transplanting. In both years, a quadratic polynomial provided the best fit to the data.

Regression equations were developed in 1980 for losses in flue-cured tobacco yield and value as functions of TMV inoculation date:

$$YL = 12.575 + 0.732T - 0.012T^2 \quad r^2 = 0.76 \quad (1)$$

$$VL = 16.557 + 0.775T - 0.014T^2 \quad r^2 = 0.68 \quad (2)$$

where YL equals percent loss in yield, VL equals percent loss in value, and T equals inoculation date in days after transplanting. Plots of residuals versus predicted values for each regression appeared to be random.

TMV incidence (percent plants infected) produced highly significant effects on yield and value but not on grade index. The relationships between TMV incidence and losses in yield and value were linear (Figs. 1 and 2). The regression equations obtained were:

$$YL = 1.696 + 0.198X \quad r^2 = 0.998 \quad (3)$$

$$VL = 2.483 + 0.226X \quad r^2 = 0.992 \quad (4)$$

where YL equals percent yield loss, VL equals percent loss in value and X equals percent TMV-infected plants in a field. Yield and value were reduced at rates of 0.198 kg/ha and 0.226 \$/ha, respectively, per 1% increase in TMV incidence. Subjective evaluations of plots of residual versus predicted values for each regression indicated no nonrandom associations.

Inoculation 7 or 35 days after transplanting reduced leaf area per plant compared with the control plots; later inoculations had no such effect (Table 2). The average area of individual leaves was reduced by inoculation 35 days after transplanting but not by any of the other treatments. The number of leaves in plots inoculated 7 days after transplanting was significantly different from those measured in all other plots, as was the number of leaves measured in the control plots.

Incidence and spread of TMV were monitored in 13 of the 20 fields surveyed in 1981 (Figs. 3 and 4). Incidence of TMV

**Table 2.** Effect of time of inoculation with tobacco mosaic virus (TMV) on leaf area and number of leaves

| Inoculation date<br>(days after<br>transplanting) | Leaf area<br>(mm <sup>2</sup> ) | Leaves<br>(no.) |
|---|---------------------------------|-----------------|
| Control   | 137,922 a                       | 18.56 a         |
| 7   | 110,789 b                       | 16.95 c         |
| 35  | 109,313 b                       | 17.84 b         |
| 49  | 128,732 ab                      | 18.06 a         |
| 63  | 122,535 ab                      | 18.35 a         |
| Waller-Duncan LSD<br>k-ratio = 100                | 21,742                          | 0.62            |

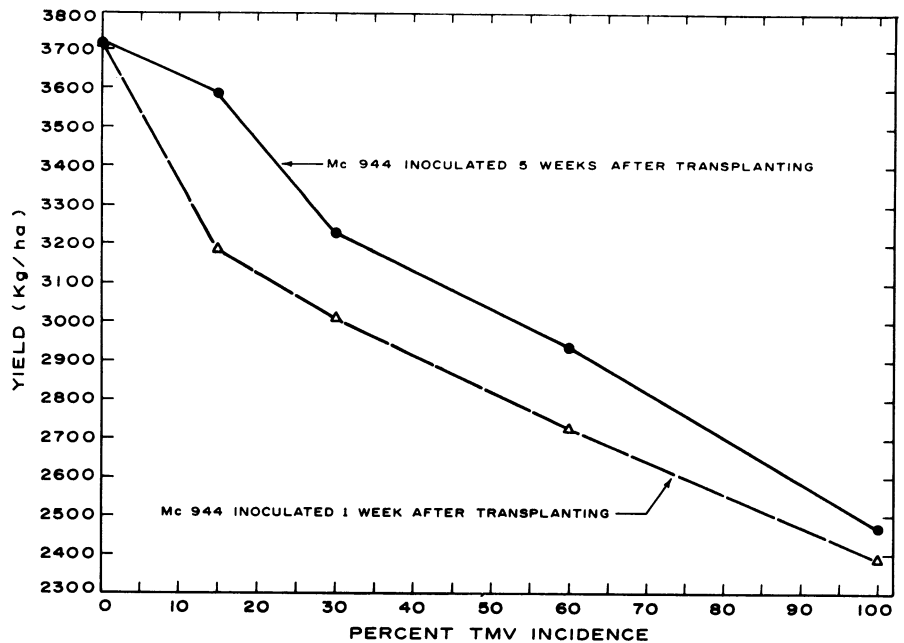
varied between 0.6 and 46%. Primary TMV infection was first observed about 3 wk after transplanting, whereas most secondary infection occurred between 4 and 7 wk after transplanting. Secondary spread accounted for over 80% of all infections observed in fields with over 12% TMV (Table 3). In all but one of the fields significantly infected with TMV, more than 90% of the infections appeared to result from secondary spread of the virus.

## DISCUSSION

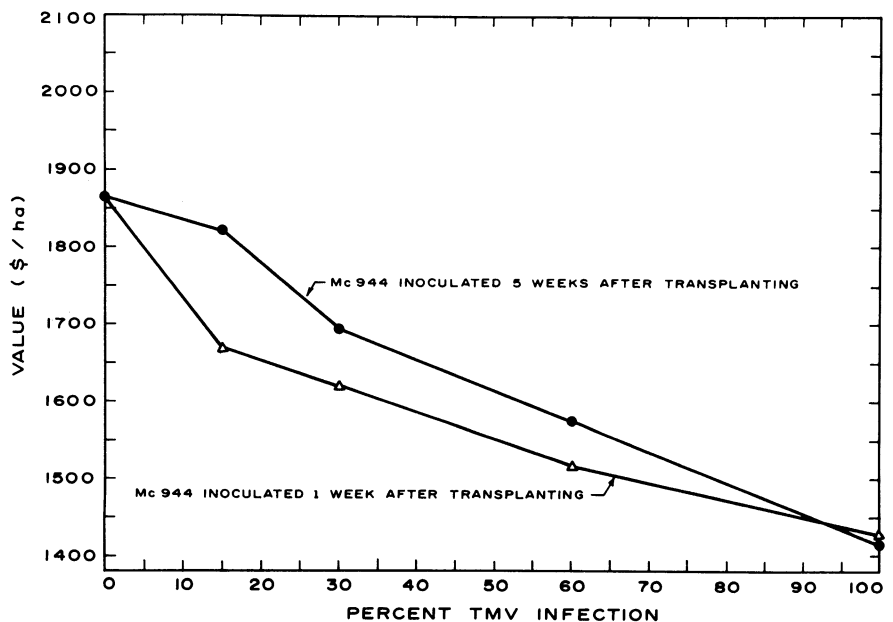
Yield and value did not differ among susceptible cultivars as a result of TMV infection, indicating that a TMV crop

loss model can be applied to a wide range of TMV-susceptible cultivars.

Location-treatment and year-treatment interactions were significant. These results are not unreasonable because of the differences in environmental factors between the two test sites as well as between years. The Whiteville location has very sandy soils, generally high temperatures and relative humidities, and frequent rainfall. The Reidsville location has heavy clay soils and is usually cooler and dryer than the Whiteville location. The beginning of the 1981 growing season was very dry throughout North Carolina and this drought could have influenced the effects of TMV on the crop.



**Fig. 1.** Yield as a function of tobacco mosaic virus (TMV) incidence for McNair-944 tobacco at Whiteville, NC, in 1981.



**Fig. 2.** Value data versus tobacco mosaic virus (TMV) infection for McNair-944 tobacco at Whiteville, NC, in 1981.

Chaplin (2) concluded that TMV infection did not affect the number of leaves produced per plant. In this investigation, TMV inoculation 7 days after transplanting reduced the number of leaves per plant measured at the topping stage although the number of leaves per plant did not always differ when counted at earlier points in the season. Although inoculation with TMV produced differences in yield or leaf weight, it did not consistently produce differences in leaf area. TMV perhaps causes leaves to be thinner or less dense or to contain less water, thus reducing yield.

Previous work on TMV-induced crop losses in tobacco has focused on the date of inoculation alone. Incidence of the

virus was not considered. One reason for this approach may have been the difficulties involved in controlling TMV infection in the field, especially in small plot tests. Special precautions must be taken to minimize spread of the virus among treatments as well as within particular specified incidence treatments.

The yield and value reductions observed in this study were well below those reported by several other workers (8,10,14,15,17) but similar to those reported by Chaplin (2). This difference in results may be attributable to differences in the precision of the different estimates or to differences in cultural practices, climate, and/or cultivar used in the various tests. The

patterns exhibited by the effects of inoculation date on yield, grade index, and value were also unexpected. Earlier infection with TMV did not always result in more severe effects on tobacco growth, yield, and value. Losses caused by TMV inoculation 7 and 35 days after transplanting were not different from each other but were different from the controls. Inoculation date, therefore, does not appear to be as important a consideration in assessing crop loss due to TMV as we had anticipated.

Gooding (3,7) reported that TMV-infected crop debris in the soil is the main source of primary TMV infection in North Carolina. It has also been observed that primary infection is generally restricted to very small proportions of the plants in infected fields and that symptoms of these infections were visible 3-4 wk after transplanting (3,4,7). Gooding (3) estimated that 90% of the TMV infection of tobacco in North Carolina results from secondary spread of the virus from such infections. The results of this study tend to support this observation (Figs. 1 and 2; Table 3).

Date of inoculation and percent incidence appeared to be highly correlated variables for the mosaic disease of tobacco. Because the effects of inoculation date were not always significant and the majority of TMV infections appeared to result from secondary spread of the pathogen anyway, percent TMV incidence should be a more sensitive variable than date of infection for assessing this disease and its effect on tobacco production. A single assessment of TMV incidence made at some critical point in the season should provide sufficient information to assess losses. Secondary spread probably occurs most often through the contact of healthy and infected plants with field equipment. If this contact occurs primarily between 4 and 8 wk after transplanting when the crop is growing rapidly and cultural operations are performed frequently, such spread should usually occur within the same time frame.

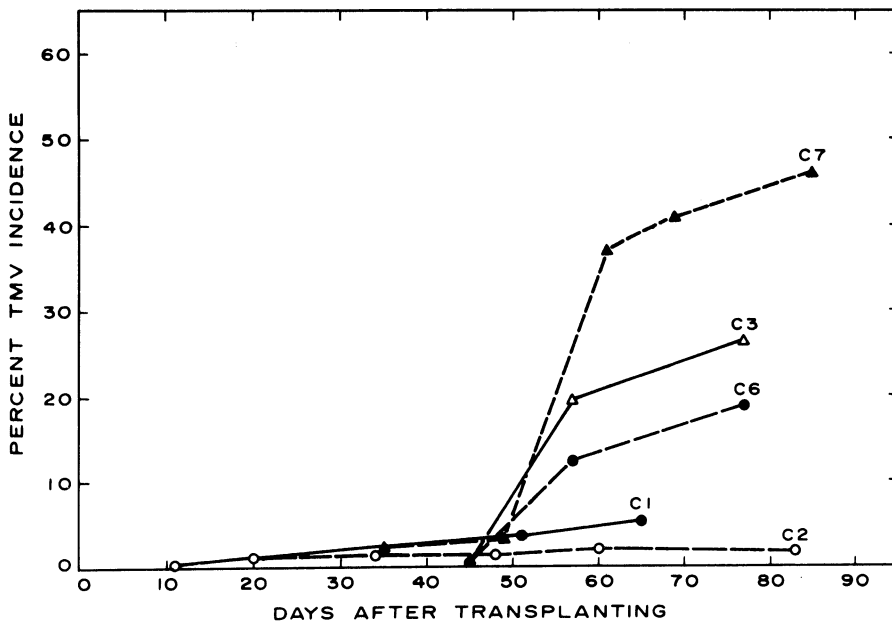


Fig. 3. Spread of tobacco mosaic virus (TMV) in five tobacco fields in Columbus County, NC, in 1981.

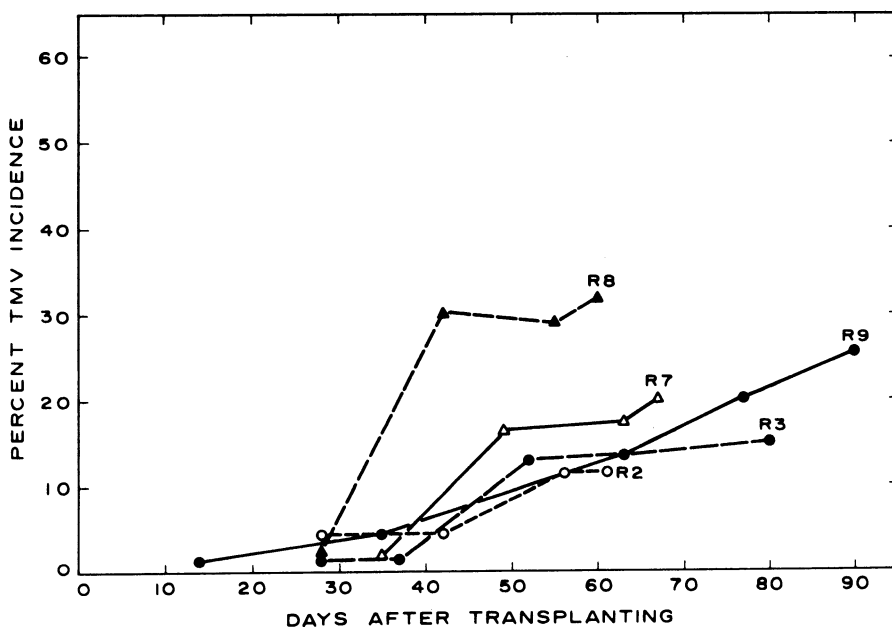


Fig. 4. Spread of tobacco mosaic virus (TMV) in five tobacco fields in Rockingham County, NC, in 1981.

Table 3. Patterns of spread of tobacco mosaic virus (TMV) in 10 tobacco fields in two counties in North Carolina in 1981

| Field   | Total TMV infection     |                                 |
|---------|-------------------------|---------------------------------|
|         | Final TMV incidence (%) | 6-11 wk after transplanting (%) |
| C1      | 5.2                     | 87.5                            |
| C2      | 2.0                     | 33.3                            |
| C3      | 26.4                    | 97.6                            |
| C6      | 18.9                    | 96.6                            |
| C7      | 46.2                    | 94.5                            |
| R2      | 11.6                    | 61.1                            |
| R3      | 15.0                    | 91.7                            |
| R7      | 20.1                    | 90.6                            |
| R8      | 31.8                    | 91.8                            |
| R9      | 25.3                    | 82.0                            |
| Average | 20.3                    | 82.7                            |

If most of the TMV infection in North Carolina occurs at a similar growth stage and the incubation period for TMV is 10–14 days (3), symptoms of most TMV infection should be visible about 6–10 wk after transplanting. The apical buds of the flue-cured tobacco crop are removed (topped) about 10 wk after transplanting and no new leaves are added to the crop after that time. TMV symptoms also “fade” after this point in the season because of physiological changes in the crop. Thus, the optimal point in the tobacco growing season to assess the amount and severity of TMV in the field is between 6 and 10 wk after transplanting and preferably between 8 and 10 wk in order to observe all infections (4).

Regression models were developed to describe percent differences (losses) in yield and value caused by TMV rather than yield and value per se because one of the objectives of this research was to develop a method to expand flue-cured tobacco disease incidence sample-survey results into estimates of statewide loss caused by TMV (6). On an individual field basis, an estimate of yield may be preferable to a prediction of percent loss, but the focus of this project was on crop production losses rather than crop production itself. The dependent variables of the regression models reflect this approach.

The intercepts for the yield loss equations did not equal zero. The regressions could have been manipulated so that the intercepts would equal zero but we decided that such an approach would not improve the accuracy or usefulness of the models in predicting loss. Student's *t* values for the intercepts of the regressions for yield and value losses were 4.98 ( $P=0.038$ ) and 2.95 ( $P=0.0985$ ), respectively.

The regression equations for losses in value assume that value is unaffected by supply/demand fluctuations or other market forces. This virus appears to affect yield only when present in a very large proportion of the crop. TMV is

estimated to infect about 5% of the tobacco crop in North Carolina (3). It is unlikely that TMV incidence would ever be high enough to seriously affect the supply of flue-cured tobacco to the point that tobacco value would be increased.

Models relating crop losses to inoculation date were developed to fit the data from both locations of this investigation even though interactions occurred between location and inoculation date. The pattern of yield losses was similar at both locations and although the severities of losses at the two locations of this study were significantly different, the regression models accounted for most of the variation in the combined data as reflected in the regression coefficients obtained for each model.

The results of this study indicate that losses in flue-cured tobacco yield, quality, and value caused by TMV are not as large as had been generally believed but are still important enough to require careful management. A single assessment of TMV incidence alone made between 8 and 10 wk after transplanting each season appears to be the best method for assessing tobacco mosaic in the field for purposes of tobacco disease loss assessment. Tobacco disease surveys conducted across North Carolina will provide these assessments of TMV incidence. Survey results can be entered into the appropriate regression equation and accurate objective estimates can be obtained for losses in flue-cured tobacco yield or value caused by TMV. Such estimates will be useful for evaluating research and disease management strategies for TMV in flue-cured tobacco as well as serving as the basis for econometric trade-off models for this disease (1).

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