AN ASSESSMENT SYSTEM FOR ESTIMATING THE DISTRIBUTION AND INCIDENCE OF TOBACCO VIRUSES IN NORTH CAROLINA [1]

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A system for assessing the distribution and incidence of tobacco viruses is given. Essentially, one in 1 x 10^5 plants in several areas of tobacco production in North Carolina comprises the sample and the data are extrapolated to the total crop to determine overall incidence. Virus incidence on tobacco in North Carolina was estimated from data obtained from 1977-79. Incidence on flue-cured tobacco for the three years was 1) tobacco mosaic virus 3.89, 5.60, and 8.48%, 2) potato virus Y 0.99, 0.54, and 1.65% and 3) tobacco etch virus 0.05, 0.17, and 0.68%. Incidence on burley tobacco was 1) tobacco vein mottling virus 3.19, 12.16, and 24.32% and 2) tobacco etch virus 0.09, 7.56, and 4.29%. Mixed infections and incidence of alfalfa mosaic, cucumber mosaic, peanut stunt, tobacco ringspot and tobacco streak viruses were less than 0.05%.

INTRODUCTION

Knowledge of the distribution and incidence of diseases and their dynamics in a crop is basic to developing disease control strategies, estimating disease losses and establishing research priorities. Ideally, disease progress should be recorded throughout the growth period of all plants in the population. Such ideal situations seldom exist, so priorities must be established on the basis of information desired and availability of resources for data collection.

An epidemic of tobacco vein mottling virus on burley tobacco (60% of the crop estimated to be infected) and potato virus Y on flue-cured tobacco (10% of the crop estimated to be infected) in 1976 gave impetus to implementing a long range project to develop a system, with statistically definable parameters, for monitoring the distribution and incidence of tobacco viruses in North Carolina. Objectives during the first three years (1977-79) of this project were 1) to evaluate sampling procedures and 2) to obtain data on distribution and incidence of viruses required to develop a monitoring system. The sampling scheme used was designed to obtain data on spread of PVY (4). Therefore, we are not reporting measures of precision, such as confidence limits, for disease distribution and incidence.

MATERIALS AND METHODS

Virus identification: Viruses were identified by field symptoms or by biological and serological assay.

Most of the viruses found on tobacco in North Carolina can be identified by field symptoms alone by one proficient in the art. Proficiency, however, requires knowledge of varietal and environmental effects on symptoms. Where data are reported in this study to be based on field symptoms, approximately 90% of the identifications were based on symptoms alone with the remainder confirmed or established with biological and serological assay. The latter procedure was applied where

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Contribution received July 11, 1980. Tob. Sci. XXIV 162-165, 1980.

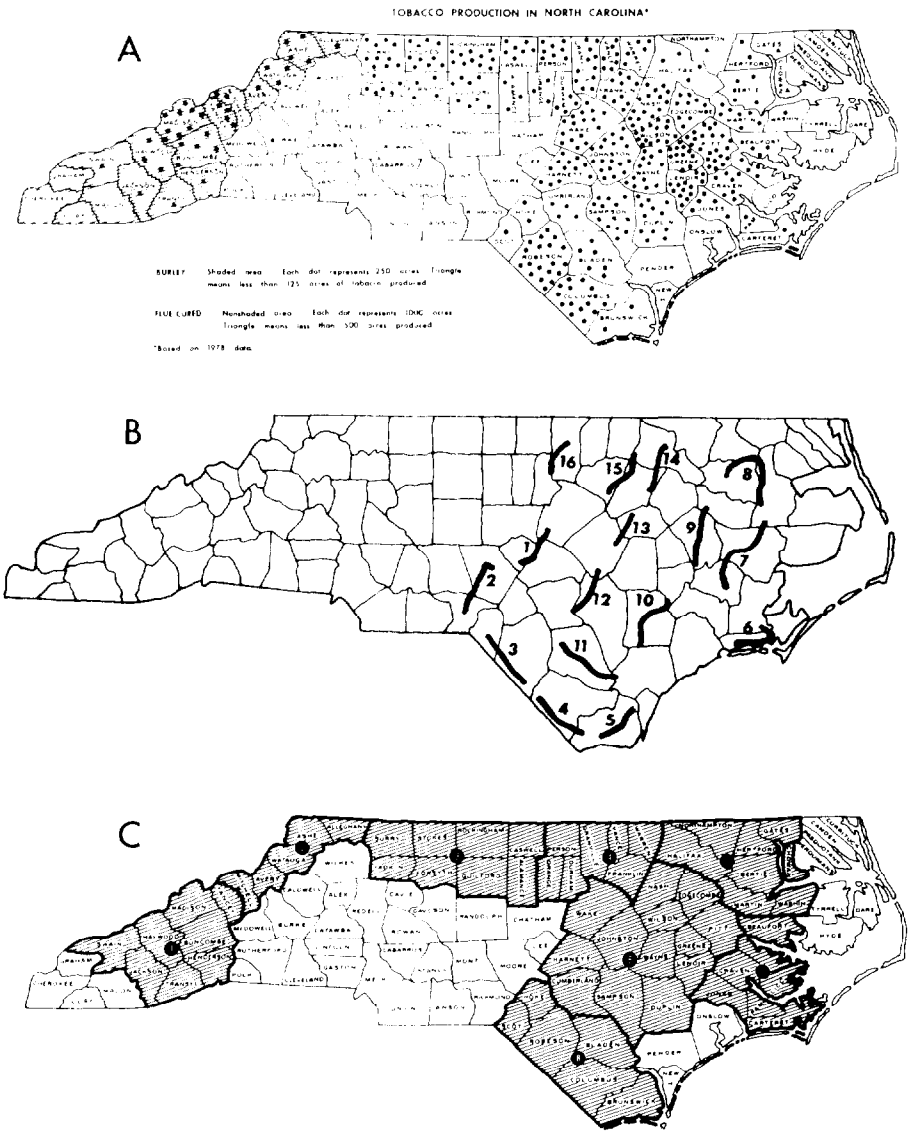

Figure 1. A - Tobacco production in North Carolina. B - Sample locations. C - Areas used in weighing incidence data.

symptoms were atypical such as in mixed infections.

Identifications based on assays involved use of an indicator plant and serological techniques. Juice from a leaf was extracted using a mechanical roller-press (6), diluted 1:10 with 0.1 M K_2HPO_4-KH_2PO_4(pH 7.2) and applied to the interveinal area on two leaves of *Nicotiana tabacum* cv. Burley 21 with a cotton swab previously dipped in 600 mesh carborundum. Tobacco mosaic virus (TMV) was recorded as present when local lesions (LL) developed on the inoculated leaves. Plants developing systemic symptoms were assayed serologically to identify viruses other than TMV (3).

Tissue from inoculated leaves with LL's (TMV) or from systemically infected leaves (other viruses) was dried (9) to maintain isolates for strain identification and other studies.

Geographic areas sampled and sampling techniques: Flue-cured tobacco is produced from the coast to the mountains of North Carolina (**Fig. 1-A**). Variations in climate, soils and cropping systems in this area create a potential for ecological niches for various pathogens, including viruses. Primary sampling emphasis in this study was, therefore, given to geographical coverage rather than intensity of tobacco culture. Ten fields in each of 16 locations were used for data collection (**Fig. 1-B**). Fields in each location were adjacent to a section of a secondary road about 25 miles in length. Prior to sampling, each field in a location (25-50 fields) was numbered and ten fields were randomly chosen for sampling. Growth stage of the plants at time of observation usually was early to late flower (60-70 days after transplanting). Virus incidence in each field was determined both by symptom expression and by random assay. Incidence based on symptom expression was determined by observing 100 plants in each of ten rows in the most accessible corner of each field. Observations were made on every fourth row so that total area observed in each field was about 1/4 ha. The random assay was based on virus incidence in ten plants randomly selected from the area surveyed. An apical leaf on each plant was assayed biologically and serologically as previously described.

Burley tobacco is produced only in the mountains of North Carolina but wide variations in climatic conditions, soil types and cropping systems, as with flue-cured tobacco, give rise to potential ecological niches. Virus observations were made in nine counties where 95% of the burley crop is produced. Sixteen fields were observed each year and the number of fields per county was proportional to the acres produced. Fields were selected based on accessibility rather than randomly but virus occurrence in the fields was unknown prior to sampling. The average size of each field sampled was approximately 1/3 ha, which is about the average size of burley fields. Virus incidence was recorded on every fourth row in each field based on symptoms. Fields where symptoms were not distinctive were randomly assayed (25 pls) and virus identity determined with indicator plants and serology.

Table 1. Distribution and incidence of tobacco mosaic virus, potato virus Y and tobacco etch virus in flue-cured tobacco from 1977-1979 in North Carolina

Location[a]	Virus Incidence (%) Tobacco Mosaic Virus 1977	1978	1979	Potato Virus Y 1977	1978	1979	Tobacco Etch Virus 1977	1978	1979
Area 3	-	-	-	-	-	-	-	-	-
Area 4	4.14	8.40	10.63	0	0.01	0	0.02	0.14	0.01
Area 5	2.12	1.88	0.03	0	0.10	0.14	0	0	0.02
Area 6	5.41	6.17	11.47	0.88	0.06	1.75	0.10	0.26	0.83
Area 7	11.25	16.10	13.94	5.36	8.22	13.22	0.01	0.40	4.49
Area 8	1.85	2.54	7.34	2.21	0.16	0.76	0.01	0.09	0.38
Mean[b]	3.89	5.60	8.48	0.99	0.54	1.65	0.05	0.17	0.68

[a] Area 3 - no data in this survey but expected to be similar to area 4. Refer to Fig. 1.

[b] Mean weighted to reflect proportion of flue-cured crop produced in each area.

RESULTS

Virus identification: Viruses detected on flue-cured tobacco were tobacco mosaic virus (TMV), potato virus Y (PVY), tobacco etch virus (TEV), tobacco ringspot and peanut stunt viruses. Incidence of the latter two and mixtures of viruses was less than 0.05% so is not reported under distribution and incidence data. Incidence of strains of PVY is reported elsewhere (4).

Viruses detected on burley tobacco were tobacco vein mottling virus (TVMV), TEV, PVY, alfalfa mosaic, cucumber mosaic, tobacco ringspot and tobacco streak viruses. Incidence of the latter four and mixtures was less than 0.05% and is not reported under distribution and incidence data.

Virus distribution and incidence: Virus distribution and incidence in the locations observed on flue-cured tobacco varied primarily by location with TMV but by location and time with PVY and TEV. Integration of these results with intensity of cultivation by county resulted in six geographic areas that are used to summarize the distribution and incidence data (**Fig. 1**) (**Table 1**).

Incidence based on symptoms was consistently lower than incidence based on random assays for TMV and was usually lower for PVY and TEV. Mean incidence over the three years in all sample locations was 1) Symptoms: TMV (7.06%), PVY (1.64%) and TEV (0.35%) and 2) Assay: TMV (23.69%) PVY (1.96%) and TEV (0.48%).

Virus distribution and incidence in the locations observed on burley tobacco were more uniform than on flue-cured tobacco but diferences that were observed and topographical considerations resulted in the burley production area being divided into two divisions for data reporting (**Fig. 1**) (**Table 2**).

DISCUSSION

These data primarily were collected to serve as a basis for development of a system with definable statistical parameters for estimating distribution and incidence of tobacco viruses. Reliable data on distribution and incidence will serve as a basis to 1) to establish what viruses are present, their effects on current and potential losses when disease incidence is combined with other potential spread and loss parameters and 2) to extend knowledge on the ecology of the viruses by establishing correlations among distribution and incidence in different geographical areas with such factors as alternate host crop and indigenous host occurence, vector populations, resistant or susceptible germplasm deployment and cultural practices.

All of the viruses detected in this study previously have been reported from North Carolina (2, 11). Potato virus X and tomato ringspot viruses have been reported on tobacco in North Carolina but were not detected in this study. An alternative to the assay procedure used in this study for virus identification would be to bypass the inoculations to B-21 and use only serological techniques. This option was not used because 1) detection of previously unreported viruses would be more cumbersome and 2) for unknown reasons (perhaps chemicals

Table 2. Distribution and incidence of tobacco vein mottling and tobacco etch viruses in burley tobacco from 1977-79 in North Carolina.

Location[a]	Virus Incidence (%) Tobacco Vein Mottling Virus 1977	1978	1979	Tobacco Etch Virus 1977	1978	1979
Area 1	2.32	9.13	20.69	0.14	11.02	5.36
Area 2	5.09	18.73	32.13	0	.09	2.00
Mean[b]	3.19	12.16	24.32	0.09	7.56	4.29

[a] Refer to Fig. 1.

[b] Mean weighted to reflect proportion of burley crop produced in each area.

applied to the crop) we occasionally have difficulties with serological tests with field-collected samples (3).

Although we considered this a pilot study, at least two interesting correlations emerged concerning virus distribution and incidence. Tobacco mosaic virus incidence in the different areas increased when fields were continuously planted in tobacco but decreased when tobacco was in rotation with other crops. This emphasizes the importance of soil-borne inoculum of TMV (5). Potato virus Y incidence was positively correlated with inoculum from potatoes (8) in area 7 (**Table 1**). The sporadic occurrence of PVY in area 6 may be due to the sporadic introduction of inoculum from potatoes, tomatoes or peppers in this area (7).

The differences obtained between virus incidence, especially TMV, based on field symptoms and random assay is not surprising but these data give an estimate of the magnitude of the differences. Tobacco mosaic virus primarily is considered transmitted by mechancal means. Conditions are created during two main periods of tobacco culture for spread. The first period occurs 4-5 weeks after transplanting when the crop is cultivated for agronomic reasons. Virus transmitted during this period results in symptoms on plants 1-2 weeks later. The second period starts when the flower buds are removed and continues through harvesting. Symptoms developing from infections occurring when the flower buds are removed are very mild because most of the leaves are mature and even these symptoms frequently are obscured as a result of chemicals applied to prevent axillary bud development. The higher incidence of PVY and TEV detected by assay vs symptoms primarily is attributed to late infections but other factors, as with TMV, may have had an influence. The higher incidence of TMV detected by the assay procedure would make this the preferable technique for studies such as correlation of quantity of soil-borne inoculum (stalk and root debris) with primary infection in the follwing crop (5). An objective of this project, however, is to develop a reliable system for determining virus incidence that can be used in estimating losses. Studies with at least two tobacco viruses, TMV and PVY, indicate that little or no losses occur when infection occurs at or about the time of flower bud removal (1, 10). Although virus incidence increases after this time, data obtained at flowering can also be used to correlate distribution and incidence with ecological factors.

The greatest difficulty experienced during this study was obtaining fields at the optimum growth stage (mid-flowering) for data collection. The random selection of fields in each location for sampling was statistically sound but resulted in some fields being observed before or, usually, after mid-flowering. Sampling techniques in future studies will be designed so that data can be obtained from fields in the optimum stage of growth.

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