Distribution, Incidence, and Strains of Viruses in Burley Tobacco in North Carolina

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ABSTRACT

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Burley tobacco fields were surveyed for viruses in five counties in North Carolina in 1984. Data were recorded from about 800 plants ($\bar{x}$) in each of 10 fields in each county. Virus incidence based on the approximately 40,000 plants observed was 25.7% for tobacco vein mottling virus (TVMV), 7.1% for tobacco etch virus (TEV), 0.2% for alfalfa mosaic virus, 0.1% for tobacco ringspot virus, $<0.1\%$ for tobacco streak virus (TSV), $<0.01\%$ for potato virus Y (PVY), and $<0.01\%$ for peanut stunt virus. Viruses except PVY and TSV occurred in all areas in the burley production region. TVMV existed as a single strain, based on symptoms induced on the differential tobacco germ plasm, but TEV isolates were differentiated into mild, moderate, and severe strains.

A breeding program to improve burley tobacco (*Nicotiana tabacum* L.) cultivars for production in North Carolina was initiated by the second author in 1984. One of the objectives of this program is to develop virus-resistant tobacco cultivars because viruses are recognized as causing significant losses on the crop (4,5,11). A survey was conducted in 1984 to establish the identities of the viruses currently causing these losses and to determine the strain composition of tobacco vein mottling virus (TVMV) and tobacco etch virus (TEV), previously reported to be the most important viruses of burley tobacco in North Carolina (4).

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MATERIALS AND METHODS

Geographical area sampled and sampling technique. Burley tobacco is produced in the mountains of North Carolina that extend from the Virginia to South Carolina border. Five counties representing different geographical areas of production were used to assess virus incidence and to collect isolates of TVMV and TEV. The southern counties, with Graham the epicenter and surveyed county, produce about 10% of the crop and were designated production area I. Three counties in the central part of the production zone, Buncombe, Madison, and Yancey, the epicenter and surveyed counties and their four contiguous counties, were designated production area II. About 65% of the crop is produced in this area. The northern counties, with Watauga the epicenter and surveyed county, produce about 25% of the crop and were designated production area III. Virus incidence was determined in 10 fields in each of the five counties selected for this study.

Fields to be assessed were selected with the assistance of the burley tobacco extension agent in each county. Each county consists of several distinct valleys with more than 75% of the tobacco in

each county located within two to five of these valleys. The number of fields assessed in each valley was proportionate to the amount of tobacco produced in the county. Data were obtained from randomly selected fields in each valley.

The number of plants on which virus incidence was recorded in a field varied according to its size. All plants in every fourth row were assessed in fields of less than 1 acre, every eighth row in fields of 1–2 acres, every 12th row in fields of 2–3 acres, and every 16th row in fields of 3–4 acres. Field size ranged from about 0.2–3.5 acres ($\bar{x}$ = 0.4 acres). Data were recorded from about 800 plants ($\bar{x}$) in each field, so virus incidence in each county was based on about 8,000 plants.

Virus identification. Viruses were tentatively identified based on symptoms on plants in the field. The identity of the virus associated with each type of symptom was established serologically (2).

Assay for virus strains. Isolates for strain identification of TVMV and TEV were taken from random plants with virus symptoms from fields used to establish virus incidence. The collection thus consisted of isolates from each of four plants in 10 fields in each of the five surveyed counties (200 total isolates). Virus isolates collected from the field were assayed (2) for all viruses known to occur in burley tobacco in North Carolina (4). Eighty-six of the isolates were TVMV, 36 were TEV, and the remainder were other viruses or mixtures of viruses. Twenty-five randomly chosen TVMV and TEV isolates were compared for the symptoms they induced on different tobacco germ plasm. The germ plasm consisted of three commercial tobacco cultivars, Ky 14, Burley 49 (B49), and Ky 14XL8, that differ in their reactions to these viruses (5,6,9) and three entries that have been reported resistant to one or both of these viruses, Greenville 107 (7,8), V20 (1), and Havana 307 (13).

One isolate each of TVMV (NC 148-P) and TEV (NC-19-P), previously purified and characterized (6,10,15), were used as a basis of comparison among the isolates in this study. Also, the strain of TEV reported by Simons (12) was included.

Isolate comparisons were conducted in a greenhouse (26 ± 5 C) in 10-cm-diameter clay pots. Entries were the same age and similar in size when inoculated. At the time of inoculation, the largest leaf on Ky 14 was 8–10 cm long. The two largest leaves on each plant were inoculated with a cotton swab dipped in 600-mesh Carborundum, then in leaf juice from an infected B21 plant macerated in 0.05 M Na_2HPO_4-KH_2PO_4 buffer, pH 7.2 (1 g of tissue:2 ml of buffer). Symptoms were recorded 4 wk after inoculation, using a disease severity index of 1–5 (1 = mild symptoms and 5 = severe symptoms). Three plants of each tobacco entry were inoculated with each isolate in two trials.

RESULTS

The most important viruses detected, based on incidence, were TVMV and TEV. Alfalfa mosaic virus (AMV), tobacco ringspot virus (TRSV), tobacco streak virus (TSV), potato virus Y (PVY), and peanut stunt virus (PSV) also were detected. The distribution and incidence of viruses in the counties sampled, except PVY and PSV, whose incidences were less than 0.01%, are summarized in Table 1.

The different isolates of TVMV could not be differentiated as distinct strains based on symptoms on the different tobacco germ plasm used. None of the isolates of TVMV caused veinal or interveinal necrosis on any of the entries. Isolates did not differ antigenically from TVMV type isolate NC 148-P using the agar-gel double-diffusion technique (2) and NC 148-P antiserum. Entries Greenville 107, V20, and Havana 307 were infected by all isolates of TVMV but were tolerant in their reactions compared with commercial entries Ky 14, B49, and Ky 14XL8.

The TEV isolates constituted a continuum from mild to severe based on the disease severity index averaged across the three commercial cultivars. The isolates caused various degrees of stunting and mosaic symptoms but no veinal or interveinal necrosis. Most of the isolates caused only mild symptoms on resistant entries Gr 107, Ha 307, and V20. Isolates did not differ antigenically from TEV type isolate NC-19-P using the agar-gel double-diffusion technique (2) and NC-19-P antiserum. There was no correlation between the geographic origin of the isolates and the severity of symptoms they caused.

DISCUSSION

The relative incidence of viruses in burley tobacco found in this study was similar to that found in 1977–1979 (4). Similar data were also found in a survey of burley tobacco viruses from 1981 to 1983 (G. V. Gooding, *unpublished*). The relative incidence of the viruses remained the same during this 8-yr period, but the amount of virus from year to year varied greatly. For example, the incidence of the most prevalent virus, TVMV, varied from 2.3% (1977) to 33.6% (1981). TVMV and TEV are the only viruses currently considered to cause sufficient losses on burley tobacco to justify breeding for resistance to them (11). This could, of course, change in the future because of the dynamic nature of some of the viruses. This is especially applicable to PVY (3), and it may be significant that more of this virus was found in 1984, even though the incidence was low, than during the previous 20 yr (G. V. Gooding, *unpublished*).

The occurrence of TVMV, AMV, TRSV, and PSV was not associated with any geographic area of burley tobacco production in North Carolina. Incidence of TEV, in both this and previous studies, was highest in the southern area and lowest in the northern area of production. TSV primarily was found in Madison County. In the 20 yr prior to 1984, PVY was identified only twice on burley tobacco from all sources, including surveys and samples submitted by county agents. PVY was found on only a few plants in fields in Yancey County included in this survey. There was an outbreak of the virus in several fields in one area of the county, however, and incidence in one field exceeded 50%.

It is considered fortunate for the breeding program that the most important virus, TVMV, apparently consists of one predominant strain for which there is tolerant germ plasm (7,9).

TEV apparently consists of a population of isolates that vary from mild to severe based on the symptoms they cause on commercial cultivars of burley tobacco. The number of definable strains probably would be limited only by the number of commercial entries and the precision of severity measurement. We consider it sufficient for our needs to divide the isolates into three strains based on the disease index across the commercial cultivars used in this study.

We use the term "mild strain" for isolates causing a mean disease index of 2.0 or less, "moderate strain" for isolates

Table 1. Distribution and incidence of viruses in burley tobacco in North Carolina in 1984

		Virus incidence (%)[a]									
		TVMV[b]		TEV		AMV		TRSV		TSV	
Area	County	Range[c]	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	Graham	3.2–63.0	21.1	0–67.2	22.1	0–0.5	0.1	0–0.2	<0.1	0	0
2	Buncombe	9.8–84.7	39.3	0–71.6	11.7	0–0.1	0.1	0	0	0	0
2	Madison	0.4–66.4	22.2	0–7.5	0.9	0–1.3	0.2	0–1.5	0.3	0–2.0	0.3
2	Yancy	0.2–54.4	14.1	0–3.5	0.3	0–0.2	0.1	0–0.1	<0.1	0	0
3	Watauga	2.0–54.4	31.8	0–3.8	0.7	0–1.4	0.3	0–0.3	0.1	0	0
Mean			25.7		7.1		0.2		0.1	<0.1	

[a] Data obtained from 10 randomly selected fields in each county. About 800 plants ($\bar{x}$) were observed in each field. Virus incidence in each county was therefore based on data from about 8,000 plants. Average time of observations was at the early flowering stage of plant development.
[b] TVMV = tobacco vein mottling virus, TEV = tobacco etch virus, AMV = alfalfa mosaic virus, TRSV = tobacco ringspot virus, and TSV = tobacco streak virus.
[c] Range represents lowest and highest virus incidence recorded in a field in a county.

causing a mean disease index of 2.0–4.0, and "severe strain" for isolates causing a mean disease index higher than 4.0. Thus defined, the mild strain consisted of two isolates, the moderate strain 18 isolates, and the severe strain five isolates. Isolate NC19-P, which is considered our "type isolate," would thus belong to the "moderate strain," and Simon's isolate (12), to the "severe strain." Simon's isolate is valuable for field studies because its restricted transmission (12) reduces its chance of movement to "virus-free" plots.

Tobacco mosaic virus (TMV), which historically has been associated with tobacco, was not detected in this survey. The reason for lack of detection of TMV is that essentially all cultivars currently used in the production of burley tobacco contain the resistance from *N. glutinosa* L. (14).

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