The Role of Chemical Soil Treatments in the Control of Nematode-Disease Complexes of Tobacco

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Disease complexes involving interrelationships between plant-parasitic nematodes and other soil-borne pathogens are indeed complex, not only in etiology and pathogenesis, but also in epidemiology. Plant pathologists are familiar with the disease triangle depicting a dynamic interrelationship between a single host, a single pathogen, and the environment. In disease complexes, other dimensions are added which may decisively influence the course of an epidemic. Plant pathologists are concerned with how these associations develop also with the resulting disease severity and crop loss. Powell (10) found that attention must be focused upon interacting pathogens, and that satisfactory control may require the use of several complementary practices. Thus, control may be more difficult, uncertain, and costly than for diseases caused by a dominant primary pathogen.

In a recent review, Pitcher (9) characterized various types of interrelationships between nematodes and other kinds of pathogens. He emphasized that most research has been exploratory and descriptive, often conducted in artificial greenhouse and laboratory environments. In his view, knowledge of plant pathogen interactions is in its infancy, and further major advances will require a multidisciplinary approach in studies conducted in the field.

There is a vast literature reporting the use of nematicides and fungicides against a primary target organism without consideration of their activity against other pathogens present in the biosphere. The need for multipurpose or broad spectrum biocides has been recognized and intensively studied. An example is the comprehensive laboratory and field evaluation by Baines et al. (1) of the nematocidal and fungicidal properties of several chemicals and mixtures of chemicals for control of the citrus nematode (*Tylenchulus semipenetrans*), *Phytophthora citrophthora*, *P. parasitica*, and other organisms that may cause decline in young citrus trees. Another is the use of methyl bromide alone and in mixtures applied under a plastic cover for the control of weeds, soil borne plant pathogens, and insects in tobacco plant beds (18). This has been a standard practice for tobacco growers in North Carolina for nearly 15 years. Similar treatments are used extensively in high-value crops, including woody ornamentals, nursery stocks, turf, flowers, and small fruits. However, their use is not practical for most field and vegetable crops because of high cost.

Experimental use of multipurpose chemical treatments selectively effective against various kinds of pathogens are useful in assessing effects of disease complexes upon their hosts.

Many federal and state research projects on plant disease complexes are currently active, and represent a wide range of problems. For example, there are nine research projects on disease complexes in eleven western states and Hawaii supported by Regional Project W-56. At least 10 other programs are underway in various parts of the United States. These are concerned with either nematode-nematode, nematode-fungus, or nematode-virus associations. Increased understanding of the nature of pathogen interrelationships and their effects on disease development is the primary objective. The influences of abiotic factors and host resistance in disease complexes are also being investigated. Relatively few are concerned with the use of chemical treatments for the control of pathogen complexes, the subject of this presentation.

Some problems of flue-cured tobacco in North Carolina.—Diseases of several kinds have threatened tobacco culture since colonial times. However, the localization and intensification of production during the past 100 years and continuous monoculture have caused most tobacco fields in North Carolina and neighboring states to be infested with various combinations of pathogens which provide the potential for a wide variety of disease complexes. These problems are of both academic and economic importance. Three of major concern are (i) root knot, caused by several species of *Meloidogyne;* (ii) southern bacterial wilt, caused by *Pseudomonas solanacearum;* and (iii) black shank, caused by *Phytophthora parasitica* var. *nicotianae.*

Although root knot was widespread, it was not regarded as a major problem until the 1940's. Bacterial wilt was first noticed about 1880. By the 1930's, it had become so destructive in certain sections that tobacco production became unprofitable. The presence of black shank in North Carolina was first confirmed in 1931. The spread of this disease across the state placed additional burdens upon the tobacco industry. The timely development of varieties resistant to wilt and black shank averted disaster. When these varieties came into widescale use, however, the importance of interrelationship between root-knot nematodes and other pathogens was recognized (7, 14), confirming the earlier findings of Tisdale (16) and focusing upon root knot as a major component of the disease complexes.

Annual disease losses in flue-cured tobacco in North Carolina were reduced from over $50 million to less than $20 million during the past 15 years (21). Disease control was achieved through an integrated program involving resistant varieties, nematicides, crop rotations, and certain cultural practices. Even so, heavy losses still occur, and growers fear unpredictable epidemics which may occur despite conscientious control efforts.

Experimental procedures.—To evaluate integrated disease control, experiments were conducted in "problem" fields possessing certain combinations of pathogens. To accomplish this, "Research On Wheels" was started in 1964. Designed to include the entire state, this program involves growers, county Extension agents, chemical and tobacco companies, the federal tobacco grading service, the Crops Research Division, ARS, USDA, and Extension and research staffs at North Carolina State University including statisticians, engineers, chemits, soil scientists, crop scientists, entomologists, nematologists, and plant pathologists.

The primary variables included various combinations of host resistance, chemical treatments, and crop rotations. Secondary variables within certain of the main treatments were (i) varieties having different levels of disease resistance; (ii) chemicals or mixtures of chemicals having selective effect against the target organisms; (iii) rates and methods of application of chemicals; and (iv) different kinds of alternate crops in tobacco rotations. At least three replications were used, and plots were one forty-fifth acre in size. Each type of experiment was conducted in at least two and usually five or six separate locations in the State to provide a range of soil types, weather conditions, and disease situations. Many experiments now underway will be continued for a 6-year period to evaluate crop rotation effects.

General management procedures were standardized for all plots within a given experiment, including land preparation, fertilizer application, transplanting, cultivation, insect control, topping, sucker control, harvesting, curing, and grading. Detailed records were kept on crop history, soil type, treatment conditions, operations schedules, and weather conditions.

Many kinds of data were used to evaluate treatments. Stand counts, crop response ratings, and counts of plants showing disease symptoms were made periodically throughout the growing season. In certain tests, insect damage data were taken. Soil samples for nematode assay were taken from each plot before treatment, in midseason, and after harvest. The roots of a prescribed number of plants in each plot were examined after harvest and scored for root galling and decay. Symptom severity classes ranging from 0 to 5 facilitated the calculation of disease indices on a 0 to 100% scale. The leaves were harvested and cured according to a prearranged schedule, and the identity of each lot was maintained by the use of color-coded tags. Each lot of cured leaf was officially graded by a professional grader and weighed, providing data on leaf quality and value. Cured leaf samples were analyzed for certain constituents of the cured leaf related to smoking quality, for chemical residue analysis, and for smoke taste tests. Certain evaluations, particularly the analyses of chemical residues and smoking quality, are incomplete.

Black shank studies.—Widescale evaluation of the effects of combined treatments for the control of black shank in flue-cured tobacco was undertaken in 1963 (17). One series (A) of tests was located in fields where black shank caused heavy losses in 1962. A duplicate series of treatments (B) was located in fields nearby where black shank was destructive in 1961. The latter fields were planted to other crops, such as cotton, corn, and fescue in 1962, providing a crop-rotation variable. A black shank-susceptible variety, Hicks, and a resistant variety, N.C. 2326, were used. Maneb was broadcast and disked into the soil at the rate of 72 lb./acre. Where no control practices were used, black shank damage was most severe and crop value was lowest (Table 1). Where the combination of all three practices was employed, best control and highest crop values were obtained. The resistant variety was the most important disease control factor. However, the performance of both the resistant and susceptible varieties was improved by crop rotation and to a lesser extent by fungicidal treatment.

Two experiments conducted in 1964 (20) demonstrated the relationship between black shank and root knot and the importance of controlling both pathogens in a disease complex. Experiments were located in fields in which black shank-resistant varieties were damaged severely in 1963, and assays showed that moderate to high populations of root-knot nematodes were present in both fields. In the first test, varieties Speight G-5 (low black shank resistance) and NC 2326 (moderate resistance) were used. The chemical treatments included maneb and the nematicide-fungicide formulations Vorlex (Morton Chemical Co.: methyl isothiocyanate 20% and chlorinated C_3 hydrocarbons 80%), and Telone PBC (Dow Chemical Co.: 1,3-dichloropropene and related C_3 hydrocarbons 80%, chloropicrin 15%, and propargyl bromide 5%).

The fungicide (maneb) had no apparent effect upon the incidence of root knot, but reduced the black shank index and increased the value of the crop (Table 2). Vorlex and Telone PBC controlled both pathogens better than maneb, and improved crop performance. A

TABLE 1. The influence of host resistance, crop rotation, and fungicidal treatment, alone and in all combinations, on the incidence of black shank and on tobacco performance in North Carolina[a]

Var.[b]	Rotation	Disease count[c]		Crop value/acre	
		Check	Maneb[d]	Check	Maneb[d]
		%	%	$	$
Hicks (S)	None	80	70	312	591
NC 2326 (R)	None	15	9	991	1,250
Hicks (S)	2-Year	41	21	895	1,238
NC 2326 (R)	2-Year	5	1	1,314	1,497

[a] Summary of six tests in 1963.
[b] Both varieties are similar in agronomic characters and yield potential: S = susceptible; R = resistant.
[c] Plants killed by black shank before the end of harvest.
[d] Broadcast application at the rate of 72 lb./acre. Approximate cost of material: $85/acre.

TABLE 2. The influence of chemical soil treatments upon the incidence of black shank (BS) and root knot (RK) and upon the performance of two root-knot-susceptible tobacco varieties differing in black shank resistance in 1964 in North Carolina

Var.[b]	Treatment		Disease index[a]		Crop value/ acre
	Material[c]	Rate/acre	BS	RK	
					$
S.G-5 (L)	Check		89	70	561
	Maneb	72 lb.	53	68	789
	Vorlex	8 gal	41	18	1,122
	Telone PBC	20 gal	17	12	1,120
NC 2326 (M)	Check		73	69	579
	Maneb	72 lb.	60	70	861
	Vorlex	8 gal	17	27	971
	Telone PBC	20 gal	3	22	1,031

[a] Based upon a scale of 0 (no disease) to 100 (maximum disease). BS = black shank; RK = root knot.
[b] S. G-5 (Speight's G-5) is a higher yielding variety than NC 2326. L = low resistance; M = moderate resistance.
[c] Approximate costs of materials in dollars per acre: Maneb = 85; Vorlex = 55; Telone PBC = not available.

TABLE 3. The influence of chemical soil treatments on the incidence of black shank and root knot and upon the performance of tobacco variety NC 2326[a] in 1964 in North Carolina

Treatment[b]				
Material	Rate/acre	Black shank[c]	Root-knot index[d]	Crop value/acre
		%		$
Check		24	44	851
Penphene	3 lb.	28	21	919
DD	10 gal	12	14	1,177
Telone PBC	10 gal	0	1	1,306

[a] NC 2326 is root-knot susceptible and moderately resistant to black shank.

[b] All chemicals were applied in the row 3 weeks before transplanting.

[c] Based upon the plants killed by black shank before the end of harvest.

[d] Based upon a scale of 0 (no root knot) to 100 (maximum root galling).

general biocide was superior to a fungicide alone. In the second test, the superiority of Telone PBC over the nematicides, DD, and Penphene (Pennsalt Chemical Co.; tetrachloro thiophene), is evident (Table 3). Control of the root-knot nematode decreased the incidence of black shank greatly and increased returns per acre. It also demonstrated the role of the root-knot nematode in the disease complex.

Bacterial wilt studies.—Long-range studies involving the evaluation of interactions of host resistance, crop rotation, and chemical treatment were established in southern bacterial wilt-root knot problem fields in 1965 (21). The 1967 data for two varieties at one location are presented in Table 4. The incidence of wilt was only moderate in NC 95 (highly resistant to both pathogens), even without rotation or chemical treatment. Crop values, however, were increased significantly by crop rotation, but not by chemical treatment. Speight G-36, which is highly resistant to wilt but susceptible to root knot, benefitted from crop rotation. However, chemical treatment decreased the incidence of wilt and root knot and increased crop values significantly only when tobacco followed tobacco. It is evident that root-knot control is an important factor in wilt control, and that a previous crop of fescue is more effective in controlling wilt than Vorlex treatment.

DISCUSSION AND SUMMARY.—Reduction of initial inoculum levels of pathogens involved in a complex is the principal objective in the use of rotation and chemical soil treatments. Whether emphasis is placed upon control of nematodes, some other pathogen, or both may depend upon the nature of their interrelationship. Slack (15) indicated that the presence of the nematode may be either obligatory or complementary. This, in turn, may depend upon host genotype and upon the circumstances under which the complex develops.

The disease complexes in flue-cured tobacco involving root knot and black shank provide an excellent illustration of the kinds of interactions to be found between associated pathogens. Varieties are now available which are either susceptible or resistant to both pathogens or resistant to one and susceptible to the other. Both pathogens are likely to be present in North Carolina tobacco soils. Inoculum levels, however, may vary widely between and within fields. If the host variety is susceptible to both pathogens, severe damage may result unless both pathogens are controlled. Black shank can cause heavy loss even in the absence of root knot. Hence, with regard to the development of black shank in a susceptible variety, the presence of the nematode is considered complementary, and a nematicide would have little effect on disease development.

If the tobacco variety is root-knot susceptible and black shank resistant, a condition that characterizes most varieties in North Carolina, control of the nematode is important. Nematode infection of the resistant host causes increased susceptibility to the fungus, apparently by interfering with the resistance mechanism. If the nematode is absent, little, if any, disease occurs. It has been demonstrated in greenhouse experiments (12, 14) that the nematode is obligatory for disease development in resistant varieties. It does not imply, however, that soil treatment with a standard nematicide

TABLE 4. The interrelationships between host resistance, crop rotation, and chemical soil treatment in the control of southern bacterial wilt and on tobacco performance in 1967 in North Carolina

Var.[a]	Previous crop	Chemical treatment[b]	Bacterial wilt[c]	Root-knot index[d]	Crop value/acre
			%		$
N.C.95 (H-H)	Tobacco	None	19	9	1,193
	Tobacco	Vorlex	15	0	1,155
	Fescue	None	9	0	1,534
	Fescue	Vorlex	3	0	1,647
S.G-36 (H-O)	Tobacco	None	69	57	616
	Tobacco	Vorlex	45	1	1,017
	Fescue	None	21	3	1,384
	Fescue	Vorlex	9	0	1,475
LSD = .05			21	14	349
LSD = .01			28	20	470

[a] H-H = high resistance to both southern bacterial wilt and root knot; H-O = high resistance to southern bacterial wilt and susceptible to root knot.

[b] Vorlex was applied as a row treatment at the rate of 8 gal/acre.

[c] Percentage of plants killed by southern bacterial wilt before the end of harvest.

[d] Based upon a scale of 0 (no galls) to 100 (maximum galling of roots).

will afford adequate protection in fields infested with both pathogens.

In early tests (8), root-knot nematode control by fumigating the soil with either DD (Shell Chemical Co.; 1,3-dichloropropene, 1,2-dichloropropane, 3,3-dichloropropene, 2,3-dichloropropene, and related C_3 hydrocarbons), or Dowfume W-85 (Dow Chemical Co.; 83% ethylene dibromide) delayed the onset of the black shank epidemic and reduced the severity of the disease in varieties having moderate to high black shank resistance, but did not give an acceptable degree of control. Similar results were obtained in field plots infested with root-knot nematodes and the *Fusarium* wilt fungus, *Fusarium oxysporum* var. *nicotianae* (6, 7). Examination of the roots of plants in these plots revealed that the incidence of root knot was reduced substantially by the chemical treatments employed. It appears that much lower preplant populations of root-knot nematodes are needed to establish the disease complex than are necessary to damage the plants by nematode activity alone.

If the host is resistant to both pathogens, chemical controls may not be needed. The flue-cured tobacco variety NC 95 has high level resistance to black shank, *Fusarium* wilt, and southern bacterial wilt. It also has the hypersensitive type of resistance to the southern root-knot nematode, *M. incognita*. However, it is susceptible to other *Meloidogyne* species such as *M. hapla*, *M. arenaria*, and *M. javanica*. Moreover, resistance-breaking biotypes of *M. incognita* have been reported (13). These circumstances may, therefore, limit the usefulness of varieties having this type of resistance. For example, studies in Florida (5) show that NC 95 suffers severe black shank damage in the presence of the black shank fungus and *M. javanica*. In such instances, the presence of the nematode is obligatory, and high level control of root knot appears to be necessary. This may be achieved by the use of crop rotation followed by treatment with a nematicide or by more effective methods of chemical treatment.

The role of the southern root-knot nematode in disease complexes involving either black shank (14), southern bacterial wilt (3), or *Fusarium* wilt (4) is well documented. Undoubtedly there are others less obvious than these. Recent studies by Powell (11) and his students have shown that root-knot infections can predispose tobacco roots to decay by certain soil-inhabiting fungi which, in the absence of root knot, are relatively harmless. Clayton et al. (2) observed that most of the damage caused by root knot occurs late in the season when heavily galled roots decay. However, the onset and severity of decay are unpredictable. The etiology often is obscured by the multiplicity of organisms that can be isolated from decayed roots at the end of harvest.

Some difficulties.—In evaluating chemical treatments for disease control in the field, many difficulties and uncertainties may be encountered. Some of them can be avoided or minimized by careful site selection, sound experimental design, use of proper equipment, coordination and timely execution of field operations, and competent supervision. Others, such as the vagaries of the weather, cannot be controlled. Some of the most troublesome problems are related to variability in soil types related to natural terrain and in horizontal distribution of pathogen populations. In some tests, statistical analysis of data shows relatively high variation coefficients, and large differences between treatment means are required for significance. Also, there are occasional discrepancies or reversals in the performance of a given treatment from one location to another. Within-field variations in the horizontal distribution of plant parasitic nematodes are great and must be taken into account. No doubt this also applies to other kinds of pathogens. Variations in pathogen virulence are also important. When the populations of two interacting pathogens vary both quantitatively and qualitatively, the relative contribution of each organism to the disease complex is difficult to determine. In most instances, therefore, caution must be exercised in predicting the outcome or even in interpreting the results of field studies.

The need for improved techniques for estimating pathogen population densities is recognized. Although useful methods for determining nematode populations are available, they have many limitations. At present we cannot determine inoculum levels of either the wilt bacteria or the black shank fungus in field soils with a degree of accuracy or reproducibility that such studies require. In a certain sense, a field experiment may be regarded as a bioassay on a large scale, but less extensive and more rapid assay procedures are needed.

In the control of disease complex problems of flue-cured tobacco, chemical treatments thus far have played a supplementary role (19). These problems are so acute and destructive that chemical treatment alone has been inadequate. Resistant varieties occupy the dominant position, followed by crop rotation. On most farms, the use of varieties having high resistance to several pathogens plus the use of a suitable cropping system has given satisfactory disease control and yield. Many farmers, however, cannot practice crop rotation consistently because of decisions related to the efficient use of facilities and land resources. As a result, certain compromises must be made. There is a strong trend back to monoculture and an increasing demand for a disease control program that would permit such a change. Increased mechanization of production and harvesting operations has contributed greatly to these new attitudes. Hence, there is considerable interest in the development of control programs involving the use of resistant varieties and chemicals.

Promising new chemicals.—Some of the new, highly active nematocides such as Temik (Union Carbide Corp.; 2-methyl-2-[methylthio] proprionaldehyde-*O*-[methylcarbamoyl] Oxime) and formulations of materials having both fungicidal and nematicidal value may find a useful place in such a program. Vorlex, Vorlex 201 (Morton Chemical Co.; methyl isothiocyanate 17%, chlorinated C_3 hydrocarbons 68%, and chloropicrin 15%), Terr-o-cide (Great Lakes Chemical Corp.; ethylene dibromide 40%, chloropicrin 15%, and inert 45%), and experimental compound No. 2680 (Dow Chemical Co.; 1-3 dichloropropene and related chlorinated C_3 hydrocarbons 85% and chloropicrin 15%) show unusual promise. The agricultural chemicals industry is active in the development of new, highly active nematicides, fungicides, and broad spectrum biocides. The outlook for further advancement in this field is encouraging.

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