

Yellow Net Virus Disease of Flue-cured Tobacco

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INTRODUCTION

TOBACCO yellow net virus (TYNV) was first reported in 1959 from the Nuwara Eliya District. The causal virus was characterized as transmissible by the aphid vector *Myzus persicae* Sulz. but not by manual inoculation of sap from diseased plants (2). Since its appearance, the disease commonly occurs in the north-eastern parts of the hill-country where bulk of the flue-cured tobacco is grown. Sporadic outbreaks of the disease have been noticed in the Matale District but the virus has not assumed epidemic importance as in the higher altitudes.

Reliable estimates of crop losses caused by TYNV are yet unavailable, chiefly because no systematic disease surveys have been conducted and because of the complexity of determining losses in the field. Reduction in the yield of the leaf harvested from infected plants is, however, thought to be negligible as the main effect of the virus on its host is the production of discontinuous yellow or chlorotic bands on the leaf blade without drastically reducing the leaf size or its weight. TYNV, unlike the Leaf Curl of tobacco does not produce enations or malformations on the leaf blade or cause violent distortion of the veins. TYNV infected plants continue apparently normal vegetative growth without exhibiting any prominent signs of stunting or reduction in their leaf-producing capacity. Infected leaves exhibit pale to bright yellow or chlorotic discontinuous vein-bands along tertiary veins and those of the lower order. Leaf texture is smooth and soft, not brittle and puckered as with Leaf Curl virus infection. The infected leaves are commercially unacceptable and frequently graded inferior in quality thereby causing economic loss indirectly to tobacco growers.

In the present paper the results of a field survey of TYNV and investigations on its control are described. Detailed studies on characterization of the virus and its vector relationships will be reported elsewhere.

DISEASE SURVEY

A survey of TYNV in growers crops was conducted in the 1963-64 cropping season with the objects of estimating seedbed infection and determining whether seedbed plants constituted a major source of infection and subsequent spread of the disease in a transplanted crop; of ascertaining the progress and severity of the disease at different stages of crop growth, and finally of investigating the epidemiology of the disease and possible relationship between aphid colonization and increase in TYNV incidence.

In the field survey estimates of TYNV were made at 3 stages of the crop, namely (I) seedbed stage, (II) active vegetative stage (2-4 weeks after transplanting), and (III) productive stage (8-10 weeks after transplanting). This periodic survey enabled a close study of the progress of infection from the seedbed until about the final stages of the crop.

(I) *Infection in the seedbed*: Seedbeds for detailed studies were selected in a random manner from lists of registered growers furnished by tobacco extension officers and designed to include one-tenth of the nursery area in Karandagolla and Walapane extension ranges. The distribution of seedbeds, virus infection and aphid colonization of seedbed plants in the two ranges are illustrated in Table 1.

Seedbeds examined were conspicuously free of visually recognizable symptoms of TYNV. A great majority of seedbeds were free of the aphid vector; this was particularly evident in commercial nurseries managed by the Ceylon Tobacco Company. An explanation is that seedbeds owned by the Ceylon Tobacco Company are regularly sprayed with insecticides to prevent aphid colonization and that the general field hygienic practices are superior. Some seedbeds owned by tobacco-growers were infested with the aphid vector, potentially viruliferous and capable of spreading the disease among seedbed plants. As examples, all seedbeds examined at Watumulla and 1 out of 4 seedbeds at Bogamuwa were infested with aphids.

Although visually recognizable symptoms of TYNV were not encountered during this survey, the occurrence of severe virus infection of seedbed plants has been noticed in other localities during certain years. Seasonal and possible local variations, and availability of viruliferous aphids may account for some of this variability. Infected seedbed plants carrying recognizable yellow-net symptoms or symptomless latent infection should be regarded as a potentially important source of spread of the disease. This view is further supported by the presence of colonies of *M. persicae* in 22 per cent.

TABLE 1.—Distribution of Tobacco Seedbeds Surveyed for TYNV Disease and Aphid Infestation

<i>Karandagolla Range</i>					<i>Walapane Range</i>				
<i>No. of Seedbeds inspected</i>	<i>Location</i>	<i>Owned By</i>	<i>%TYNV</i>	<i>No. of Seedbeds Infested with aphids</i>	<i>No. of Seedbeds Examined</i>	<i>Location</i>	<i>Owned By</i>	<i>%TYNV</i>	<i>No. of Seedbeds Infested with aphids</i>
4	Karandagolla	Grower	0	0	8	Nildandahinna	Grower	0	0
3	Makuruppa	Grower	0	0	1	Kalaganwatte	Grower	0	0
4	Bogamuwa	Grower	0	1	2	Harasbedda	Grower	0	0
1	Pannala	C.T.C.	0	0	5	Watumulla	Grower	0	5
1	Mailapitiya	C.T.C.	0	0	4	Kumbalgammuwa	Grower	0	0
					1	Munwatte	Grower	0	0
					1	Kalaganwatta	C.T.C.	0	0

C.T.C. = Ceylon Tobacco Company

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of the seedbeds examined. Absence of systematic aphid control in seedbed plants and lack of field sanitation in the establishment of seedbeds could therefore largely contribute to the introduction of the virus from an external source.

Infection of TYNV could be readily introduced to seedbeds by the arrival of winged aphids flying from an infected source. Once a viruliferous aphid alights on a seedbed plant, the spread of the disease among the other plants in the seedbed could occur with remarkable rapidity because a single viruliferous aphid is capable of infecting several plants in succession without having to feed on a new virus source and because of the ease with which new progenies of wingless aphids could crawl from one seedbed plant to another chiefly due to the high density of plants in the seedbed.

(II) *Infection in the transplanted crop*: By a process of random sampling representative holdings were selected from lists of registered growers in a manner to include each overseer unit; 61 growers-holdings representing 478 acres were thus surveyed in Karandagolla and Walapane extension ranges. This method not only permitted the study of widely scattered crops in the north-eastern parts of the hill-country but also enabled the survey of a minimum of 10 per cent. of the representative crop. A total of 400 plants distributed randomly in 4 widely scattered areas in each 8-acre growers crop was critically examined; each sample comprised of 4×100 sub-samples, the latter being always in a contiguous row. The percentage plants infected with TYNV and the severity of aphid infestation were recorded at each assessment. The severity of aphid infestation was estimated on a category scale from 0-3 where 0 = no aphids; 1 = few scattered aphids per leaf (mild infestation); 2 = 25-50 aphids per leaf (moderate infestation); and 3 = more than 50 aphids per leaf (severe infestation).

TYNV incidence and aphid infestation were measured in the transplanted crop at 2 stages in its development; the results are summarized in Table 2.

Except in Ambanella, Marassana, Mailapitiya and Malulla, crops in other areas included in the survey showed infection 2-4 weeks following transplanting; a high incidence, in particular, occurred in Watumulla, Kalaganwatta and Padiyapellella. The disease was widely distributed throughout the two extension divisions despite its low initial incidence. For practical virus control, therefore, both extension divisions should be recognized as one contiguous infected zone. Natural aphid colonization of the transplanted crop in its early

TABLE 2.—Actual Acreage Infected with TYNV in Walapane and Karandagolla Ranges

Extension Range	Overseer Unit	No. of Holdings Surveyed	Total Acreage Surveyed	First Assessment			Second Assessment		
				Mean % Plants Infected	Actual Acreage Infected	Aphid Infestation	Mean % Plants Infected	Actual Acreage Infected	Aphid Infestation
Walapane	Nildandahinna	4	32	0.13	0.015	2	1.31	0.419	2
	Ambanella	6	48	0.00	0.000	2	0.003	0.001	1
	Watumulla	3	24	0.83	0.199	2	2.08	0.499	2
	Kalaganwatta	5	40	0.80	0.320	2	2.30	0.920	1
	Padiyapelella	7	56	0.75	0.420	2	2.50	1.400	2
Karandagolla	Marassana	6	48	0.00	0.000	2	0.71	0.341	1
	Mailapitiya	4	32	0.00	0.000	2	1.06	0.339	2
	Hanguranketa	5	40	0.15	0.060	2	0.60	0.24	1
	Karandagolla	13	104	0.06	0.062	2	1.03	1.07	2
	Dehiye	4	32	0.13	0.015	2	0.81	0.259	1
	Matulla	4	32	0.00	0.000	2	0.75	0.24	2

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TABLE 3.—Estimated Acreage Infected with TYNV in Walapane and Karandagolla Ranges

<i>Extension Range</i>							<i>First Assessment</i>				<i>Second Assessment</i>			
							<i>Actual Acreage Diseased</i>		<i>Estimated Total Acreage diseased</i>		<i>Actual Acreage Diseased</i>		<i>Estimated Total Acreage Diseased</i>	
Walapane	..	..	..	2,000	..	200	..	0·954	..	9·54	..	3·239	..	32·39
Karandagolla	..	..	..	1,776	..	288	..	0·137	..	0·84	..	2·489	..	15·35

stages was extremely high, possibly favoured by the warm dry climatic conditions and by the vigorously growing succulent foliage. Pronounced increase in virus incidence during the later stages of the crop was combined with its rapid spread into disease-free regions. This is accounted for by the steady increase in the inoculum in scattered areas and by the abundance of natural aphid populations.

The estimated acreage infected with TYNV in each extension division is given in Table 3. The disease was predominantly more serious in Walapane extension division, which may be attributed to consistently even distribution of the primary inoculum and subsequent secondary spread through aphids, especially from older plantings to younger fields. The survey results do not particularly pose the seriousness of the disease, but it must be concluded that widespread epidemics have been noticed during certain years and that the disease looms large as a threat to tobacco production in the north-eastern parts of the hill-country.

SOURCES OF TYNV INFECTION

The major sources of TYNV infection in the field are alternate host plants, infected self-sown or volunteer tobacco plants, escapes and secondary growths from preceding season's crop remains. Besides providing a continuous reservoir of the virus, they also constitute potential sources on which aphids easily persist during the off-season. Thus, in harbouring both the virus and its vector the alternate hosts and infected crop residues constitute a serious hazard for they alone provide real sources of re-infection of the tobacco crop each successive season.

Alternate host plants.—*Solanum nigrum* (Kalukamberiya) is a common weed abundant in tobacco fields and is naturally infected with TYNV. The virus was also experimentally found to infect several other solanaceous plants such as *Solanum villosum*, *S. nodiflorum*, *Nicotiana glutinosa*, *N. rustica* and *N. repanda*. Except in *S. nodiflorum*, typical yellow-net symptoms are produced on all other alternate hosts. The virus infects *S. nodiflorum* systemically producing localized faint yellow or chlorotic blotches, often distributed irregularly on the leaf blade. In contrast to plant species in which definite visual symptoms are expressed, the common ornamental Bachelor's Button (*Gomphrena globosa*) was experimentally demonstrated to carry symptomless infection (1).

These results clearly show that TYNV is not host specific, and that several plant species can provide sources of TYNV for the new tobacco crop. Of these alternate hosts *S. nigrum* is of major

importance because of its common occurrence as a weed in the north-eastern parts of the hill-country ; in contrast, *G. globosa* is cultivated in home gardens, often remote from tobacco plantations and is therefore unlikely to be a serious source of TYNV for the new tobacco crop.

In experimental inoculations TYNV failed to infect chilli, tomato, brinjal, potato, bandakka, cucumber, *Ageratum conyzoides*, *Datura stramonium*, *Solanum capsicastrum*, *Chenopodium amaranticolor*, *Physalis peruviana* and *P. floridana*. Of these chilli, tomato, brinjal and potato are usually cultivated in the north-eastern regions of the hill-country while *Ageratum conyzoides*, often infected with a virus disease resembling yellow net of tobacco, is a widely occurring weed. From a practical aspect of TYNV control, cultivation of chilli, tomato, brinjal, potato and cucumber in tobacco-growing areas presents no hazard.

Volunteer plants and secondary growths.—Secondary growths from the stumps of old abandoned plants and volunteer or self-sown plants harbour the virus during the off-season. They survive the dry yala season, especially when growing along water-courses and channels and would provide sources of the virus for the new tobacco crop. These sources of TYNV, however insignificant they may appear, cannot be dismissed as unimportant because from small initial infection the disease could develop into a serious epidemic, particularly due to the high vector efficiency in the transmission of the virus from diseased to healthy plants.

INTRODUCTION OF TYNV INFECTION INTO A CROP

The disease is introduced into a crop (a) by planting diseased seedlings originating from infected seedbeds, and (b) by migratory winged aphids flying from an infected source on which they have previously fed. Both methods are highly effective in distributing the virus over extensive areas in the north-eastern parts of the hill-country.

Infection through seedbeds.—The field survey revealed a general freedom of seedbeds from any visual symptoms of virus infection. However, late infection of seedbeds and the occurrence of latent infection with no evidence of visual symptoms near transplanting time or even at transplanting cannot be fully dismissed. The seedling plants with their greater exposed leaf area near transplanting time provide increased chances for the aphid immigrants to settle, also exposing thereby the seedling plants to increased infection. Evidence in support of the view that seedling plants constitute an important

source of distribution of the initial infection can be further found in (i) definite visual symptoms of seedbed infection noticed during certain seasons, (ii) widespread vector infestation of seedbeds, being as high as 22 per cent. of seedbeds included in the survey, and (iii) the development of clear yellow net symptoms within 2-4 weeks after transplanting when the incubation period for the appearance of initial symptoms of chlorotic specks alone is 7-9 days.

Infection through migrating aphids.—TYNV infection could be introduced into a crop by winged immigrants of *M. persicae* which have previously fed on diseased tobacco plants or alternate hosts like *S. nigrum* and *G. globosa*. Sources of the vector are also abundant in the north-eastern parts of the hill-country. Besides tobacco, crops like cabbage, potato, etc., that are widely grown in the area, are readily colonized by the vector and would therefore serve as alternate sources for the multiplication of winged aphids. Flying of winged aphids from these alternative hosts or carriage by wind to great distances, almost covering the entire tobacco-growing region can be expected to occur commonly.

After the initial infection is introduced, subsequent spread within the crop occurs with remarkable rapidity. The reasons for this explosive spread of infection are first, as revealed in the survey and illustrated in Table 2, tobacco crops are heavily colonized with the aphid vector in 2-4 weeks after transplanting and secondly, the virus being persistent, a single aphid can serially infect several plants without feeding on fresh source of the virus.

TESTS ON SUSCEPTIBILITY OF TOBACCO VARIETIES

Cultivation of resistant or tolerant tobacco varieties in areas like Karandagolla, Walapane, Hanguranketa and Nildandahinna where the disease is endemic constitutes the most effective method of combating TYNV infection. Therefore, with a primary object of determining their relative tolerance or susceptibility forty-eight commercially established tobacco varieties were inoculated under insect-proof conditions. Viruliferous apterae or late instar nymphs of *M. persicae* were transferred to test seedlings, 15 per plant, and allowed an infection feeding period of 24 hours, at the end of which the aphids were killed by spraying with malathion and the test plants placed in an insect-proof house.

All varieties tested developed systemic infection and no recognizable differences existed in the severity of the disease reaction within the population of any single variety. However, a wide reaction spectrum

on different varieties was conspicuous and thus enabled the differentiation of varieties on the basis of severity or tolerance to infection. Tobacco varieties tested were thus rated according to the following severity reaction.

- (1) Highly tolerant.—Disease expressed as discontinuous, faint yellow blotches along tertiary veins and veins of lower order. The blotches are 2-4 mm. in diameter, non-spreading and oval to round in shape.
- (2) Moderately tolerant.—The disease appears as faint-yellow discontinuous bands over 4 mm. in length along the tertiary veins and veins of the lower order.
- (3) Susceptible.—Severe reaction with extensive yellow bands along the veins. They spread and coalesce producing characteristic yellow-net symptoms.

The varieties tested are given below according to their tolerance or susceptibility.

Highly tolerant : Burley 2, Oxford 1, Oxford 1-81, Vamorr-50 and Kirigasaku-Ha.

Moderately tolerant : White Gold, Virginia Gold, Burley 11 a, Burley 11 b, Bel-2-69, Dixie Bright 28, Dixie Bright 102, H-246-11 b, Rg, Diquescene, Sumatra, and Matsukawa-Ha.

Susceptible : Golden White, Golden Cure, Golden Harvest, Golden Special, Golden Boom, Coker 139, Coker 140, Coker 157 Hicks, Coker 187, Dixie Bright 101, Dixie Bright 244, Oxford-3, H-274-11, Virginia 21, Vesta 30, Bottom Special, Beinhart 1000, Bright Yellow, Greider, S-1A, Swarr Hibsham, Hicks Broad Leaf, Dixie Bright 402, Tobacco Introduction 706, Connecticut Broad Leaf, Delcrest, Petite Havana, Akrad-Samsoun, Check-el-bent, Harrisons' Special and North Carolina-95.

No variety was immune or exhibited symptomless reaction to TYNV. However, Burley 2, Oxford 1, Oxford 1-8-1, Vamorr-50 and Kirigasaku-Ha showed a high degree of exploitable tolerance to systemic infection. They will, therefore, serve as potential sources of tolerance to TYNV and could be exploited with advantage in a breeding programme. The two commercial flue-cured tobacco varieties, namely, Harrisons' Special and North Carolina 95 are highly susceptible to TYNV and should be replaced with tolerant types in areas where the disease becomes epidemic and constitute a major threat to tobacco production.

METHODS OF CONTROL

Until truly immune or highly resistant and commercially acceptable tobacco varieties are developed maximum efficiency in the control of TYNV must inevitably be accomplished by an integrated approach through seedbed sanitation, destruction of alternate hosts, infected secondary growths from crop residues and volunteer plants, avoidance of staggered planting, elimination of the vector and cultivation of tolerant varieties. If applied collectively and concurrently, these control measures will greatly reduce the virus incidence; however, no one method applied singly and separately will substantially benefit a program of virus elimination or even cause a reduction in its incidence mainly because of the high vector efficiency in transmitting the virus and because few infected plants would be sufficient to initiate an epidemic.

Seedbed sanitation : Although visual TYNV infection is uncommon, available evidence emphasizes the existence of symptomless or latent infection in seedbed plants consequent to the relatively long incubation period of the virus within its host. This view is strongly supported by the evidence of vector colonization of seedbeds and by relatively early expression of yellow-net symptoms in transplanted crops. Dissemination of TYNV through seedbed plants therefore could be greatly reduced by the avoidance of vector colonization of seedbeds. Regular spraying of insecticides, commencing relatively early after the emergence of seedlings and especially before the arrival of winged aphids, and the establishment of seedbeds in moist and exposed locations that are unfavourable for aphid colonization constitute the important methods of aphid control.

Eradication of alternate hosts and secondary growths : Alternate hosts, especially *S. nigrum*, and to a lesser degree *G. globosa*, volunteer tobacco plants, abandoned tobacco crops and secondary growths from crop residues regularly harbour the virus during the off-season. Post-harvest destruction of these sources of infection will not only arrest the continuity of TYNV during the off-season but will also greatly reduce the viruliferous aphid populations carried on them. Rigid inspection of the tobacco fields immediately following the final harvest combined with a carefully planned programme of eradication of alternate hosts will prove to be of great value in virus control in tobacco production. At the commencement of each cultivation season, destruction of potential virus reservoirs, especially around seedbeds will delay the onset of infection and thereby reduce the cost of spraying insecticides on seedbed plants.

Avoidance of staggered planting: Tobacco-growing in the North Eastern regions of the hill-country is seasonal and confined to the rainy Maha season. However, due to the traditional practice of staggering the sowing time of seedbeds, late plantings are exposed to greater infection and often show pronounced increase in TYNV incidence relatively early during vegetative growth. Given in Table 4 are the times of seedbed sowing, transplanting and harvesting in the two extension divisions included in the survey.

TABLE 4.—Existing Times of Seedbed Establishment, Transplanting and Harvesting in TYNV Endemic Area

<i>Extension Range</i>		<i>Seedbed Establishment</i>	<i>Transplanting</i>	<i>Harvesting</i>
Walapane	..	.. October– December	.. November– January	.. February– June
Karandagolla	..	.. October– November	.. November– December	.. February– May

In Walapane, seedbeds are continually established during the months of October to December, in contrast to a more restricted period in the Karandagolla division. Consequent to this, late plantings in Walapane are liable to greater risk of infection because of the build up of TYNV and its vector populations in the early planted crops. Support for this view is found in survey results (Table 2) where a greater incidence of TYNV occurred in Walapane division. For practical virus control, however, both Walapane and Karandagolla extension divisions must be recognized as one contiguous endemic zone and minor adjustments in planting time could greatly suppress the disease incidence. Establishment of tobacco seedbeds within a period of 4 weeks, or with greater advantage in 2 weeks in both Walapane and Karandagolla divisions will be by far the most effective system.

Varietal tolerance: Tobacco varieties like Oxford 1, Vamorr-50, etc., exhibited a highly tolerant reaction to experimental inoculation. They could therefore be grown in endemic areas or exploited in a breeding programme for TYNV tolerance. In the selection of a tolerant variety, however, agronomic qualities and yield characteristics should receive examination.

DISCUSSION

The demonstration that in tobacco plantations initial infections occur apparently in the seedbed, poses special problems in the control of TYNV epidemics. It appears therefore that the primary aim in

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control must be the eradication of the potential sources of infection and prevention of introducing the virus into seedbed by migratory aphids. Good plantation hygiene is therefore of great importance and must include attention to many details, e.g., eradication of alternate hosts, volunteer plants and escapes and uniformity in planting of tobacco in the infected zone considered as one contiguous entity.

Initial infection is introduced into seedbeds or transplanted crops by viruliferous winged aphids flying from an infected source. Control measures must therefore also be concerned with preventing aphid colonization; for this purpose early spraying specially of seedbed plants, preferably with systemic insecticides is of the utmost importance.

Commercially cultivated tobacco varieties are highly susceptible to TYNV infection, and must be replaced with tolerant types in epidemic conditions. Agronomic characters, yield of leaf and other commercial requisites should be satisfied in introducing tolerant varieties. Much might also be accomplished by breeding for disease-tolerant mechanism.

SUMMARY

Survey has demonstrated the wide occurrence of TYNV in the major flue-cured tobacco producing areas of the hill-country. Infections are apparently initiated from the seedbed; vector colonization of seedbed plants, and appearance of recognizable yellow-net symptoms early in the transplanted crop in particular support this view.

Sources of inoculum are many and varied; alternate hosts, especially *Solanum nigrum* and *Gomphrena globosa*, and infected tobacco plants obviously harbour the virus during the off-season. Initial infections could be attributed unequivocally to winged aphids flying into disease-free crop from such infected sources.

Many solanaceous plants were experimentally infected with the virus; it failed to infect the commonly cultivated solanaceous crops, as well as cucumber, bandakka and *Ageratum conyzoides*. The last named is a common weed in tobacco-producing areas and frequently found to be infected with yellow-net-like virus disease.

Forty eight tobacco varieties have been evaluated for their relative resistance to TYNV. None proved to be highly resistant; however, Burley 2, Oxford 1, Oxford 1-8-1, Vamorr and Kirigasaku-Ha exhibited high tolerance to systemic spread of the disease.

Control measures applicable to the North Eastern parts of the hill-country are discussed. Uniformity in planting time, vector control and scrupulously rigid destruction of the off-season carriers of the virus are measures recommended to prevent epidemics of TYNV infection.

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