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Pesticide-treated vs. "Pesticide-free" Tobacco

I. Tobacco Production and Leaf Analysis*

by T. C. Tso, James F. Chaplin, K. E. LeLacheur and T. J. Sheets

U.S. Department of Agriculture, Science and Education Administration, Beltsville Agricultural Research Center, Beltsville, Maryland, U.S.A.

U.S. Department of Agriculture, Science and Education Administration, Oxford Tobacco Research Laboratory, Oxford, North Carolina, U.S.A.

Agriculture Canada, Research Branch, Research Station, Charlottetown, Prince Edward Island, Canada

and

Pesticide Residue Research Laboratory, School of Agriculture and Life Science, North Carolina State University, Raleigh, North Carolina, U.S.A.

Many chemicals are used for pest control and for growth regulation in tobacco production. Some of these chemicals are applied to the soil and some directly to the tobacco plant. Certain chemicals may leave high levels of residue on cured tobacco leaf, and some residues have been found in the mainstream of cigarette smoke.

Various studies have been conducted to examine the nature of pesticide residue in relation to the plant and environmental conditions, as well as the levels of pesticides on tobacco and in tobacco smoke, including their pyrolytic products (1). All these studies, however, were made with pesticide-treated or contaminated tobacco. Recent concern about the tobacco smoking and health problem makes it necessary to compare pesticide-treated and pesticide-free tobaccos in respect of leaf composition, smoke constitution, and biological activity. The inherent problem of this project is obvious: it is difficult to produce sufficient quantities of tobacco that is free of pesticide residues, since in every area of the earth where tobacco is normally produced it is already contaminated to some degree, in soil, air, or water. Through much searching and consultation, it was generally agreed that the experimental plot for pesticide-free tobacco production should meet three basic qualifications: [a] No crops grown or no chemicals applied to the field for at least 10 years, [b] Constant free air movement, with air current coming from clean open space, preferably from the ocean, and [c] Sufficient rainfall for water supply during the growth period so that irrigation would not be necessary. In addition, there should be experienced personnel for tobacco growing and facilities for flue-curing.

A location was selected at Heatherdale, Prince Edward Island, Canada, to study the effects of pesticides vs. pesticide-free tobacco on leaf composition, smoke components and biological activities of the smoke. This report deals with tobacco growing and leaf analysis.

MATERIALS AND METHODS

1. *Soil:* An area of 1.62 ha was used for the study, where constant northeasterly air movement from the open ocean was available. Approximately 0.91 ha was used to grow pesticide-treated (T) tobacco, and 0.71 ha for "pesticide-free" or untreated (UT) tobacco. These two plots were separated by a shelter belt of spruce forest that prevented possible cross contamination. The soil type in both fields was a fine sandy loam.

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Table 1. Soil fertility and pesticide residue analyses for pesticide-treated (T) and untreated (UT) plots.

Component*		Treated tobacco (T)	Untreated tobacco (UT)
Nitrate production (NO_3)	(ppm)	12.4	12.7
Organic matter	(%)	2.9	2.9
pH		5.7	5.5
Phosphorus (bray), P_2O_5 equivalent	(kg/ha)	610	717
Potassium, K_2O equivalent	(kg/ha)	157	174
Calcium	(kg/ha)	673	336
Magnesium	(kg/ha)	157	28
Aldrin	(ppm)	0.03	< 0.01
Dieldrin	(ppm)	0.32	< 0.02
Endrin	(ppm)	< 0.03	< 0.03
p,p'-TDEE	(ppm)	< 0.02	< 0.02
o,p'-TDE	(ppm)	< 0.01	< 0.01
p,p'-TDE	(ppm)	< 0.01	0.01
o,p'-DDE	(ppm)	< 0.01	0.02
o,p'-DDT	(ppm)	0.02	0.02
p,p'-DDT	(ppm)	0.04	0.09

* Full chemical names of the pesticides are given in Table 5.

Plot T was used to grow strawberries in 1970, but had not produced tobacco before 1974. It had reverted to the natural grass species. Plot UT had not been broken for many years and had a thick sod of quack grass, bluegrass, and bent grass species in 1973. Both plots were plowed in the fall of 1973; soil samples were analyzed and the results are shown in Table 1. The small amount of pesticide residue found in the UT plot was expected and the levels were considered to be insignificant. The aldrin and dieldrin carry-overs in Plot T were probably from chemicals applied to the strawberry crop. Chemical uptake seems to be within expected limits considering the amounts applied to Plot T.

2. *Crop Production: Nicotiana tabacum* cv. Virginia 115 was used for the study, and seedlings were produced in an unheated polyethylene greenhouse in 1974. Seed was planted April 2, 1974, on a medium of peat moss. Greenhouse beds received 2(N)-16(P)-6(K) fertilizer at a rate of 0.54 kg/m^2. No other treatments were applied. Seedlings grew slowly because of abnormally cool weather in April and May but were healthy. Plants for both the T and UT plots were transplanted on June 18. Plants were transplanted mechanically in rows 117 cm apart, the plants spaced 56 cm apart (15 300 plants/ha). Fertilizer was applied at 1233 kg/ha of 2(N)-18(P)-8(K) in two bands 7.6 cm from the seedlings just before transplanting.

Both the T and UT areas had been cultivated in May, 1974; because of the heavy infestation of quack grass, a third cultivation was considered necessary in early June. The third cultivation held the quack grass under reasonable control for the growing season. The crop was cultivated four times and hand-hoed once. The final cultivation was the formation of a broad hill to control any remaining quack grass. Tobaccos in the T and UT plots were topped at 15 to 16 leaves on August 19 and the UT plants were hand-suckered on September 5.

In accord with the purpose of the study, most pesticides used for tobacco production were applied to the T plot. In addition, DDT was applied although it is no longer used in tobacco production. The description of chemicals, rates, and dates of application are shown in Table 2.

The tobacco on both plots developed normally but had slightly too much nitrogen for early maturity. This was especially evident in the T plot. Tobacco in the UT plot ripened well, and the bottom leaves cured well. Because of the necessity of separate curing facilities, both lots of tobacco were left until 8 to 9 bottom leaves were ready to harvest. These leaves were harvested and cured separately, making two full runs in a three-bay, bulk-curing barn. Curing results were better with the UT tobacco than with the T material. The comparatively poor curing of T tobacco was probably due to a combination of excess nitrogen and the fact that the UT plot was well sheltered and able to retain more heat. Although the bottom 8 to 9

Table 2. Description of chemicals, rates and date of application for pesticide-treated plot (T).

Pesticide formulation+	Rate and kind of application	Date
Chlorpyriphos (Lorsban, 25% w.p.*)	Approx. 0.84 kg/ha (in transplanting water)	18 June 1974
Trichlorfon (Dylox, 80% w.p.)	2.80 kg/ha (overall spray)	24 June 1974
Diphenamid (Enide, 50% w.p.)	4.48 kg/ha (overall spray)	25 June 1974
Methomyl (Lannate, 90% a.i.**)	1.68 kg/ha (overall spray)	3 August 1974
DDT (50% w.p.)	2.80 kg/ha (overall spray)	4 August 1974
Carbaryl (Sevin, 50% w.p.)	2.24 kg/ha (overall spray)	4 August 1974
C-10 fatty alcohol (Contak)	9.33 liters/ha (overall spray)	17 August 1974
MH (MH-30, 30% a.i.)	16.33 liters/ha (overall spray)	31 August 1974

\+ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina State University and does not imply its approval to the exclusion of other products that may also be suitable.

* wettable powder.

** active ingredient.

Table 3. Temperature and precipitation during the 1974 growing season.

Month	Temperature (°C)						Precipitation (cm)	
	Maximum		Minimum		Mean			
	1974	Long-term average	1974	Long-term average	1974	Long-term average	1974	Long-term average
May	10.1	14.8	1.4	3.5	5.9	9.2	9.12	7.44
June	21.0	20.1	9.6	9.0	15.8	14.5	3.89	6.38
July	21.9	24.5	12.0	13.5	17.6	19.2	6.83	6.20
August	23.8	23.5	13.5	13.4	18.5	18.5	5.46	7.67
September	18.2	19.5	9.6	9.8	13.8	14.6	12.73	7.90

Table 4. Leaf composition of pesticide-treated and untreated tobacco.

		Treated tobacco (T)	Untreated tobacco (UT)	Averaged from number of collaborators
Moisture	(%)	3.76	3.65	3
Sand	(%)	0.76	0.21	2
pH		6.00	5.81	1
Ash	(%)	15.2	12.3	4
Alkalinity of water-soluble ash (ml 0.1 N HCl)		5.76	4.56	3
K	(%)	2.55	2.65	2
Na	(%)	0.10	0.08	1
Ca	(%)	3.21	2.47	2
Mg	(%)	1.28	0.61	3
Mn	(%)	0.02	0.04	1
Cl	(%)	0.47	0.45	3
NO_3	(%)	0.29	0.25	7
NH_3	(%)	0.12	0.09	7
α-amino N	(%)	0.54	0.42	7
Total N	(%)	2.66	2.65	7
Total sugars	(%)	8.90	12.2	2
Reducing sugars	(%)	6.98	9.4	6
Starch	(%)	0.46	3.33	2
Cellulose (holo)	(%)	34.8	39.6	1
Nicotine	(%)	2.07	2.00	9
Total volatile bases (TVB)	(%)	0.62	0.51	8
TVB/nicotine		0.30	0.26	8
Water-soluble acids (ml 0.1 N NaOH)		3.10	3.25	2
Malic acid	(%)	9.80	6.29	2
Citric acid	(%)	2.78	1.19	2
Oxalic acid	(%)	2.13	1.31	2
Total polyphenols	(%)	5.33	5.75	2
Chlorogenic acid	(%)	3.34	3.96	2
Rutin	(%)	1.02	1.24	1
Phytosterols	(mg/g)	1.50	1.47	1
Waxes	(%)	1.96	1.70	1
Oven volatiles	(%)	4.6	4.0	1
Petroleum-ether extracts	(%)	3.05	2.71	2
Hexane solubles	(%)	2.55	2.29	1
Neophytadiene	(%)	0.15	0.09	1
Glycerine	(%)	0.10	0.18	1

leaves of each lot were harvested at the same time, care was taken to label racks of each priming so that stalk positions were kept separated.
The bottom leaves were harvested on September 10. Six to seven leaves remained in stalk positions usually considered to be the 4th and 5th priming. Frost occurred on September 25 and affected the remaining tobacco. Damage was not obvious on the morning of September 26, but clearly visible within 2 days. The remaining leaves were harvested because of the requirements of the project. Curing was difficult because of the lack of moisture in the frozen leaf, but no serious leaf breakdown occurred.

3. *Environmental Conditions:* The average monthly temperature and precipitation during the tobacco growing period, together with long-term averages, are listed in Table 3. Although there were no drastic changes in comparison with long-term averages, the comparatively lower temperature and higher moisture in September of 1974, together with an early frost, might have had some effects on the leaf quality of the last priming of both T and UT tobaccos.

4. *Leaf Analysis:* A total of 2250 kg of experimental tobacco was produced. After stemming, a net weight of 703 kg lamina from the treated (T) plot and 854 kg from the untreated (UT) plot were obtained. Each sample was thoroughly mixed for cigarette manufacture, and a representative subsample from each was withdrawn for leaf analysis. Leaf analysis was conducted through collaboration of 16 research laboratories involving 5 countries. (One composite result involved seven collaborators.) Each laboratory used its own analytical method, continuously employed for its specific need, and selected the variables for analysis in which it was interested. There was no attempt to assign one unified method, nor to dictate the variables to be analyzed for by all collaborators. Since the main objective of the study was to compare treated and untreated samples, results so obtained from these collaborators were adequate and generally agreeable. Methods of analysis for each variable from each of the 16 collaborators are therefore too involved to be described in this report. For general reference, one may consult [a] Leaf analysis of first experimental cigarettes (2), [b] USDA Technical Bulletin No. 1551 (3), or [c] Tobacco, Method of Analysis (4).

RESULTS AND DISCUSSION

Analyses of leaf components were conducted by different collaborators. The data are shown in Table 4. Some of the variables were determined by only one laboratory, others involved 8 or 9 laboratories. Each laboratory may have made several determinations on a single variable. The reported data are averages of all collaborators. Thirty-five variables were examined.
Pesticide-treated samples appeared to have a higher sand, ash, and malic and citric acid content than untreated ones, but the untreated samples had higher total and reducing sugars, and starch and cellulose contents than treated ones. The total as well as individual nitrogenous fractions did not differ widely between the two treatments; in fact, the nicotine levels were almost identical.
Levels of 28 pesticides were determined (Table 5). It is quite evident that in treated tobacco, considerable amounts of DDT isomers and maleic hydrazide (MH) were present. Other pesticides showed little or no detectable residue, which indicated no apparent differences between treated and untreated tobaccos.
These two tobacco samples were made into experimental cigarettes for smoke analysis and for biological assay. Detailed information on this will be presented in Part II of this study.

SUMMARY

A special study was conducted with the aim of evaluating the effects of pesticide treatment on tobacco in comparison with tobacco not treated with any pesticide. For the purpose of growing these tobaccos, experimental plots were selected on Prince Edward Island, Canada, where contamination of air, soil and water was at a minimum. The tobacco leaf was analyzed for 35 components and 28 pesticide residues. These samples are to be used for smoke analysis and bioassay. The results will be reported in a later publication.

ZUSAMMENFASSUNG

Durch einen Vergleich von Tabaken, die mit und ohne Anwendung von Pestiziden gewachsen waren, wurde untersucht, welche Wirkung Pestizide auf die Tabakpflanze ausüben. Die Tabake wurden in einem Gebiet in Kanada (Prince Edward Island) gezogen, in dem die Verunreinigung des Bodens, der Luft und des Wassers sehr gering ist. Das Blattgut wurde auf 35 Inhaltsstoffe und auf Rückstände an 28 Pestiziden untersucht. Es sollen auch Rauchanalysen und biologische Versuche durchgeführt werden, über die in einer späteren Publikation berichtet werden wird.

RÉSUMÉ

Une étude spéciale a été effectuée afin d'établir une comparaison entre des tabacs traités aux pesticides et des tabacs n'ayant subi aucun traitement. Pour cultiver ces tabacs, on a choisi des parcelles expérimentales sur l'Île du Prince Édouard (Canada) où la contamination de l'air, de la terre et de l'eau est minime. La feuille de ces tabacs a été analysée sur 35 composants et sur les résidus de 28 pesticides. Ces échantillons seront soumis également à des analyses par fumage et à des essais biologiques; les résultats feront l'objet d'un rapport ultérieur.

Table 5. Pesticide levels (ppm) in leaf samples of pesticide-treated and untreated tobaccos.

	Treated tobacco (T)	Untreated tobacco (UT)	Averaged from number of collaborators
p,p'-TDEE [1-chloro-2,2-bis(4-chlorophenyl)ethylene]	0.19	0.02	2
o,p'-TDE [1,1-dichloro-2-(1-chlorophenyl)-2-(4-chlorophenyl)ethane]	0.25	0.01	5
p,p'-TDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane]	1.25	0.02	5
Total TDE [sum of p,p'-TDEE, o,p'-TDE, and p,p'-TDE]	1.69	0.05	
p,p'-DDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene]	1.06	0.02	5
o,p'-DDT [1,1,1-trichloro-2-(1-chlorophenyl)-2-(4-chlorophenyl)ethane]	2.36	0.02	5
p,p'-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane]	19.97	0.06	4
Total DDT [sum of p,p'-DDE, o,p'-DDT, and p,p'-DDT]	23.39	0.10	
Total TDE + DDT [sum of total TDE and total DDT]	25.08	0.15	
Toxaphene [chlorinated camphene containing 67–69 % chlorine]	< 0.3	< 0.3	2
Endrin [1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-*exo*-5,8-dimethanonaphthalene]	0.01	< 0.01	2
Dieldrin [1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene]	0.04	0.01	3
Endosulfan I [α-isomer of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo[e]dioxathiepin-3-oxide]	< 0.02	< 0.02	2
Endosulfan II [β-isomer of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo[e]dioxathiepin-3-oxide]	< 0.02	< 0.02	2
Endosulfan sulfate [6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide]	< 0.05	< 0.05	2
Total endosulfan [sum of endosulfan I, endosulfan II, and endosulfan sulfate]	< 0.09	< 0.09	1
α-BHC [α-isomer of 1,2,3,4,5,6-hexachlorocyclohexane]	0.01	0.01	1
Lindane [γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane]	0.01	0.01	1
Heptachlor [1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-*endo*-methanoindene]	0.01	0.01	1
Aldrin [1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-5,8-*exo*-dimethanonaphthalene]	0.01	< 0.01	1
Heptachlor epoxide [1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan]	0.01	< 0.01	1
Parathion [O,O-diethyl O-(4-nitrophenyl) phosphorothioate]	< 0.05	< 0.05	1
Carbaryl [1-naphthyl methylcarbamate]	0.40	< 0.10	1
Diazinon [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate]	0.01	0.01	1
Methyl parathion [O,O-dimethyl O-(4-nitrophenyl) phosphorothioate]	0.02	0.01	1
Malathion [S-(1,2-di(ethoxycarbonyl)ethyl) O,O-dimethyl phosphorothioiothioate]	0.05	0.03	1
Trichlorfon [dimethyl (1-hydroxy-2,2,2-trichloroethyl) phosphonate]	< 0.10	< 0.10	1
Monocrotophos [dimethyl *cis*-1-methyl-2-methyl carbamoyl vinyl phosphate]	< 0.20	< 0.20	1
Diphenamid [N,N-dimethyl-2,2-diphenylacetamide]	< 0.10	< 0.10	1
MH [1,2-dihydropyridazine-3,6-dione]	91.00	< 5.00	4

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Authors' address:

U.S. Department of Agriculture,
Agricultural Research,
Beltsville Agricultural Research Center,
Plant Genetics and Germplasm Institute,
Tobacco Laboratory,
Beltsville, Maryland, 20705, U.S.A.