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A NOTE ON THE PRESENCE OF ANTIBACTERIAL SUBSTANCES IN TOBACCO PLANTS

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SUMMARY: The juice pressed from twelve varieties of tobacco, and materials extracted with ether from these plants, frequently displayed antibacterial activity against several species of bacteria. Some varietal difference in activity towards *Ps. tabaci* was observed, and there is a suggestion of some correlation between this and resistance to 'Wildfire'.

ANTIBACTERIAL activity has been recorded from many plant species. Sherman & Hodge (1936) found it in several plant juices and Osborn (1943), who examined 2,300 plant species, found that 134 gave inhibitory extracts. Particularly active were members of the Ranunculaceae, a fact confirmed by Seegal & Holden (1945). Examinations have also been carried out by Sanders, Weatherwax & McClung (1945), Little & Grubough (1946), Carlson, Bissell & Mueller (1946), Carlson & Douglas (1948) and Carlson, Douglas & Robertson (1948). The latter tested members of the Solanaceae.

In all this work, emphasis has been placed on activity against human pathogens. Little & Grubough (1946), however, found their extracts to be also active against a number of phytopathogenic bacteria and obtained an indication that, in the case of corn, resistance to the bacterial wilt due to *Bacterium stewartii* (*Xanthomonas stewarti*) may be due to the presence of antibacterial substances, as these were found in the juice of resistant varieties.

EXPERIMENTS AND RESULTS

The plant juice was obtained by macerating leaves and whole shoots from 4–5 month old plants grown in a greenhouse and filtering off the debris. The clear juice was used without sterilization. Ether extracts were prepared by adding 5 ml of pure ether to 5 g of macerated plant material, storing for 24 hr at 6° and filtering to obtain a clear extract.

The plant juices were examined by the Oxford cup method of assay and the ether extracts by the paper disc method (Vincent & Vincent, 1944; Epstein *et al.* 1944). The test organisms were *Staphylococcus aureus* and *Escherichia coli*, together with the plant pathogens *Agrobacterium tumefaciens*, *Corynebacterium fascians*, *Erwinia carotovora*, *Pseudomonas tabaci* and *Xanthomonas campestre*.

Petri dishes of 6 in. diameter were used; 60 ml of nutrient agar seeded with the test organism were poured into each, allowed to set, and the 'plates' left at room

temperature for 1 hr. Sterile porcelain cylinders were then placed on the agar and filled with the plant juice. For the ether extracts, sterile 8 mm filter paper discs were dipped in the extract, allowed to drain for 5 sec and immediately placed on the agar surface. All 'plates' were incubated at 30° and examined after 24 and 48 hr.

Table 1. *The occurrence of antibacterial activity in leaf and shoot juice and in ether extracts* of leaves of twelve varieties of tobacco*

Variety	Antibacterial activity† against					
	E. coli	*A. tumefaciens*	*C. fascians*	*X. campestre*	*Ps. tabaci*	*Staph. aureus*
Bonanza	—	—	—	P (+)	+	P
Broad leaf	—	P	—	—	+(P)	P
C 7	—	P (−)	P	− (+)	+	P
Delcrest	—	—	—	+	—	—
Gold Dollar	—	P	+ (−)	P (−)	+	− (+)
Harrison's Special	—	—	—	− (P)	+	P (+)
Hickory Prior	—	—	—	—	—	—
Hicks' Special	—	P	—	P	P	—
Jamaica Wrapper	—	− (+)	+ (−)	—	+	P
Oxford 26	P (+)	− (+)	—	P (+)	+	P (+)
Special 400	—	—	—	P	+	P
White Stem Orinoco	—	—	—	− (P)	+	P

* Symbols in brackets indicate results from tests with ether extracts. These are shown only where they differed from those with expressed juice.

† Inhibition zones: +, zone of complete inhibition; P, zone of partial inhibition; −, no inhibition of any type.

The results, shown in Table 1, revealed the presence of antibacterial substances in the juices and ether extracts of the majority of the varieties tested. Inhibition of *E. coli* was restricted to the juice and ether extract of the variety Oxford 26, but some inhibitory activity against *Staph. aureus* was evident in 9 of the 12 varieties examined. Inhibition of all the plant pathogens was occasioned by one or other of the varieties, and the effects were most pronounced against *X. campestre* and, especially, *Ps. tabaci*. Inhibition of the latter organism is of special interest as a possible factor in resistance to the 'Wildfire' disease, and it will be seen that two varieties, Hickory Prior and Delcrest, yielded no inhibitory material and a third variety, Hicks' Special, gave rise only to partial inhibition. Precise information concerning the natural resistance of tobacco varieties to 'Wildfire' appears to be lacking, but Hickory Prior is known to be highly susceptible and the resistance of Hicks' Special and Delcrest very much less than that of the other varieties tested. It appears therefore that a correlation may exist between resistance to 'Wildfire' and antibacterial activity, though more information on resistance to 'Wildfire' is required before this can be confirmed.

The conditions under which the plants were grown may have affected the production of the inhibitory substances. The work was carried out over three years, one batch of plants of each variety being grown in a greenhouse during each summer, and the results in Table 1 represent the combined results from the three series of tests. Although the plants were greenhouse grown, the external conditions had a pronounced influence on the rate and type of growth. Two of the three summers

were dull and cold and the leaf tissue was less fleshy and yielded less juice than in the third summer, which was sunny and warm. The sizes of the inhibition zones were likewise affected; they were smaller when the plants were grown under dull and cold conditions, and it thus appears that the conditions of growth affected the production of the antibacterial substances. It will therefore be necessary, in any further study of the connection between antibacterial activity and disease resistance, to examine plants grown under normal conditions of cultivation, when their resistance to 'Wildfire' could also be determined.

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