PHYSIOLOGICAL STUDIES ON THRIPS IN RELATION TO TRANSMISSION OF TOMATO SPOTTED WILT VIRUS

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Summary

The hydrogen ion concentration of the midguts of larval and adult *Thrips tabaci* and *T. imaginis* is between pH 5·0 and 5·6. The oxidation-reduction potential at these values is between + 0·184 and + 0·262 V. There is thus no difference between thrips that are vectors and those that are not vectors of the virus causing tomato spotted wilt. Furthermore, the pH and Eh conditions in the midgut of larval *T. tabaci* are unsuitable for long survival of the virus.

The larval midgut of *T. tabaci* is less thoroughly tracheated than that of the adult. The larval *T. tabaci* ingests a smaller amount of plant tissue than the adult. This quantity averages about $8{\cdot}0 \times 10^{-5}$ mg per insect per minute, when the insects were exposed to the leaf for a 30-min feeding period. The entire epithelium of the midgut of larval and adult *T. tabaci* absorbs iron and copper ions.

These data are discussed in relation to theories to account for the ability of larval and the inability of adult *Thrips tabaci* to transmit tomato spotted wilt virus.

I. Introduction

A peculiar feature of the transmission of tomato spotted wilt virus is the fact, originally discovered by Bald and Samuel (1931) and independently by Linford (1932), that the virus must be acquired by the thrips when in the larval stage. The virus may be transmitted by the adults produced from larvae that have fed on an infected plant, but not by thrips that fed on infected plants only after they had reached the adult stage. This has been confirmed by Smith (1932) and Moore (1933).

Various suggestions concerning the mechanism of this inability of the adult thrips to acquire the virus have been put forward. Smith (1932) suggested that the anatomy of the adult and larva may be sufficiently different to account for the failure of the adult to transmit; Linford (1932) considered it possible that the virus might be digested by the adult but not by the larva; and Bawden (1950) suggested that the adult gut may be impermeable to the virus. None of these suggestions has been tested experimentally. However, Sakimura (1947) concluded that larvae and adults feed on the same tissues of the plant and considered that the differences between the mouth-parts of the larvae and adults were insufficient to account for the differences in viral transmission.

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Tomato spotted wilt virus is rapidly destroyed by oxidation (Best 1939*a*). This fact, together with the observed correlation between midgut tracheation and the oxidation-reduction potential (Eh) in the insect gut (Day 1951), suggested that the virus might be destroyed in the gut of the adult thrips but not in that of the larva. It seemed worth while, therefore, to determine whether there was any difference between the pH and Eh conditions in the gut of the larval and adult thrips.

The amount of material ingested by larval and adult thrips and permeability of the midgut to iron and copper ions were also investigated.

II. Methods

A culture of *Thrips tabaci* Lind., a vector of tomato spotted wilt, was maintained on onion plants in the laboratory throughout the year. *Thrips imaginis* Bagn., a non-vector species, was collected from rose hips or dandelion flowers as required.

Midgut tracheation was studied by the methods of Hagmann (1940) and Wigglesworth (1950). Both were suitable but not all specimens were satisfactory whatever method was used.

The pH and Eh of the gut were determined by feeding about 10 thrips on each of a series of indicators in 5 per cent. sucrose solutions. Sakimura and Carter (1934) had shown that thrips would feed through a plastic membrane, and the solutions in plastic containers were therefore separated from the insects by the commercially available plastic membranes used by Day and Irzykiewicz (1953). The insects were generally left to feed overnight, but occasionally a period of 3 days was necessary when insufficient indicator was ingested during the shorter period. They were then dissected in saline and the presence of the indicators was determined in the isolated midgut. The presence of indicators that were colourless or yellow was determined by adding appropriately buffered solutions to the drop of saline in which the dissection was performed, to produce a more readily detectable colour. Similarly colour changes of oxidation-reduction indicators were checked in alkaline saline by the addition of potassium persulphate as an oxidizing agent.

The amount of material ingested was examined by feeding thrips on solutions containing ^{32}P; for details of the technique, see Day and Irzykiewicz (1953).

Absorption of iron and copper was studied by the methods of Waterhouse (1940) and Waterhouse (1945) respectively.

The tomato spotted wilt virus used consisted of a mixture of strains cultured in Bonny Best tomato plants.

III. Observations

(*a*) *Eh and pH of Midgut Contents*

The hydrogen-ion concentrations of the midgut contents of the larval and adult *T. tabaci* and *T. imaginis* were determined by the experiment detailed in

Table 1. It will be observed that the pH was between 5·0 and 5·6 for larvae and adults of both species.

Similarly, an approximate value for the oxidation-reduction potential of the midgut contents was determined by the use of the indicators listed in Table 2. Again there was no detectable difference between larvae and adults and between the vector and non-vector species. In all, the potential lay between a minimum of + 0·184 and a maximum of + 0·262 V.

TABLE 1

THE pH OF CONTENTS OF MIDGUT OF *THRIPS TABACI* AND *THRIPS IMAGINIS*

Indicator	*Thrips tabaci*		*Thrips imaginis*	
	Adults	Larvae	Adults	Larvae
Thymol blue	>2·2	>2·2	>2·2	>2·2
	<8·6	<8·6	<8·6	<8·6
Bromphenol blue	>4·3	>4·3	>4·3	>4·3
Phenol red	<7·4	<7·4	<7·4	<7·4
Bromthymol blue	<6·5	<6·5	<6·5	<6·5
Bromcresol purple	<6·0	<6·0	<6·0	<6·0
Chlorphenol red	<5·6	<5·6	<5·6	<5·6
Bromcresol green	>5·0	>5·0	>5·0	>5·0
pH range	5·0-5·6	5·0-5·6	5·0-5·6	5·0-5·6

(*b*) *Tracheation of Midgut*

Examination of tracheal impregnations of larval and adult *T. tabaci* showed that tracheation of the midgut was not particularly thorough. There were no anastomoses, as in the cockroach midgut (see Day 1951), and by no means every epithelial cell was tracheated. It was apparent, however, that the adult midgut was more completely tracheated than that of the larva.

These observed differences could not be correlated with a difference in oxidation-reduction potential of midgut contents. Undoubtedly the small size of these insects contributes to the maintenance of the relatively oxidizing conditions in the midgut contents in the absence of a highly developed tracheal supply.

(*c*) *Quantity of Material Ingested by Larval and Adult Thrips*

Observation had suggested that larval thrips were responsible for greater tissue damage to host plants than adult thrips. It was thought that differences in amount ingested might possibly explain differences in the transmission of tomato spotted wilt. The following experiments demonstrated that this suggestion was unfounded.

Larval and adult *T. tabaci* were fed on 5 per cent. sucrose solution containing ^{32}P. The duration of feeding was observed through a transparent plastic

membrane and an accurate estimate of the rate of ingestion could therefore be made. The insects were starved for from 30 to 60 min before being permitted to feed, and in view of the short time of feeding excretion of ^{32}P was probably very low in these instances. The results (Table 3) show that adults ingested more than larvae, and that the amount ingested by larvae averaged $1{\cdot}2 \times 10^{-3}$ and by adults $2{\cdot}0 \times 10^{-3}$ cu. mm. per insect in 1 min.

TABLE 2

THE OXIDATION-REDUCTION POTENTIAL OF THE CONTENTS OF THE MIDGUT OF *THRIPS TABACI* AND *THRIPS IMAGINIS*

Indicator	E'_0 of Indicator (V)		Colour of Indicator		Colour of Indicator in Gut of Thrips			
					Thrips tabaci		*Thrips imaginis*	
	pH 5·0	pH 5·6	Oxidized Form	Reduced Form	Adults	Larvae	Adults	Larvae
Indigo disulphonate	−0·010	−0·045	Blue	Yellow	Blue	Blue	Blue	Blue
Indigo trisulphonate	+0·032	−0·004	Blue	Yellow	Blue	Blue	Blue	Blue
Indigo tetrasulphonate	+0·065	+0·029	Blue	Yellow	Blue	Blue	Blue	Blue
Methylene blue	+0·101	+0·066	Blue	Colourless	Blue	Blue	Blue	Blue
Thionine	+0·138	+0·106	Blue-purple	Colourless	Blue-purple	Blue-purple	Blue-purple	Blue-purple
Toluylene blue	+0·221	+0·184	Blue	Colourless	Pink*	Pink*	Pink*	Pink*
1: Naphthol-2-sodium sulphonate-indo 2,6-dichlorphenol	+0·262	+0·223	Blue in alk. red in acid	Colourless	Colourless	Colourless	Colourless	Colourless

*In spite of the anomalous colour, it was shown that the dye was present in the midgut in the oxidized form.

Lower figures were obtained for insects feeding on a leaf of Chinese cabbage (*Brassica chinensis* L.) for 30 min (Table 4), because the insects did not feed for all of the time and possibly also because some of the isotope was excreted. Adult female *T. tabaci* weighed about $2{\cdot}8 \times 10^{-2}$ mg, and ingested roughly 17 per cent. of their weight per hour. The Chinese cabbage leaf was made radioactive by standing in White's nutrient solution containing ^{32}P for 24 hr. Again, adults ingested more than larvae, but the differences between larvae and adults were clearly unrelated to inability of the adult thrips to acquire virus.

(*d*) *Midgut Permeability*

Data to test Bawden's (1950) suggestion that the adult midgut might be impermeable, but that the larval gut could be penetrated by the virus, were sought by two methods.

(i) An attempt was made to puncture the midgut with a fine steel needle sharpened under a magnifier on a hard Arkansas oilstone. The operation was performed on 188 adult *T. tabaci*, held under carbon dioxide anaesthesia, that had fed for 24 hr on tomato plants infected with tomato spotted wilt virus. After 24 hr 20 adults remained alive on the infected tomato plant. These were transferred to six seedlings of *Datura stramonium* and seven seedlings of tomato. Eight adults survived on eight of these test plants for 14 days but no plant became infected with the disease.

TABLE 3

RATE OF INGESTION OF 5 PER CENT. SUCROSE SOLUTION BY ADULTS AND LARVAE OF *THRIPS TABACI*

Insect Stage	Feeding Time (min) (*a*)	Ingested ^{32}P (counts/min) (*b*)	^{32}P Content in 1 cu. mm. Sucrose (counts/min) (*c*)	Rate of Sucrose Ingestion per Insect (cu. mm./min) $\left(\frac{b}{a \times c}\right)$	Mean Rate of Ingestion of Solution (cu. mm./insect/min)
Adult	6·25	72	9,600	$1{\cdot}2 \times 10^{-3}$	
Adult	7·50	272	9,600	$3{\cdot}8 \times 10^{-3}$	
Adult	17·50	534	12,600	$2{\cdot}4 \times 10^{-3}$	$2{\cdot}0 \times 10^{-3}$
Adult	16·00	97	12,600	$0{\cdot}5 \times 10^{-3}$	
Adult	15·50	440	12,600	$2{\cdot}3 \times 10^{-3}$	
Larva	11·00	81	9,600	$0{\cdot}8 \times 10^{-3}$	
Larva	5·33	63	9,600	$1{\cdot}2 \times 10^{-3}$	
Larva	8·66	128	12,600	$1{\cdot}2 \times 10^{-3}$	
Larva	14·00	99	12,600	$0{\cdot}6 \times 10^{-3}$	$1{\cdot}2 \times 10^{-3}$
Larva	5·00	111	12,600	$1{\cdot}8 \times 10^{-3}$	
Larva	17·75	388	12,600	$1{\cdot}7 \times 10^{-3}$	

(ii) Any physiological difference in absorption between the larval and adult thrips midgut would be of interest. An interesting system in the larval blowfly midgut concerns the absorption of ions of copper and iron (Waterhouse 1940, 1945). The second method of studying midgut permeability was therefore to feed solutions containing increased iron and copper salts through plastic membranes to larval and adult *T. imaginis* and *T. tabaci* for 48 hr. The dissected midguts were then examined after application of the appropriate histochemical tests for detection of these metals. All cells of the midgut stained evenly in larvae and adults of both species and no region appeared to be especially concerned with the absorption of these metallic ions.

There was thus no evidence from these experiments that differences existed between the permeability of the midgut of larval and adult thrips.

IV. Discussion

The suggestion that variation in pH may determine the distribution of virus of tomato spotted wilt in various tissues of the plant has been made by Best (1939*c*); there were logical reasons for expecting that pH, Eh, or both would be concerned in determining the distribution of the virus in the insect vector. The evidence presented above proves that there are no differences in pH or oxidation-reduction conditions in the midguts of adult or larval thrips or of a species of thrips incapable of acting as a vector of the virus, and that the explanation of the inability of the adult *T. tabaci* or of adult and larval *T. imaginis* to acquire the virus must be sought elsewhere.

Table 4

RATE OF INGESTION OF LEAF MATERIAL BY ADULTS AND LARVAE OF *THRIPS TABACI* WHEN LEFT ON ^{32}P LEAF FOR 30 MIN

Insect Stage	Feeding Time (min) (*a*)	Ingested ^{32}P (counts/min) (*b*)	^{32}P Content in 1 mg Leaf Tissue (counts/min) (*c*)	Rate of Ingestion of Leaf Tissue per Insect per Min (mg/min) $\left(\frac{b}{a \times c}\right)$	Mean Rate of Ingestion of Leaf Tissue (mg/min)
Adult	30	376	67,400	$1{\cdot}9 \times 10^{-4}$	
Adult	30	97	67,400	$0{\cdot}5 \times 10^{-4}$	
Adult	30	86	67,400	$0{\cdot}4 \times 10^{-4}$	
Adult	30	240	67,400	$1{\cdot}2 \times 10^{-4}$	
Adult	30	123	67,400	$0{\cdot}6 \times 10^{-4}$	
Adult	30	149	67,400	$0{\cdot}7 \times 10^{-4}$	$1{\cdot}0 \times 10^{-4}$
Adult	30	102	67,400	$0{\cdot}5 \times 10^{-4}$	
Adult	30	454	67,400	$2{\cdot}2 \times 10^{-4}$	
Adult	30	186	67,400	$0{\cdot}9 \times 10^{-4}$	
Adult	30	241	67,400	$1{\cdot}2 \times 10^{-4}$	
Larva	30	95	67,400	$0{\cdot}5 \times 10^{-4}$	
Larva	30	261	67,400	$1{\cdot}3 \times 10^{-4}$	
Larva	30	104	67,400	$0{\cdot}5 \times 10^{-4}$	
Larva	30	56	67,400	$0{\cdot}3 \times 10^{-4}$	
Larva	30	83	67,400	$0{\cdot}4 \times 10^{-4}$	$0{\cdot}6 \times 10^{-4}$
Larva	30	130	67,400	$0{\cdot}6 \times 10^{-4}$	
Larva	30	136	67,400	$0{\cdot}7 \times 10^{-4}$	
Larva	30	127	67,400	$0{\cdot}6 \times 10^{-4}$	
Larva	30	61	67,400	$0{\cdot}3 \times 10^{-4}$	

More surprising is the demonstration that the conditions of pH and Eh in the gut of the larval *T. tabaci*, which is capable of acquiring the virus of tomato spotted wilt, are quite unsuitable for the maintenance of the virus. Thus Best

(1939*b*) has shown that infectivity of tomato spotted wilt is greatly reduced at pH 5·1 and it is surmised from his data on oxidative destruction of tomato spotted wilt virus (Best 1939*a*) that an oxidation-reduction potential of $> +0{\cdot}100$ V would result in fairly rapid destruction of the virus. However, it is appreciated that measurement of this potential does not necessarily indicate the oxidizing power of the midgut contents. Nevertheless, the results suggest the possibility that the tomato spotted wilt virus may be protected from destruction in the midgut of the larval thrips and that such a protecting mechanism may be absent from the midgut of the adult. However, an attempt to test this hypothesis gave no support to the suggestion.

Two possible mechanisms remain to explain the differences between the ability of larval and adult thrips to acquire the virus of tomato spotted wilt from infected plants. There may be differences between the midgut permeability of the two stages, or the virus may not be able to multiply in the tissues of the adult, whereas it finds suitable conditions in the larva.

V. Acknowledgments

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VI. References

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