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ESTIMATION OF VARIATIONS IN SURFACE WAX INDUCED BY *PIPALDIPLOSIS PIPALDIPLOSIS* MANI IN THE LEAVES OF *FICUS RELIGIOSA L.*[1]

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ABSTRACT

Pipaldiplosis pipaldiplosis Mani is gall forming insect affecting the leaves of *Ficus religiosa L.*

Qualitative and quantitative estimation of variation in leaf surface wax induced by this insect was undertaken. The comparative analysis of wax deposition in the normal leaf surface, in infected leaf and the gall surface revealed changes in the wax components. Therefore, it can be summarised that the waxes present on the healthy as well as on the diseased leaf surfaces play an important role in limiting the insect infestations. Otherwise it would have been very severe and damaging too.

INTRODUCTION

The nature of leaf surface is an important factor in leaf infections. The outermost coating of the leaves, the cuticle, is made up of waxes. Waxy nature of leaf surface is water repellant and important as the drop retention makes the leaf susceptible for variety of infections. The waxy leaf may detract some types of insects, while on the other hand it may be very much luring to others. Anstey and Moore (1954) reported that a glossy leaved mutant of sprouting broccoli (*Brassica oleracea* var. *clalica*) is more susceptible to the cabbage beetle (*Phyllotreta albionica*) than the normal waxy plants. On the other hand, Thompson (1963) noted that the normal waxy plants in field populations of narrow stem kale (*B. oleracea* var. *acephala*) had large colonies of the cabbage aphid (*Brevicoryne brassicae*) whereas non waxy plants were not colonised. Way and Murdie (1965) also reported that a non-glaucous strain of brussel sprout was more resistant to the cabbage aphid than the normal glaucous but relatively more attractive to another aphid *Myzus persicae*. Leaves of *Rubus phoenicolasius* are unattractive to the raspberry beetle (*Byturus tomentosus*) being heavily waxed and the wax being richer in acidic substances than other *Rubus* waxes (Martin and Juniper, 1970). The effects of insect attack on the cuticle are often marked. Sometimes, excess of wax production occurs at and around the places of the insect attack. Bystrom *et al.* (1968) reported that excess of wax development occurs around the punctures of aphids. The changes in waxes are very significant and lead to lower down the insect infestation and decrease its spread (Anstey and Moore, 1954).

So far, there are no reports of the

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work related to the interaction of leaf surface and gall making insect (*Pipaldiplosis pipaldiplosis* Mani). The present work deals with the qualitative and quantitative response of leaf surface wax to this parasitic insect which causes gall formation due to browning swelling of the midrib and other side veins (Fig. I).

MATERIAL AND METHODS

The normal leaves with no apparent symptoms and infected leaves of the same size and age with variable stages of gall formation were selected so as to detect overall effect and differences as compared with normal ones.

Extraction of wax was done from samples of 10 leaves each of normal and infected ones separately by immersing the leaves in 25 ml of cold chloroform according to Martin and Batt (1958) and drying in oven at 35°C till constant weight was achieved. The solvent containing the wax was filtered by centrifugation at 3000 r.p.m. for 20 minutes at 5°C to make it free from insoluble impurities and later placed in hot air oven at 30°C to evaporate the solvent for about 48 hours. The containers with the dry matter were weighed at room temperature. In another estimation galls were dissected out, further washed with water gently and subjected to air-drying for removal of extra water, at room temperature, and the same procedure was adopted as for normal and infected leaves. Comparative surface wax components in the three cases is given in Table I.

The samples for qualitative analysis were prepared by dissolving out the dry matter obtained from leaf washings for quantitative observations in 5 ml of chloroform and were applied to the starting line of the thin layer plates of Kieselgel G (E. Merck AG). These loaded plates were developed by Adsorption thin layer chromatography technique of Holloway and Challen (1966) in an air tight chamber saturated for two hours prior to use. The developing solvent was prepared by mixing benzene and chloroform in the ratio 7 : 3 (V/V). The developed plates were fan dried and then sprayed with saturated solution of antimony trichloride in chloroform to detect the spots on the chromatograms. The uniformly sprayed plates were dried at 120°C in hot-air-oven as a result of which the spots were visible. The Rf. value of wax constituents was calculated and identified with those of known ones (Table II).

RESULTS AND DISCUSSION

The observations indicate that the amount of leaf surface wax per gram dry weight varies considerably in all the samples i.e. normal, infected (leaves with galls) and dissected galls. The total surface wax in 10 normal leaves calculated per gram dry weight was 132.100 $\times 10^{-4}$ grams while in the case of infected

TABLE I

QUANTITY OF LEAF-SURFACE WAX AND THEIR RELATIVE DIFFERENCES FROM NORMAL LEAF

Normal Leaf Surface Wax	Diseased leaf Surface Wax	Difference	Surface wax on galls
332.100×10^{-4}	209.100×10^{-4}	123.00×10^{-4}	111.520×10^{-4}

1. The quantity of wax is in gms/gram dry weight of leaf.
2. Results are the mean of ten replicates.

TABLE II

QUALITATIVE VARIATIONS IN WAX COMPONENTS

S. No.	Wax Components	Normal Leaf	Infected Leaf	Diseased part of the leaf only
1.	Ursolic Acid ..	+	+	+
2.	n-Fatty Acids (Rf. 0.0503) ..	+	+	—
3.	n-Monounsaturated Acid (Rf-0.094)	—	+	+
4.	Sterols (Rf-0.147)	+	+	+
5.	n-Hydroxy Acid (Rf-0.025) ..	+	+	+
6.	n-Alkyl Ketone (Rf-0.772) ..	+	+	—
7.	n-Primary Alcohol (Rf-0.196) ..	+	—	—
8.	n-Secondary Alcohol (Rf-0.501) ..	+	+	+
9.	n-Alkene (Rf-0.37)	+	+	—
10.	B-amyrin (Rf-0.22)	—	+	+
11.	Unknown-1 (Rf-(0.367) ..	+	+	+
12.	Unknown-2 (Rf-0.307)	—	+	+
13.	Unknown-3 (Rf-0.685) ..	+	+	—

(+)=Present and (—) Absent.

leaves the dry weight was 209.100×10^{-4} grams. The galls showed 111.520×10^{-4} grams of wax deposition per gram dry weight. The difference in surface wax depositions between normal ar d infected leaves was found to be 123.00 $\times 10^{-4}$ grams. Thus, infected leaves show decrease in wax quantity in spite of the increase in dry weight which was found to be 5.1348 grams as against 4.2210 grms of normal leaves. The substantial increase in dry weight is certainly due to the galls present on midrib and other veins. In all, thirteen constituents were isolated (Table II).

Insect infected leaf-surface wax has all the constituents which are present in the normal leaf except the n-Primary

Fig. 1. Showing galls on the leaf of *Ficus religiosa* L.

Alcohol. In addition it has few more viz. Unknown-2. Rf. .307 ; B-amyrin Rf. .22 ; and Monounsaturated acid, Rf. .094 which are not present in the normal one. The surface-wax on dissected galls alone was also taken into account just to confirm about the changes in gall-surface wax itself. The gall surface wax shows the presence of eight constituents. Monounsaturated Acid, B-amyrin and Unknown-2 which are absent in normal leaves, show their presence exclusively. On the other hand, Fatty Acids, Sterols, n-Hydroxy Acid, n-Secondary Alcohol, and Unknwon-1, common to infected leaf-surface wax are also present.

Thus, surface wax of galls contains three such components which are not present in normal leaf. It may be concluded that the insect attack alters the plant metabolism of the infected cells resulting in distinct changes in leaf-surface wax both qualitatively and quantitatively.

The insect disturbs the defence mechanism by reducing the surface wax which is about 2/3 of the normal leaf and reduced to only 1/3 on the gall surface. This decrease in the wax surface renders the host leaf more prone to the insect attack. Besides, the insect attack also brings about changes in the wax constituents as is revealed by the presence of n-monounsaturated acid and B-amyrin which are absent in the wax of a normal leaf.

The insect attack not only brings about decline in wax content but also disturbs its composition. Normally, as pointed out by Martin and Juniper (1970), wax composition is genetically controlled by specific genes but here this is induced by insect attack.

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