Studies on the Substances of Leaves Affecting Positive and Negative Phagoactivities of the Domestic Silkworm

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ABSTRACT   In this dissertation, the studies on the effects of the substances of leaves on the positive and negative phagoactivities of the silkworm, *Bombyx mori* L., were carried out in detail.   The residual, nonpolar, and polar substances of the leaves of mulberry, fig, hackberry, Japanese oak, and heaven-tree were fractionated, analysed, and assayed by using new-hatched larvae of the F_1 of Gunko x Manri for the phagoactivity.   From series of the experimental results, five important new facts are epitomized as follows:

(1)   A complete positive phagoactivity of the silkworm was a complemental action, elicited by the functional components of the mulberry leaves as the following classification:

  (A)   Basal components-water, residual components (cellulose, protein, and starch), and a gelatinizer (agar) for solidification of the diet to form an even stratum.

  (B)   Water-soluble components (polar phagostimulants)—(a) saccharides (a mixture of fructose, sorbitol, glucose, inositol, and sucrose);   (b) inorganic salts (a mixture of KCl, $NaNO_3$, K_2SO_4, Na_2SO_4, KH_2PO_4, K_2HPO_4, $MgCl_2$, $CaCl_2$, and $Fe_2(SO_4)_3$;   (c) salts of organic acids (probably neutral K and Na salts of malic, chlorogenic, fumaric, phthalic, and protocatechuic acids;   (d) flavonoid pigments (quercetin and quercitrin).

  (C)   Ethyl ether-soluble components (nonpolar phagostimulants) - (a) fatty acids (linoleic and linolenic acids);   (b) phytosterol (β-sitosterol);   (c) volatile alcohol (trans-2-hexenol);   (d) carotenes (probably α, β, and γ).

(2)   The polar phagostimulants were active and the nonpolar phagostimulants were inactive when fed to the silkworm in the combination of the basal components.   But feeding with the polar and nonpolar phagostimulants, and the basal components in the combination was indispensable for the manifestation of a complete positive phagoactivity of the silkworm.

(3)   The effect of mulberry leaves was higher than that of fig leaves in terms of the positive phagoactivity of the silkworm, mostly due to phagostimulating actions by the higher contents of five saccharides (especially inositol and sucrose) and fatty acids (especially linoleic and linolenic acids).

(4)   As for the negative phagoactivity of the silkworm, it was elucidated that the effect of phagostimulants on the phagoactivity was completely depressed by the phagorepellents in hackberry, Japanese oak, and heaven-tree.   However, the repellent actions of either polar or nonpolar phagorepellents were drastic enough to inhibit the stimulant actions of the polar phagostimulants when coexisted.

(5)   The polar and nonpolar phagorepellents present in the leaves of hackberry, Japanese oak, and heaven-tree could be adsorbed by active cahrcoal; the former were presumed to be flavonoid

pigments having Rf values of 0.29 and 0.37 (paper chromatography, developed by butanol-acetic acid-water, 4:1:5 v/v), and the latter were presumed to be some isomers of the xanthophyll.

Introduction

The species specificity of the eulepidopterous insects in terms of the selection of host-plants has been ascribed to the actions of attractants and other relevant chemical factors in the green leaves (DETHIER, 1947, 1954, 1970). Up to the present, the mechanism for specific relation between mulberry leaves and the larvae of the silk-worm, *Bombyx mori* L., has not yet been elucidated, although an interesting speculation has been proposed that the feeding behavior of the domestic silkworm is controlled by three factors in the mulberry leaves, viz:

(1) Attractants—citral, linalol, linalyl acetate, and terpinyl acetate;

(2) Biting factors—main factors (β-sitosterol, isoquercitrin, and morin) and co-factors (inositol and sucrose);

(3) Swallowing factors—cellulose, silicate, and potassium phosphate (HAMAMURA, 1959, 1963–1964).

It has been further reported that the biting factors are β-sitosterol, isoquercitrin, and morin and the swallowing factors are cellulose, silicate, sucrose, inositol, and potassium phosphate (HAMAMURA, 1970).

Some phagostimulants for the larvae of the silkworm were recently discovered, e.g., quercetin (NAITO, 1968a-b), n- and neochlorogenic acids, free fatty acids, and neutral salts of $KOH + NaOH + H_2PO_4$ (LIN et al., 1970, 1971a-b). However, the activities of these substances in combination were inferior to that of the fresh mulberry leaves or the powder of mulberry leaves in terms of (A) the phagoactivity (the activity of feeding of the newly-hatched larvae of the domestic silkworm) and (B) normal growth of the whole larval stage of the domestic silkworm.

In nonpolar substances of the mulberry leaf, some components have been demonstrated, such as volatile alcohols (benzyl, isoamyl, phenethyl alcohols, etc.), volatile organic acids (acetic, propionic, n-butyric, isobutyric, n-caproic acids, etc.) (WATANABE, 1958; YAMAZAKI, 1967), monoterpenes (citral, linalol, linalyl acetate, and terpinyl acetate) (HAMAMURA, 1959), β-sitosterol (HAMAMURA, 1959; HAMAMURA et al., 1961; MIYAUCHI et al., 1964), campesterol (MIYAUCHI et al., 1964), n-aliphatic alcohols (NAYAR et al., 1962), and eight fatty acids (ITO et al., 1966; YAMADA et al., 1967; LIN et al., 1971). However, no study has been presented on the effects of volatile components, chlorophylls, and caroetnoids on the phagoactivity of the domestic silkworm except for β-sitosterol and fatty acids.

In polar substances of the mulberry leaf, some important factors were described previously, e.g., sucrose, inositol, and potassium phosphate as swallowing factors (HAMAMURA, 1970), isoquercitrin as a biting factor (HAMAMURA, 1970), quercetin as

a phagostimulant (NAITO, 1968a), n- and neochlorogenic acids as growth factors and phagostimulants (KATO et al., 1963–1964, 1966; LIN et al., 1970). However, according to the recent investigations by the author, there seemed to be many other phagostimulants such as flavonoid pigments, inorganic salts, salts of organic acids in the water-soluble fraction of the mulberry leaf besides the above mentioned factors.

In contrast to the positive factors, the investigations by the author strongly support the existence of the following negative factors in the none host-plants affecting the negative phagoactivity of the silkworm:

(A)   Olfactorepellents—For instance, the fresh leaves of *Cinnamomum camphora* (L.) Sieb. and *Houttuynia cordata* Thunb. cause a conspicuous repellence on the olfactoactivity of the domestic silkworm;

(B)   Gustorepellents—For instance, the fresh leaves of *Ailanthus altissima* Swingle and *Quercus variabilis* Blume cause an attractive action but reversely bring about a complete negative phagoactivity;

(C)   Olfacto-gustorepellents—For instance, the fresh leaves of *Cinnamomum camphora* (L.) Sieb. and *Houttuynia cordata* Thunb. cause a negative activities on both olfaction and gustation.

In order to determine these phagostimulants (positive factors) and phagorepellents (negative factors) affecting the host-specificity of the domestic silkworm, the systematical fractionation, purification, and bioassay of the residual, nonpolar, and polar substances of one host-plant (mulberry leaf), one of the substitutional plants (fig leaf), and three none host-plants (hackberry, Japanese oak, and heaven-tree leaves) were carried out.   In this paper, the effects of: (1) nonpolar substances including chlorophyll, carotenoids, sterols, fatty acids, and volatile components; (2) polar substances including pectin, flavonoid pigments, inorganic salts, salts of organic acids, and saccharides; (3) residual components including cellulose, protein, starch, and ashes on the phagoactivity of the domestic silkworm were assayed in detail.

Materials and Methods

Powder of Leaves

The juvenile fresh leaves of mulberry, fig, hackberry, Japanese oak, and heaven-tree were collected in the botanical garden of the Faculty of Science, Kyoto University, at 3:00 P.M. as follows:

(1)   Mulberry leaves (ML, *Morus bombycis* Koidz.), on September 6, 1970 and on May 23, 1971;

(2)   Fig leaves (FL, *Ficus pumila* L.), on September 7, 1970 and on May 24, 1971;

(3)   Hackberry leaves (HBL, *Celtis sinensis* Pers. var. *japonica* Nakai), on September 8, 1970 and on May 25, 1971;

(4)   Japanese oak leaves (JOL, *Quercus variabilis* Blume), on September 9, 1970 and
      on May 26, 1971;

(5)   Heaven-tree leaves (HTL, *Ailanthus altissima* Swingle), on September 10, 1970
      and on May 27, 1971.

These leaves were dried at room temperature for five days, pulverized in a rotary
ball-mill for one week, and the powder was passed through a round sieve of 200
mesh to separate from the coarse fragments.

Silkworm Race

      The newly-hatched larvae of the F_1 of Gunko × Manri were used throughout
the experiments.   The eggs were kindly provided by Gunze Silk Co. Ltd., Kyoto.

Basal Diet for Bioassay of Phagoactivity (BD)

      The composition of the basal diets from ED-1 to BD-9 is shown in Table 1.

Table 1.   Composition of the basal diet for bioassay of phagoactivity

Component	Dry weight (g)								
	BD-1	BD-2	BD-3	BD-4	BD-5	BD-6	BD-7	BD-8	BD-9
A	10 ml	10 ml	8 ml	8 ml	8 ml	8 ml	8 ml	8 ml	8 ml
B	—	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C	—	0.6	0.6	0.6	0.6	0.45	0.45	0.45	0.45
D	—	—	—	—	—	0.2	0.2	0.2	0.2
E	0.18	0.18	—	0.18	0.18	—	—	0.18	0.18
F	—	—	—	0.071	0.071	—	—	0.071	0.071
G	—	—	—	—	—	—	—	—	1 ml
H	0.009	0.009	—	—	0.009	—	0.009	0.009	0.009
I	—	—	—	—	0.011	—	0.011	0.011	0.011
J	—	—	—	—	—	—	—	—	4 mg
K	—	—	—	—	—	—	—	—	8 mg
L	0.2	0.2	0.2	0.2	0.2	0.15	0.15	0.15	0.15

Note: A—Water;
        B—Difco casein;
        C—Cellulose;
        D—Starch;
        E—Saccharides containing fructose (0.055 g), sorbitol (0.004 g), glucose (0.037 g), inositol
            (0.021 g), sucrose (0.067 g);
        F—Inorganic salts containing KCl (14 mg), $NaNO_3$ (8 mg), K_2SO_4 (16 mg), Na_2SO_4
            (13 mg), KH_2PO_4 (10 mg), K_2HPO_4 (10 mg), $MgCl_2$ (0.03 mg), $CaCl_2$ (0.03 mg),
            $Fe_2(SO_4)_3$ (0.05 mg);
        G—Salts of organic acids containing malic (1 g), fumaric (0.4 g), phthalic (0.3 g),
            protocatechuic (0.25 g), caffeic (0.3 g), and quinic acids (0.3 g), dissolved in 100 ml
            of distilled water, and neutralized with 3M KOH plus 1M NaOH to pH 7.0;
        H—Fatty acids containing linoleic (1.4 mg) and linolenic acids (8.1 mg);
        I —β-sitosterol;
        J —trans-2-hexenol;   K—ML carotenes fraction;   L—agar

Chemicals except for distilled water were obtained from the local industrial companies.

Method for Bioassay of Phagoactivity

Various fractions obtained from ML, FL, HBL, JOL, and HTL were dried on a water-bath, and each fraction was incorporated with the appropriate BD. For instance, the fraction of xanthophylls was incorporated with BD–5 consisting of cellulose, casein, saccharides, inorganic salts, fatty acids, β-sitosterol, and the agar-gel for laying an even physical stratum of the diet in order to examine the repellent action. The agar and water were heated together to form a complete jelly-like solution prior to the incorporation of the fractional substances to be assayed. After gelatinization of the diet in the petri dish of 6 cm in diameter, 10 newly-hatched larvae were fetched in each petri dish and kept at $27\pm1°C$ in a dark incubator. Each experiment was carried out in duplicate. To BD–8 and BD–9, 0.2% streptomycin and 0.6% sorbic acid or benzoic acid were added for the prevention of contamination by the pathogenic organisms. The number of frass excreted by the fetched larvae has been established to be one of the best parameters for indication of the phagoactivity of the larvae. The effect of substances to be assayed for the phagoactivity of the larvae was examined by counting the number of frass excreted in 18 hr or 24 hr after fetching. If the number of frass excreted by the fetched larvae in more than five per one larva per day, it should be regarded as significant in phagoactivity when compared to the control.

Fractionation of Nonpolar and Polar Substances from Powder of Leaves

(1) *Fractionation of Nonpolar Substances*

The powder of leaves was treated with ethyl ether with magnetic stirrer at room temperature and filtered through Toyo No. 2 filter paper. The filtrate showed a green color and was denoted E–1. The fraction E–1 was treated with active carbon and filtered through Toyo No. 2 filter paper. The filtrate was colorless and was denoted E–2. The fraction E–2 was shaken with 5% sodium carbonate. Two layers were formed; the ethyl ether layer was separated and denoted E–3, and the sodium carbonate layer was acidified with phosphoric acid to pH 2.0 and further shaken with ethyl ether. The ethyl ether layer was separated and denoted E–4. The sodium carbonate layer was discarded.
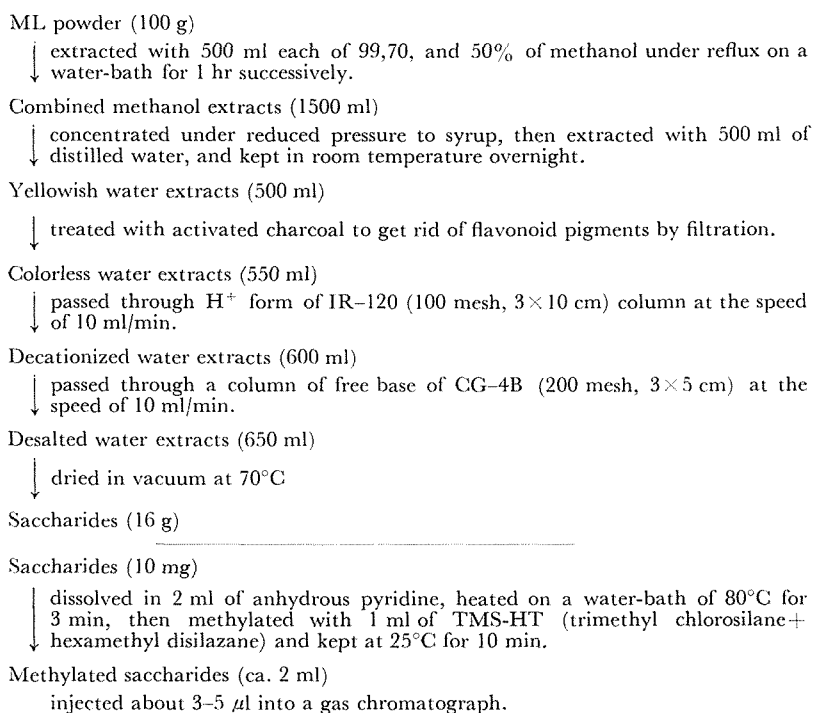
(2) *Fractionation of Polar Substances*

The powder of leaves was treated with distilled water, heated on a water-bath and was filtered by sucking. The filtrate was termed W–1. To fraction W–1 was added methanol and the precipitate was filtered by sucking. This non-pectic filtrate was termed W–2. The fraction W–2 was treated with active carbon and filtered

through Toyo No. 2 filter paper. The filtrate was colorless and was named W–3. The fraction W–3 was passed through ion-exchange resins IR–120 and CG–4B columns. The effluent was denoted W–4.

Extraction, Isolation, and Identification of Mono- and Disaccharides from Powder of Leaves

The powder of each plant was heated with 99%, 70%, and 50% methanol, on a water-bath under reflux, filtered through Toyo No. 2 filter paper by sucking. The filtrate was treated with active carbon and ion-exchange resins. The GC–4A and GC–5A Shimadzu gas-liquid chromatographs were used for the qualitative and quantitative analyses of saccharides. A detailed procedure is given in Figure 1.

Fig. 1.   Procedure for extraction, isolation, and identification of mono-and disaccharides

ML powder (100 g)
│ extracted with 500 ml each of 99,70, and 50% of methanol under reflux on a
↓ water-bath for 1 hr successively.

Combined methanol extracts (1500 ml)
│ concentrated under reduced pressure to syrup, then extracted with 500 ml of
↓ distilled water, and kept in room temperature overnight.

Yellowish water extracts (500 ml)

│ treated with activated charcoal to get rid of flavonoid pigments by filtration.
↓

Colorless water extracts (550 ml)
│ passed through H^+ form of IR–120 (100 mesh, 3×10 cm) column at the speed
↓ of 10 ml/min.

Decationized water extracts (600 ml)
│ passed through a column of free base of CG–4B (200 mesh, 3×5 cm) at the
↓ speed of 10 ml/min.

Desalted water extracts (650 ml)

│ dried in vacuum at 70°C
↓

Saccharides (16 g)

Saccharides (10 mg)
│ dissolved in 2 ml of anhydrous pyridine, heated on a water-bath of 80°C for
│ 3 min, then methylated with 1 ml of TMS-HT (trimethyl chlorosilane+
↓ hexamethyl disilazane) and kept at 25°C for 10 min.

Methylated saccharides (ca. 2 ml)
   injected about 3–5 μl into a gas chromatograph.

And the gas-liquid chromatograms of mono and disaccharides of ML, FL, HBL, JOL, and HTL are illustrated in Figures 2a-e.

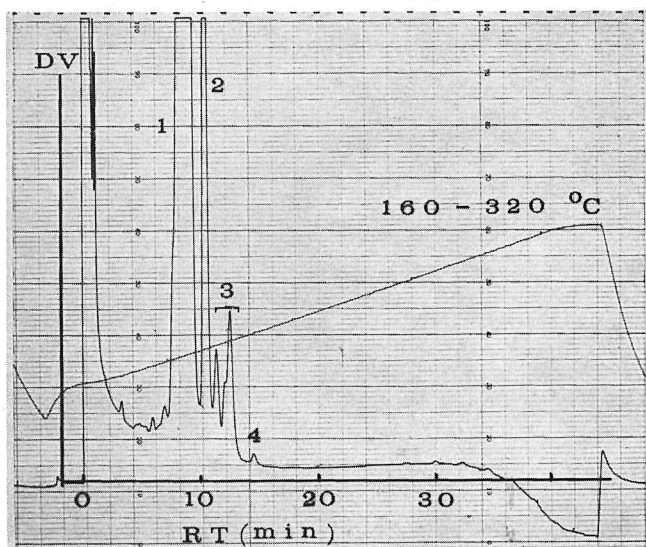*Crystallization of Inorganic Salts from Powder of Leaves*

The ML powder (300 g) was refluxed on a water-bath with 1000 ml each of 99%, 70%, and 50% methanol succeedingly, and was filtered through Toyo No. 2
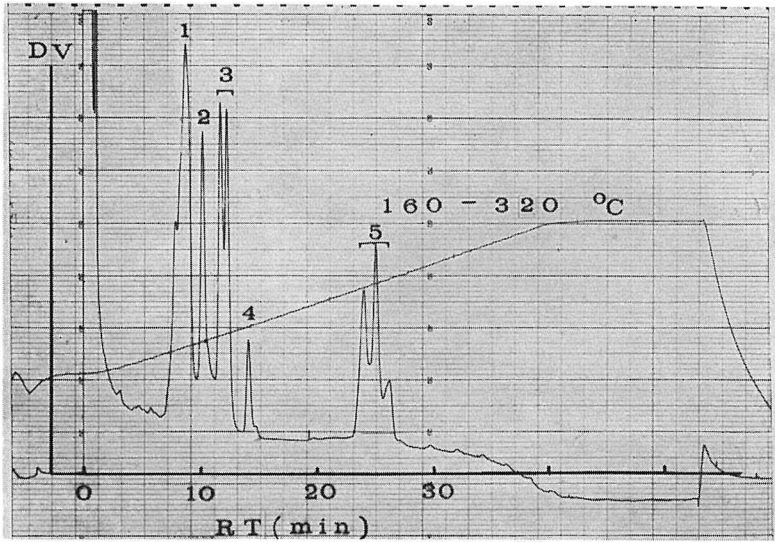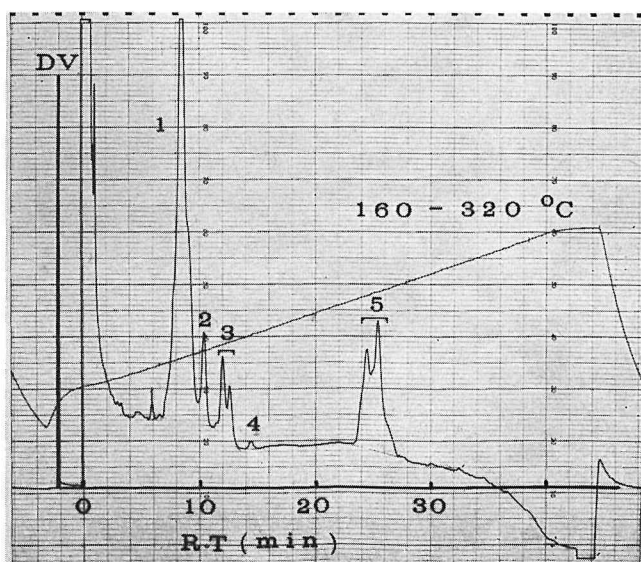
a. Mono- and disaccharides of ML, collected on September 6, 1970.
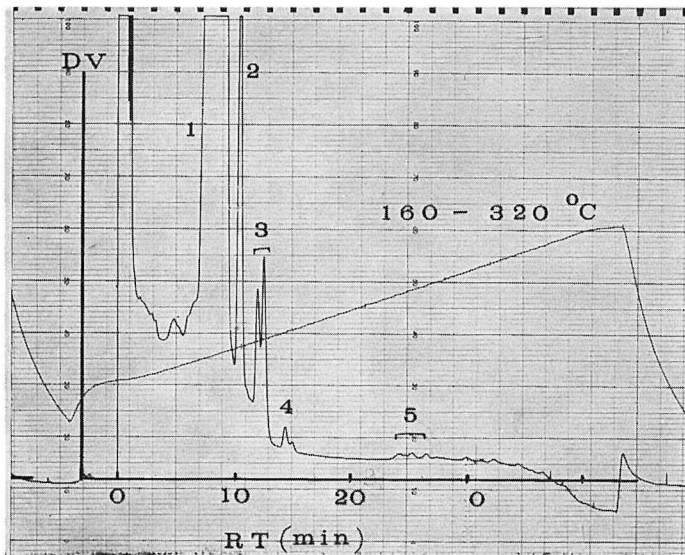
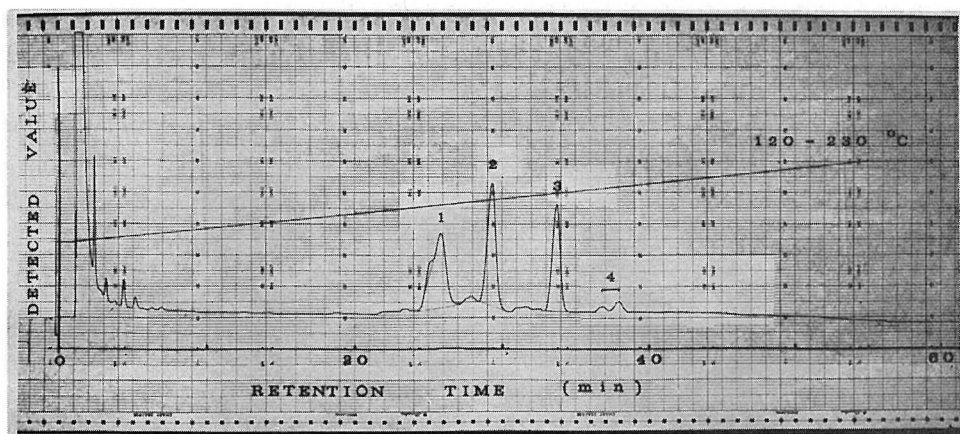b. Mono- and disaccharides of FL, collected on September 7, 1970.

c. Mono- and disacchariedes of JOL, collected on May 26, 1971.

d. Mono- and disaccharides of HTL, collected on September 10, 1970.

e.   Gas-liquid chromatogram of saccharides of HBL.

Figs. 2a-d.   Gas-liquid chromatograms of sacchariedes of ML, FL, JOL, and HTL.
Saccharides were meythylated with TMS-HT and analysed by the use of Shimadzu
GC–5A gas chromatograph as follows:
Stainless steel coulumn: 1.5 m, packed with 5% SE 30, supported by chromosorb
                                    W (80 mesh)
Column temperature: 160–320°C
Flow rate of N_2 carrier gas: 30 ml/min
Temperature programmer: 4°C/min
Chart speed: 5 mm/min
Sensitivity: FID 10^2 (m)
Peaks show: 1. fructose; 2. sorbitol; 3. glucose; 4. inositol; 5. sucrose.

Fig. 2e.   Saccharides were methylated with TMS-HT, and analysed by the use of Shimadzu
GC–4A gas chromatograph as follows:
Stainless steel column: 1.5 m, packed with 5 % SE 30 on chromosorb W (80 mesh)
Column temperature: 120–230°C
Flow rate of N_2 carrier gas: 30 ml/min
Temperature programmer: 2°C/min
Chart speed: 10 mm/min
Sensitivity: FID 10^2 (mΩ)
Peaks show: 1. fructose; 2. sorbitol; 3. glucose; 4. inositol; 5. sucrose.

filter paper.   The filtrate was combined and concentrated under the reduced pressure
to 500 ml.   To this, the same volume of ethyl acetate was added, and the mixture
was shaken vigorously and kept at 10°C for three days.   A large amount of plate
crystals containing K^+, Na^+, SO_4^{--}, PO_4^{3-}, Cl^-, and NO_3^- were separated out.
Water and ethyl acetate were decanted and were further concentrated the water
layer to 100 ml and kept overnight at 10°C.   A small amount of orthorhombic
crystals containing K^+, Na^+, and SO_4^{--} were formed.   The qualitative and quanti-
tative analyses were carried out by the conventional instrumental methods, e.g., P
and Cl analyzers, and the parctical methods (ISHIBASHI, 1953).

Qualitative and Quantitative Analyses of Fatty Acids in Powder of Leaves

Fractions E–4 containing free fatty acids were dried up under N_2 and methylated with diazomethane at room temperature to form methyl esters. The qualitative and quantitative analyses were carried out by use of GC–5A Shimadzu gas chromatograph (HASEGAWA et al., 1960). A gas-liquid chromatogram is given in Figure 3.
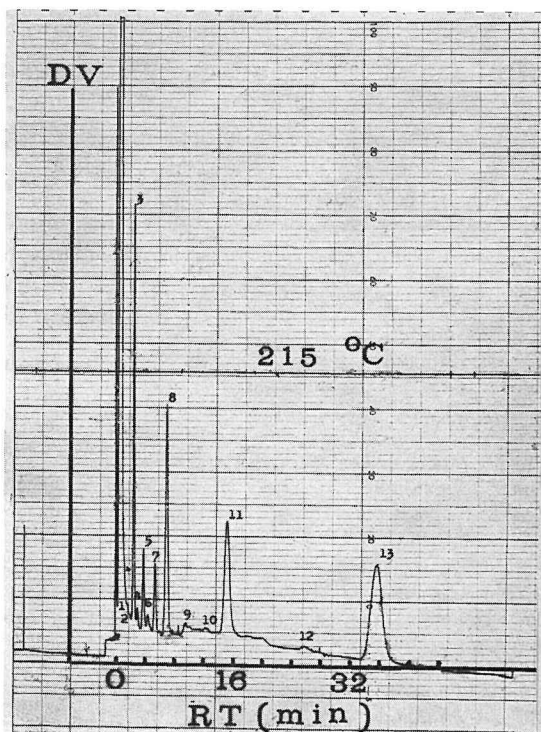

Fig. 3. Gas-liquid chromatogram of fatty acids (methyl esters) of ML. Fatty acids were methylated with diazomethane, and analysed by the use of Shimadzu GC–5A gas chromatograph as follows:
Glass column: 1.5 m, packed with 15% DEGS, supported by Neopack (60–80 mesh)
Column temperature: 190°C
Injection temperature: 230°C
Flow rate of carrier gas (N_2): 28 ml/min
Sensitivity: FID 10^2 (mΩ)
Peaks show: 1. lauric; 2. myristic; 3. palmitic; 4. palmitoleic; 5. stearic; 6. oleic; 7. linoleic; 8. linolenic; 9. behenic; 10. erucic; 11. lignoceric; 12. cerotic; 13. montanic.

Fractionation of Nonpolar Pigments from Powder of Leaves

The powder of each plant (10 g) was heated with 50% acetone (100 ml) on a water-bath under reflux for 1 hr and filtered through Toyo No. 2 filter paper. The

filtrate was shaken with the same volume of ethyl ether. Two layers were formed; the bottom layer was discarded, and the upper layer was collected and denoted NP–1. To fraction NP–1 was added the same volume of 2% KOH in methanol. After shaking and standing for 10 minutes, a small amount of distilled water was added. Two layers were formed; the bottom layer containing saponifiable pigments was discarded, and the ethyl ether layer containing carotenoids was collected and denoted NP–2. After fraction NP–2 was dried up, to it 85% methanol and
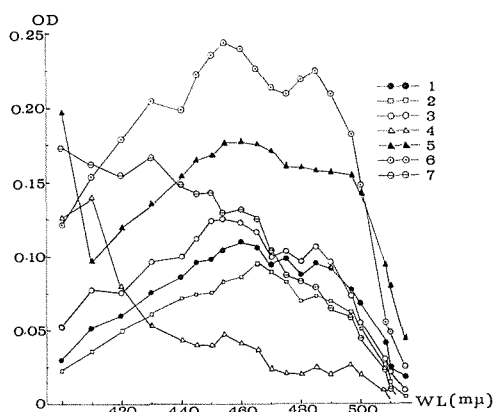

Fig. 4. Absorption spectra of fractions NP–3 in chloroform. Number shows: 1. ML; 2. FL; 3. HBL; 4. JOL; 5. HTL; 6. α-carotene; 7. β-carotene.
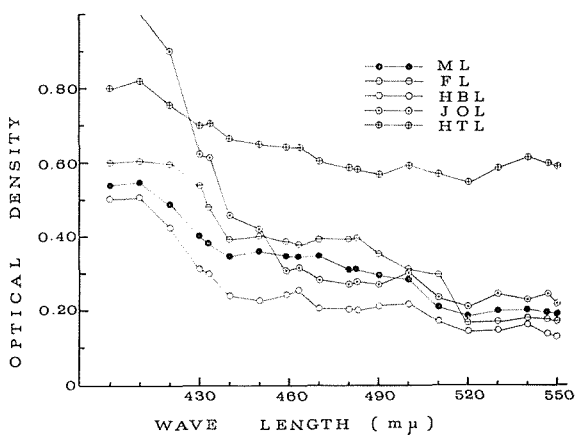

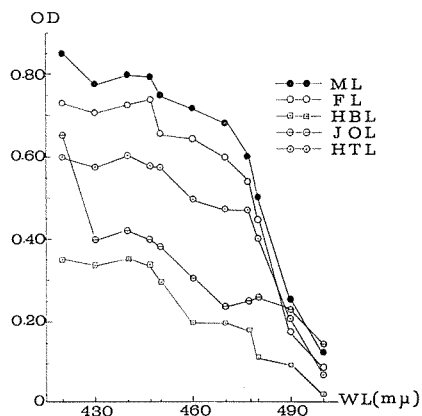Fig. 5. Absorption spectra of fractions NP-4 of ML, FL, HBL, JOL, and HTL in chloroform.

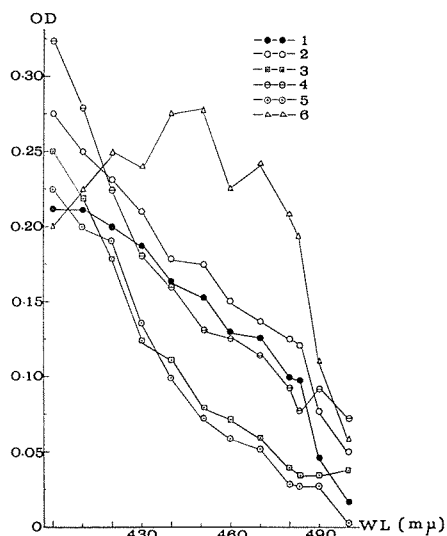Fig. 6. Absorption spectra of fractions NP–4 of ML, FL, HBL, JOL, andHTL in ethanol.

Fig. 7. Absorptoion spectra of fractions NP–4 in petroleum ether. Number shows: 1. ML; 2. FL; 3. HBL; 4. JOL; 5. HTL; 6. authentic lutein.

petroleum ether (1:1 v/v) were added. The petroleum ether layer containing caro-tenes was denoted NP–3 and the methanol layer containing xanthophylls was denoted NP–4 (BONNER, 1952). The absorption spectra of these carotenoids in organic sol-vents such as petroleum ether, chloroform, benzene, and ethanol were examined with a Hitachi M–101 spectrophotometer. The absorption spectrum of NP–3 in chloroform is given in Figure 4 and those of fraction NP–4 in chloroform, ethanol, and petroleum ether are given in Figures 5, 6, and 7 respectively.

Extraction and Identification of Flavonoid Pigments of ML, FL, HBL, JOL, and HTL

The flavonoid pigments in each water-soluble fraction were adsorbed by the active charcoal and eluted with 14% ammonia water. The eluate containing fla-vonoid pigments was concentrated under reduced pressure using a rotary evaporator for evaporation of the ammonia. These flavonoid pigments were submitted to paper chromatography. The development of the flavonoid pigments on Toyo No. 2 filter paper was carried out with butanol-acetic acid-water (BAW, 4:1:5 v/v) at room temperature. Each spot was detected with the use of Shinko ultraviolet (UV) fluorescent lamp. Rf values and colors under UV lamp of the pigments are sum-marized in Table 2.

Table 2. Paper chromatographic properties of flavonoid pigments of ML, FL, HBL, JOL, and HTL.

Number of spot		Rf value in BAW	Color under UV lamp
ML	1	0.13	Yellow (Y)
	2	0.21	Y
	3	0.26	Violet (V)
	4	0.30	V
	5	0.34	V
	6	0.60	V
	7	0.97	V
FL	1	0.08	V
	2	0.15	V
	3	0.26	V
	4	0.34	V
	5	0.44	V
	6	0.60	V
HBL	1	0.12	Y
	2	0.20	V
	3	0.29	V
	4	0.55	V
JOL	1	0.29	V
	2	0.37	Y
HTL	1	0.13	Y
	2	0.19	V
	3	0.25	V
	4	0.29	V
	5	0.35	V
	6	0.37	Y
	7	0.65	V
	8	0.97	V

Results and Discussion

Analyses of Constituents of Plant Leaves

The major components of ML, FL, HBL, JOL, and HTL which were collected on May 23–27, 1971 are listed in Tables 3a-b.   As shown in Table 3a, the quantities of water in the fresh leaves decreased in the following order: ML>FL>HTL> HBL>JOL.   In Table 3b is shown the contents of protein, cellulose, starch, ash, polar and nonpolar constituents in the plant leaves.   Protein: HBL>ML>FL> HTL>JOL; starch: HBL>FL>JOL>HTL>ML; ash: HBL>HTL>FL>ML>

Table 3. Major components of leaves of ML, FL, HBL, JOL, and HTL, collected on May 23–27, 1971.

(a) In fresh weight of leaves

Components	Contents (% in fresh wt. of leaves)				
	ML	FL	HBL	JOL	HTL
Water	80.0	72.0	57.0	54.0	70.0
Protein*	3.0	3.4	7.0	3.8	3.0
Cellulose**	4.5	3.4	8.6	10.3	4.5
Starch***	2.0	4.4	7.8	5.6	3.4
Ash	1.6	2.5	6.5	3.6	3.6
Polar substances	7.0	7.8	8.8	7.5	9.1
Nonpolar substances	1.2	1.4	1.7	1.7	1.6
Other	—	—	—	—	—

(b) In dry weight of leaves

Components	Contents (% in dry wt. of leaves)				
	ML	FL	HBL	JOL	HTL
Protein	14.0	12.0	16.5	8.2	10.0
Cellulose	22.0	12.0	20.0	22.4	15.0
Starch	9.0	15.8	18.2	12.3	11.5
Ash	8.3	9.0	17.0	4.0	12.0
Polar substances	38.2	28.0	25.3	22.5	32.0
Nonpolar substances	6.0	4.4	4.7	4.3	5.8
Other	—	—	—	—	—

* Powder of each plant was treated with distilled water and filterd by sucking. The nonpectic powder was treated with 0.5 M KOH, boiled for 10 min, and filtered by sucking. To the filtrate was added acetone to form precipitate. The precipitate was collected by filtration and centrifugation, dried in vacuum, and was analysed for N quantity by spectrophotometer (Confer Methods in Enzymology, Volume 111, Academic Press Inc., edited by Colowick et al.).

** Powder of each plant was treated with 4 % KOH, boiled for 30 min, and filtered by sucking. The residue was further treated with 17.5 % KOH, boiled for 30 min, filtered by sucking, washed with distilled water completely, and was dried in vacuum (Confer Bonner, 1952).

*** Powder of each plant was treated with distilled water and filtered by sucking. The residue was treated with 20 % HClO$_4$ for 30 min on a water-bath and filtered through Toyo No. 2 filter paper. The filtrate was treated with activated carbon, and the filtrate was allowed to react with anthrone sulfate for colorimetric analysis (Confer Methods in Enzymology, Volume 111, Academic Press Inc., edited by Colowick et al.).

JOL; polar substances: ML>HTL>HBL>FL>JOL. The constituents of ML among these plants were analyzed previously by Tanaka (1943). In quantitative analysis, each fraction was dried up in vacuum and weighed with the use of Jupiter C–1 micro-balance. Table 4 shows the contents of fractions E–1, E–2, E–3, and

Table 4.   Contents of dry substances in the nonpolar fractions of ML, FL, HBL, JOL, and HTL (% in dry wt. of powder, collected on May 23–27, 1971)

Powder of leaves	Fractions			
	E–1	E–2	E–3	E–4
ML	6.0	2.5	1.4	1.1
FL	4.4	2.0	1.2	0.8
HBL	4.7	1.2	0.7	0.5
JOL	4.3	1.5	0.9	0.6
HTL	5.8	2.0	1.0	1.0

E–4 which are components of nonpolar substances such as chlorophylls, carotenoids, sterols, fatty acids, and volatile substances.   The contents of fractions E–1 decreased in the following order: ML>HTL>HBL>FL>JOL.   The contents of fractions E–2: ML>HTL=FL>JOL>HBL.   The contents of fractions E–3: ML>FL> HTL>JOL>HBL.   The contents of fractions E–4: ML>HTL>FL>JOL>HBL. In Table 5, it demonstrates that the quantity of fraction W–1 containing a mixture of pectin, flavonoid pigments, organic and inorganic salts, and saccharides was ML> HTL>FL>HBL>JOL.   The fraction W–2 was a depectic fraction from fraction W–1.   The fraction W–3 was a colorless fraction containing organic and inorganic salts, and saccharides.   The fraction W–4 contained saccharides only.   The order of quantity was: ML>HTL>FL>HBL>JOL in fractions W–2, W–3, and W–4.

Table 5.   Contents of dry substances in the polar fractions of ML, FL, HBL, JOL, and HTL (% in dry wt. of powder, collected on May 23–27, 1971)

Powder of leaves	Fractions			
	W–1	W–2	W–3	W–4
ML	36.3	32.3	26.7	16.0
FL	26.2	22.7	17.7	8.2
HBL	23.3	20.1	13.8	5.0
JOL	21.6	17.4	12.0	4.5
HTL	30.0	26.5	19.5	9.8

The contents of mono- and disaccharides of both the feeding preferential (ML and FL) and unpreferentail (HBL, JOL, and HTL) leaves collected in May and September are listed in Table 6.   In May, the contents of saccharides of ML was as follows: sucrose>fructose>inositol>glucose>sorbitol.   In the case of FL, glucose> fructose>sorbitol>inositol>sucrose.   In the case of HBL, fructose>glucose>sucrose>sorbitol>inositol.   In the case of JOL, fructose>sorbitol>glucose>inositol (sucrose absent).   In the case of HTL, fructose>glucose>sucrose>inositol (sorbitol

Table 6.   Seasonal change of mono-and disaccharides in ML, FL, HBL, JOL, and HTL

On May 23–27	Fructose	Sorbitol	Glucose	Inositol	Sucrose	Total amount (% in dry wt. of powder)
ML	40.5	2.0	2.5	4.0	51.0	15.0
FL	30.3	4.0	64.5	0.7	0.5	8.0
HBL	85.0	4.2	5.5	0.1	5.2	5.1
JOL	92.0	6.0	1.2	0.8	0	3.4
HTL	97.0	0	1.2	0.8	1.0	10.0
On September 6–10						
ML	23.2	0.8	30.9	2.8	33.0	17.2
FL	59.8	20.0	18.0	0.8	0.3	8.5
HBL	69.7	12.0	17.0	1.1	0	6.0
JOL	85.9	0	7.0	1.1	5.8	5.8
HTL	56.7	6.2	9.4	0.5	26.0	11.4

absent).   The constituents of saccharides in September were different from those in May.   For instance, in September, the contents of saccharides of ML: sucrose>fructose>glucose>inositol>sorbitol; FL: fructose>sorbitol>glucose>inositol>sucrose; HBL: fructose>glucose>sorbitol>inositol (sucrose absent); JOL: fructose>glucose>sucrose>inositol (sorbitol absent); and HTL: fructose>sucrose>glucose>sorbitol>inositol.   As for the total amount of saccharides, the order was ML>HTL>FL>HBL>JOL in May and September.   But sugar content of each plant in September was greater than that in May.

The mono- and disaccharides of young and old fig leaves were investigated in detail.   As can be seen from Table 7, the total amount of saccharides in young leaf was smaller than that in old leaf.   Moreover, a considerable difference was found in the content of each sugar between young and old leaves (note: the young leaf was light yellow, the old leaf was dark green incolor); in the former, fructose>glucose>sorbitol>inositol>sucrose, and in the latter, fructose>sorbitol>glucose>sucorse>inositol.   The circadian change of ML at 10:00 A.M., 3:00 and 8:00 P.M. were also studied.   The analytical result shown in Table 8 proves that the content of saccharides at 3:00 P.M. was greater than that at 10:00 A.M. and 8:00 P.M.

Table 7.   Contents of sugars in young and old fig leaves collected on September 7, 1970

Foliage	Fructose	Sorbitol	Glucose	Inositol	Sucrose	Total amount (% in dry wt. of powder)
	(% in dry wt. of sugar fraction)					
Young FL (yellow)	48.0	14.5	35.5	1.3	0.6	1.3
Old FL (green)	83.6	12.2	3.2	0.1	0.9	5.5

Table 8. Mono- and disaccharides of ML collected at different daytime on September 6, 1970

Time of collection	Fructose	Sorbitol	Glucose	Inositol	Sucrose	Total amount (% in dry wt. of powder)
		(% in dry wt. of sugar fraction)				
10:00	47.2	0.3	18.6	3.3	30.6	8.8
15:00	40.5	2.0	12.5	4.0	41.0	17.2
20:00	30.0	2.5	52.0	0.5	14.5	12.6

Table 9. Constituents and amounts of fatty acids fractions of ML, FL, HBL, JOL, and HTL collected on May 23–27, 1971

Constituents	Amounts (% in dry wt. of fatty acids fractions)				
	ML	FL	HBL	JOL	HTL
Lauric	0.12	0.80	0.30	0.16	0.10
Myristic	0.12	0.40	0.10	0.08	0.05
Palmitic	15.34	29.00	4.40	6.02	1.10
Palmitoleic	3.83	0.50	0.20	0.21	0.12
Stearic	6.30	4.80	3.50	0.80	1.20
Oleic	0.76	4.50	2.50	1.40	0.01
Linoleic	6.10	5.10	1.30	1.60	2.00
Linolenic	22.38	7.30	2.50	7.08	4.10
Behenic	0.38	0.20	—	0.33	2.20
Erucic	0.12	—	—	—	—
Lignoceric	16.77	7.40	21.00	16.00	—
Cerotic	0.25	—	—	—	—
Montanic	27.53	40.00	64.20	66.32	89.22

Results of the qualitative and quantitative analyses of fatty acids of ML, FL, HBL, JOL, and HTL are given in Table 9 and Figure 3. It was found that the fatty acid fraction (E–4) of ML contained 13 fatty acids, in which the unsaturated fatty acids consisted of palmitoleic, oleic, linoleic, linolenic, and erucic acids; the saturated fatty acids consisted of lauric, myristic, palmitic, stearic, behenic, lignoceric, cerotic, and montanic acids.   Fractions E–4 of FL, HBL, JOL, and HTL also showed a similar pattern to that of ML but with different quantity and quality of components as shown in Table 9.   The free fatty acids of ML had been analysed by Ito et al. (1966), Yamada et al. (1967), and Lin et al. (1971), but they demonstrated only eight fatty acids in ML probably due to low column temperature and/or poor separation.   For example, at 190°C, it took 17 minutes (min) to detect linolenic acdi and 105 min to detect montanic acid.   However, when separated at 215°C, it took only 7 min for linolenic acid and 36 min for montanic acid under the same analytical conditions except for the temperature.   Seasonal change of ML fatty acids in

Table 10: Seasonal change of ML fatty acids

Constituents	Amounts (% in dry wt. of fatty acids fraction)	
	ML collected on September 6, 1970	ML collected on May 23, 1971
Lauric	0.08	0.24
Myristic	0.08	0.02
Palmitic	1.600	12.00
Palmitoleic	0.40	0.60
Stearic	4.00	2.20
Oleic	0.70	0.90
Linoleic	3.00	6.10
Linolenic	19.20	34.32
Behenic	0.40	0.81
Erucic	0.30	0.70
Lignoceric	15.50	16.00
Cerotic	0.20	—
Montanic	41.14	26.11

September and May was studied. The result shown in Table 10 shows that in September, the amounts of linoleic and linolenic acids were decreased, whereas, palmitic, stearic, and lignoceric acids were rather increased when compared to those in May.

The nonpolar pigments of ML, FL, HBL, JOL, and HTL were fractionated and denoted NP–1, NP–2, NP–3, and NP–4. Fraction NP–1 contained chlorophylls, carotenes, and xanthophylls. Fraction NP–2 contained carotenes and xanthophylls. Fraction NP–3 contained carotenes. Fraction NP–4 contained xanthophylls. Up to now, six isomers of carotenes have been reported (Goodwin, 1969). In xanthophylls, twelve isomers, viz: lutein, cryptoxanthin, rhodoxanthin, rubixanthin, lycophyll, zeaxanthin, flavoxanthin, capsanthin, violaxanthin, neoxanthin, lycoxanthin, and lycopene have been reported (Goodwin, 1966). Each isomer has its characteristic absorption spectrum in organic solvents such as petroleum ether, chloroform, benzene, and ethanol, etc.. The absorption spectra of fractions NP–3 and NP–4 were taken. Fractions NP–3 were apparently ascertained to contain α, β, and γ in ML; α and β in FL, HBL, and JOL; β in HTL. (Note: The absorption maxima of carotenes in chloroform: α-carotene—454, 485 mμ; β-carotene—466, 497 mμ; γ-carotene—446, 475, 508.5 mμ). Fractions NP–4 were suggested to have lutein, zeaxanthin, and flavoxanthin in ML and FL; lutein and cryptoxanthin in HBL; cryptoxanthin and rhodoxanthin in JOL; cryptoxanthin and lycophyll in HTL (Note: The absorption maxima of xanthophylls: cryptoxanthin in chloroform—433, 463, 497 mμ; rhodoxanthin in chloroform—482, 510, 546 mμ; lutein in petroleum ether—447.5, 477.5

mμ; zeaxanthin in ethanol—451.5, 483 mμ; lycophyll in benzene—456, 487, 521 mμ; flavoxanthin in chloroform—430, 459 mμ.   Confer the 7th edition of the Merck Index of Chemicals and Drugs).   The absorption spectra of fractions NP–4 are given in Figures 4, 5, 6, and 7.

The paper chromatographic properties of the flavonoid pigments of ML, FL, HBL, JOL, and HTL are shown in Table 2   It was revealed that both JOL and HTL contained two pigments having Rf value of 0.29 and 0.37.   HBL also contained a pigment having Rf value of 0.29.   ML and FL contained two pigments having Rf value of 0.34 and 0.60 that were confirmed to be quercetin and quercitrin.

Phagoactivities of the Silkworm Larvae Against Various Substances of Leaves

The phagoactivities of the silkworm larvae against nonpolar and polar fractions of ML, FL, HBL, JOL, and HTL were assayed and the results are shown in Table 11

Table 11.   Comparison of phagoactivity in fractions E–1, E–2, E–3, and E–4, of ML, FL, HBL, JOL, and HTL, collected on May 23–27, 1971

Fraction no.	Addition (g of dry wt.)		Frass on. per 10 larvae per 24 hr	
			In BD–3	In BD–4
E–1	0.1	ML	0	394
		FL	0	155
		HBL	0	0
		JOL	0	0
		HTL	0	0
E–2	0.03	ML	0	300
		FL	0	145
		HBL	0	60
		JOL	0	58
		HTL	0	120
E–3	0.02	ML	0	295
		FL	0	140
		HBL	0	151
		JOL	0	51
		HTL	0	181
E–4	0.01	ML	0	265
		FL	0	145
		HBL	0	100
		JOL	0	142
		HTL	0	114
Control			0	15

Table 12.   Comparison of phagoactivity in fractions W-1, W-2, W-3, and W-4 of ML, FL,
HBL, JOL, and HTL, collected on May 23-27, 1971

Fraction no.		Additional quantity (g of dry wt.)	Frass no. per 10 larvae per 24 hr	
			In BD-6	In BD-7
ML	W-1	0.38	120	410
	W-2	0.34	117	411
	W-3	0.28	85	353
	W-4	0.15	63	281
FL	W-1	0.28	81	310
	W-2	0.24	84	357
	W-3	0.15	48	221
	W-4	0.08	4	201
HBL	W-1	0.25	3	0
	W-2	0.20	1	0
	W-3	0.14	0	87
	W-4	0.04	0	56
JOL	W-1	0.20	0	0
	W-2	0.16	0	0
	W-3	0.11	0	36
	W-4	0.03	2	50
HTL	W-1	0.32	0	0
	W-2	0.29	0	0
	W-3	0.17	84	141
	W-4	0.08	75	132
Control			0	0

and 12.   As shown in Table 11, all of the nonpolar fractions were inactive when
coexisted with residual components (cellulose and protein), water, and agar (BD-3).
Fraction E-1 of ML caused a higher effect than that of FL when combined with
residual components (cellulose and protein), polar substances of ML (saccharides and
inorganic salts), water, and agar (BD-4).   But fractions E-1 of HBL, JOL, and
HTL caused a negative effect under the same conditions.   Fractions E-2 elicited a
positive phagoactivity when incorporated by the corresponding components of BD-4.
The order of effect was ML>FL>HTL>HBL>JOL presumably owing to the dif-
ferent quantities of fatty acids, phytosterols, and the volatile components.   However,
in this regard, it is of special interest that the phagorepellents present in fractions
E-1 of HBL, JOL, and HTL can be eliminated by the treatment with active carbon.
In fractions E-3, the unsaponifiable substances of ML, FL, HBL, JOL, and HTL

cause a positive phagoactivity in the presence of BD–4. In this fraction, β-sitosterol has been reported by HAMAMURA as a biting factor (1959, 1963–1964, 1970). In fractions E–4, the effect of ML was higher than those of FL, HBL, JOL, and HTL in terms of the promotion of phagoactivity. Although E–4 fractions of five plants contained similar fatty acids, the content of each fatty acid was quite different depending on the kinds of plants as shown in Table 9. The positive phagoactivity of the domestic silkworm against the fatty acids of ML was mostly elicited by the mixture of linoleic and linolenic acids as described previously (LIN et al., 1971a). The newly-discovered long-chain free fatty acids, i.e., lignoceric and montanic acids did not cause a promotion on the larval feeding.

Fractions W–1, W–2, W–3, and W–4 of ML, FL, HBL, JOL, and HTL were assayed using BD–6 and BD–7 as basal diets for investigations of the effect on the phagoactivities. The results given in Table 12 show that in ML and FL, the effects of fractions W–1 and W–2 were not significantly different. Therefore, the existence of pectin did not influence larval feeding. The effect of fraction W–2 was greater than that of W–3, and that of W–3 was greater than that of W–4 in terms of the promotion of phagoactivity. Moreover, the effects of the fractions W–1, W–2, W–3, W–4 of FL were lower than those of ML mostly due to lower contents of saccharides. On the contrary, in HBL, JOL, and HTL, fractions W–1 and W–2 caused a negative phagoactivity when given in combination with BD–6 and BD–7. But the fact is of great interest that fractions W–3 and W–4 of HTL caused a positive phagoactivity in the presence of BD–6, moreover, they were further promoted by the addition of the nonpolar phagostimulants in BD–7. Fractions W–3 and W–4 of HBL and JOL also caused a positive phagoactivity when given together with BD–7. As regards to these results, the following three important lines of evidence were obtained: (1) the effects of polar substances of ML and FL on the positive phagoactivity of the domestic silkworm depended upon the flavonoid pigments, organic and inorganic salts, and saccharides; (2) the effects of the inorganic salts, salts of organic acids, and saccharides in HBL, JOL, and HTL was depressed by the prevailing phagorepellents present in the same fraction; (3) the phagorepellents were speculated to be some of the flavonoid pigments that could be adsorbed by the active charcoal.

The result of bioassay of the fractions W–4 (saccharides fraction) of the leaves, collected on September 6–10, 1970, is given in Table 13. In which the effect of fraction W–4 on the phagoactivity was ML > HTL > JOL > FL > HBL when used BD–7 as a basal diet. In order to find out the effective sugar, the authentic samples of fructose, sorbitol, glucose, inositol, and sucrose were added to BD–7 at the same concentrations as those of ML saccharides. The result shown in Table 14 indicates that the sole effect of sucrose and inositol was higher than that of fructose, glucose, and sorbitol. (Note: The commercial inositol was myo-inositol and its effect was

Table 13.   Effects of fractions W–4 of ML, FL, HBL, JOL, and HTL on phagoactivity of domestic silkworm (leaves were collected on September 6–10, 10979)

Addition (g of dry wt.)	Frass no. per 10 larvae per 18 hr in BD–7
ML 0.1	270
FL 0.1	75
HBL 0.1	38
JOL 0.1	82
HTL 0.1	211
Control	0

Table 14.   Effects of commercial saccharides on phagoactivity of domestic silkworm

Addition (g of dry wt.)	Frass no. per 10 larvae per 18 hr in BD–7
Fructose (F) 0.055	19
Sorbitol (S) 0.004	17
Glucose (G) 0.037	19
Inositol (I) 0.021	72
Sucrose (Su) 0.067	82
F+G	32
F+I	96
F+Su	56
G+I	78
G+Su	72
I+Su	190
F+I+Su	274
G+I+Su	270
F+S+G+I+Su	285
Control	0

higher than that of chiro-inositol, but lower than those of epi-inositol and myo-inositol, 1:1 w/w).   Furthermore, the effect of the mixture of these five sugars was higher than the mixture of sucrose and inositol that had been described as biting cofactors (HAMAMURA, 1963–1964) and swallowing factors (HAMAMURA, 1970) respectively.

The mixture of inorganic salts of ML was assayed using BD–2 (confer Table 1). The result given in Table 15 shows that the phagoactivity of the domestic silkworm was positively raised to a large extent when enriched with inorganic salts of ML. Such effect was not only caused by K^+, Na^+, and PO_3^{4-} as stated in the previous paper (LIN et al., 1971b) but also strengthened by the actions of SO_4^{2-}, Cl^-, NO_3^-.

Table 15. Effects of inorganic salts on phagoactivity of domestic silkworm

Addition (g of dry wt.)		Frass no. per 10 larvae per 24 hr in BD–2
Wesson's salts*	0.05	323
	0.1	210
	0.2	131
ML salts	0.07	233
ML sash	0.01	46
	0.05	7
	0.1	0
Control		20

Note: * confer Kato et al., 1966.

The sole activity of the neutral K and Na salts of thirty four organic acids in terms of the phagoactivity of the domestic silkworm was reported in the previous paper (Lin et al., 1971b). The result showed that the neutral K and Na salts of phthalic, malic, acetic, gallic, protocatechuic, fumaric, caffeic, quinic, tartaric, pyromellitic, and sorbic acids were effective on the promotion of phagoactivity. Through the combinational assay of these organic acids, it was found that the mixture of salts consisting of 1% malic, 0.4% fumaric, 0.3% phthalic, 0.25% protocatechuic, 0.3% caffeic, and 0.3% quinic acids, neutralized with 3M KOH and 1M NaOH, produced a rather high efficacy on the increase of phagoactivity when compared to the basal diet (BD–8) as a control.

The phagoactivities of the domestic silkworm against the fractions NP–1, NP–2, NP–3, NP–4 of ML, FL, HBL, JOL, and HTL were assayed under the incorporation of BD–5 as basal diet. The results are given in Tables 16a-d. As shown in Table 16a, fraction NP–1 of ML caused a higher effect than that of FL, fraction NP–1 of HBL showed a much lesser activity, but those of JOL and HTL showed a negative phagoactivity. As shown in Table 16b, fraction NP–2 of ML still caused a higher effect than that of FL, fractoin NP–2 of HBL showed a rather high repellent action, but those of JOL and HTL repelled completely on the feeding of larvae. As shown in Table 16c, fraction NP–3 of ML conspicuously raised the positive phagoactivity, but those of FL, HBL, and HTL did not, probably due to the presence of less isomers of carotenes (confer Figure 4). As can be seen from Table 16d, fractions NP–4 of ML and FL did not influence phagoactivity of the silkworm, that of HBL showed a drastic repellent action, but those of JOL as well as HTL caused a negative phagoactivity. Therefore, a conclusion was drawn that the negative phagoactivity observed in the nonpolar fractions of HBL, JOL, and HTL was practically affected by the phagorepellents that might be some isomers of the xanthophyll (confer Figures 5, 6, and 7).

Table 16a. Effects of fractions NP–1 of ML, FL, HBL, JOL, and HTL on phagoactivity of
            domestic silkworm (leaves were collected on May 23–27, 1971)

Addition (g of dry wt.)		Frass no. per 10 larvae per 18 hr in BD–5
ML	0.025	221
FL	0.024	206
HBL	0.025	111
JOL	0.028	0
HTL	0.028	0
Control		145

Table 16b. Effects of fractions NP–2 of ML, FL, HBL, JOL, and HTL on phagoactivity of
            domestic silkworm

Addition (g of dry wt.)		Frass no. per 10 larvae per 18 hr in BD–5
ML	0.015	283
FL	0.014	251
HBL	0.012	50
JOL	0.018	0
HTL	0.018	0
Control		216

Table 16c. Effects of fractions NP–3 of ML, FL, HBL, JOL, and HTL on phagoactivity of
            domestic silkworm

Addition (mg of dry wt.)		Frass no. per 10 larvae per 18 hr in BD–5
ML	8	226
FL	7	98
HBL	6	88
JOL	10	120
HTL	10	35
Control		121

Table 16d. Effects of fractions NP–4 of ML, FL, HBL, JOL, and HTL on phagoactivity of
            domestic silkworm

Addition (mg of dry wt.)		Frass no. per 10 lavrae per 18 hr in BD–5
ML	7	104
FL	7	110
HBL	6	54
JOL	8	0
HTL	8	0
Control		121

The positive phagoactivity against 10 volatile components which were found in ML as major volatile components were assayed with the use of BD–5 as basal diet. The result is given in Table 17.   However, about 4 mg of trans-2-hexenol showed a topped effect.   The hexanol also promoted phagoactivity to some extent, but the compounds such as citral, linalol, eugenol, phenethyl alcohol, and terpinyl acetate caused a lethal inhibition presumably due to over threshold dose.

The effect of flavonoid pigments on the positive phagoactivity was examined when the BD–9 was used as basal diet.   The result given in Table 18 shows that the

Table 17.   Effects of major volatile components on phagoactivity of domestic silkworm

Components	Frass no. per 10 larvae per 18 hr in BD–5		
	added 1 μl	added 5 μl	added 10 μl
Benzyl alcohol	52	61	168
Isoamyl alcohol	50	72	259
Phenethyl alcohol	102	81	0
Hexanol	96	112	292
Eugenol	71	45	0
trans-2-Hexenol	109	111	420
Citral	128	108	0
Linalol	100	101	0
Linalyl acetate	90	108	0
Terpinyl acetate	120	98	0
Control	177		

Table 18.   Effects of authentic samples of flavonoid pigments on phagoactivity of domestic silkworm

Addition (mg of dry wt.)		Frass no. per 10 larvae in BD–9		
		1st day	2nd day	3rd day
Rutin (R)	5	327	349	255
Quercitrin (Q)	5	367	374	291
Quercetin (Qu)	5	385	402	279
Morin (M)	5	453	510	386
R+Q	5/each	307	285	221
R+Qu	do.	290	273	259
Q+Qu	do.	402	415	370
R+Q+Qu+M	do.	258	220	153
Control 1 (ML powder)		405	429	382
Control 2 (BD–9)		352	401	293

Note: ML powder was gelatinized by 2 % agar-gel.

effect of morin was the most obvious that was reported in the previous papers (HA-
MAMURA, 1959, 1963–1964, 1970) as a biting factor.   Quercetin also raised the phago-
activity to some extent that was described previously by NAITO who used a complex
diet containing soybean powder, vitamin mixture, and Wesson's salts, etc. (1968a).
The mixture of quercetin and quercitrin, 1:1 w/w, elicited a higher phagoactivity
than quercetin, but rutin and quercitrin were inactive.   Moreover, the addition of
morin or the mixture of quercetin and quercitrin to BD–9 produced an efficacy to
the same extent as the powder of mulberry leaves.

The residual fractions of ML, FL, HBL, JOL, and HTL which was obtained by
treatment with distilled water and ethyl ether, were assayed when BD–1 was used
as basal diet (confer Table 1).   The result shows that there still remained some
water- and ethyl ether-insoluble repellents in the residues of HBL, JOL, and HTL.
These repellents could be removed by the further treatment with 5% KOH or NaOH.
The effect of residues of ML, FL, HBL, JOL, and HTL is given in Table 19.   The

Table 19.   Effects of residues of ML, FL, HBL, JOL, and HTL on phagoactivity of domestic
silkworm

| Addition (g of dry wt.) | Frass no. per 10 larvae per 24 hr in BD–1 | |
	Residue treated with distilled water and ethyl ether (R–1)	R–1 further treated with 5 % KOH
ML 1	245	192
FL 1	80	170
HBL 1	10	121
JOL 1	0	98
HTL 1	0	135
Control	19	

Note: The leaves wete collected on May 23–27, 1971.

Table 20.   Effects of ashes of ML, FL, HBL, JOL, and HTL on phagoactivity of domestic
silkworm

| Ashes (dry wt.) | Frass no. per 10 larvae per 24 hr in BD–2 | |
	added 0.05 g	added 0.1 g
ML	4	50
FL	42	34
HBL	3	18
JOL	61	102
HTL	72	164
Control	10	

Note: Leaves were collected on May 23–27, 1971.

residue of ML showed a higher activity than those of others, presumably owing to the actions of the remaining minerals.

The positive phagoactivity against the ashes of ML, FL, HBL, JOL, and HTL was assayed using BD–2 that contained Whatman ashless cellulose, Difco casein, saccharides, fatty acids, and 2% agar gel. The result shown in Table 20 indicates that the phagoactivity was $HTL > JOL > ML > FL > HBL$.

Acknowledgements

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