Growth, development, feeding preference, and food consumption and utilization by tobacco hornworm on tobacco mosaic virus-infected and non-infected tobacco leaves*

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Summary. Plant disease may cause a variety of changes in plants which influence their suitability to insect herbivores. The reported physiological changes in tobacco due to infection by tobacco mosaic virus have the potential to influence the feeding, growth and behavior of the tobacco hornworm, *Manduca sexta*. However, feeding on foliage of systematically infected plants had no statistically significant effects on development time, mean pupal weight or the amount of foliage consumed by 4th instars. Although an initial preference was exhibited by larvae for healthy leaves after about 72 h no differences were observed. These results do not support the results of previous research on the influence of tobacco mosaic virus-infected plants on the tobacco hornworm.
Key words. Manduca sexta; tobacco mosaic virus; tobacco hornworm; plant pathogen/insect interaction.

Insect/plant interactions can be influenced by a variety of biotic and abiotic environmental factors[1]. Plant disease is an important factor which may potentially influence the interactions between non-vector herbivores and their plant hosts but it rarely has been evaluated[2–4]. Plants infected with phytopathogens undergo significant chemical and physical changes which can alter their attractiveness and suitability for insect herbivores. For example, plant diseases could alter not only an herbivore's feeding preference[2,3], but also its reproduction[3,5] and food consumption and conversion efficiency[4]. Understanding the interaction between plant diseases and insect herbivores is likely to alter current theoretical and applied aspects of insect nutritional ecology.

Insect/plant interactions can be significantly affected by the nutritional and allelochemical composition[6,7] of the plant tissues. Nutritional factors such as leaf water content[8–10] and leaf nitrogen[11] also are of major importance. Owen[12] found that TMV infection could significantly reduce leaf water content of tobacco leaves. However, in the present study the leaf water content in the TMV-infected and non-infected leaves were 18.2% and 17.7%, respectively, and the difference was not statistically significant. TMV infection also can affect the nitrogen metabolism in the infected leaves. Although there is no massive net increase in protein and nucleic acid after viral infection there is a redirection of part of that synthesis[13]. Wildman et al.[14] and Sheen and Lowe[15] reported that in infected tobacco leaves, TMV was produced at the expense of a normal nucleoprotein found in leaf cell cytoplasm, but the total nitrogen was unaffected. Commoner and Nehari[16] found that early after TMV infection there was a decline in free amino acids. However, after the initial flush of TMV synthesis the value of the amino acids approached those of the controls. The same phenomenon was observed with the amides.

Little is known about the effects of virus infection on the total alkaloid content. Infection of *Nicotiana* species with potato X virus causes the alkaloid content to increase above those in disease-free plants. After the infection proceeds, the alkaloid content of infected plants is similar or may drop below that of healthy plants[17]. Similarly, Pirone and Davis[18] reported that tobacco infected with tobacco vein mottling virus had decreased alkaloid levels compared to healthy plants. Schuster[17] also suggested that virus infection enhanced the metabolic conversion of nicotine to nornicotine, an alkaloid which is relatively less toxic than nicotine.

This study was designed to determine the effect of the tobacco mosaic virus (TMV), via the systemic infection of tobacco plants, upon the growth, development, feeding preference, and food consumption and utilization of the tobacco hornworm, *Manduca sexta*. Since the bionomics of the tobacco hornworm and the physiological and chemical changes of tobacco plants (infected with TMV) are well-documented, the tobacco/hornworm system is ideal for the study of the effect of plant pathogens on non-vector insect herbivores.

Materials and methods

Experimental plants, insects and virus. The tobacco plants, cultivar Maryland 609, were seeded in April and transplanted into the field on 22 May 1986. About one month after transplanting, 180 plants were inoculated with the tobacco mosaic virus (TMV, ATCC#21) by using a leaf rubbing method, which resulted in the systemic infection of this variety. Fourteen days after virus inoculation 150 inoculated plants showed the typical mosaic symptom. Whole plants or leaves of plants were used in the experiments described below. Another 180 plants were inoculated with distilled water as control. Only 2 plants of the latter group exhibited the TMV infection symptom and were removed from the field immediately after the symptoms appeared. Tobacco hornworm eggs were obtained from the USDA, ARS Research Center at Beltsville, Maryland, and reared on synthetic diet for 24 h prior to their placement in the field.

Larval development and pupal weight. Fourteen days after the virus inoculation, 12 TMV-infected plants (exhibiting

typical mosaic symptoms) and 12 non-infected control plants were covered with 45 cm (diameter) by 70 cm (high) cages covered with nylon net. Six tobacco hornworm larvae were placed on each caged plant within 24 h after hatching. The larvae were inspected daily (at regular intervals during the photophase) for 12 days until 1–2 days prior to the hornworm's wandering (prepupal) stage. Larvae were collected and brought back to the laboratory. 10–12 larvae were placed into each of several plastic $25 \times 32.3 \times 8.75$ cm containers with 3.75 cm of sand at the bottom in which larvae pupated. Prior to pupation, larvae were fed TMV-infected or non-infected tobacco leaves, depending on what they had fed on in the field. Pupae were weighed 10 days after pupation.

Feeding preference of first instars. Semi-circular, 9.4 cm diameter leaf disks from both TMV-infected (six weeks after virus inoculation) and non-infected plants were used in preference tests. Two leaf semi-circular disks, one from a TMV-infected plant and another from a non-infected plant, were put on the bottom of plastic 10×4.4 cm cups, covered with transparent plastic lids to maintain humidity. One tobacco hornworm egg was placed into each cup on either the infected leaf half (group 1) or the disease-free half (group 2). The leaf pieces were changed every 24 h and the larvae or unhatched egg was replaced on the same type of leaf it was found on prior to the leaf change. In each of the two groups (eggs on the infected half vs eggs on the non-infected half), 20 eggs were used. The whole experiment was conducted twice. All cups were checked every 12 h for 4 days to determine the location of larvae, i.e. on which leaf semi-circle they occurred. The latter represented our measure of preference.

The feeding preference of the fourth instars. One fourth instar was placed in cups containing disks prepared as described above. In each of two groups, 20 larvae were reared from hatch to the 4th stadium on either TMV-infected or non-infected tobacco leaves prior to preference tests to determine the role of previous feeding experience. During the test, leaf pieces were changed three times in a period of 48 h, when about 3/4 of the area of either of the two leaf pieces had been consumed. The leftover leaf pieces were compared with their original area to measure the area eaten by the larvae. The area eaten from TMV-infected leaf pieces (D) and non-infected leaf pieces (H) were recorded separately and an index H/(H + D) was used to represent the larval feeding preference for healthy (non-infected) tobacco leaves. The test was conducted twice.

Growth, food consumption and utilization of the fourth instars. Thirty days after the virus inoculation, inner leaves were collected from both TMV-infected and non-infected tobacco plants, stored in refrigerator at 2–4 °C and used to feed larvae. The leaf TMV content was determined using microanalytical techniques described by Taniguchi[19].

24-h-old 1st instars were divided into four treatment groups of 10 larvae each and fed TMV-infected or non-infected tobacco leaves, respectively, as follows:

(1) H-H; larvae were fed non-infected leaves during the first four stadia,
(2) D-H; larvae were fed TMV-infected leaves during the first three stadia, but fourth instars were fed non-infected leaves,
(3) D-D; larvae were fed TMV-infected leaves during the first four stadia, and
(4) H-D; larvae were fed non-infected leaves during the first three instars, but fourth instars were fed TMV-infected leaves.

To make wet weight to dry weight conversions two groups of 5, 6-h-old 4th instars which had been fed either TMV-infected or non-infected leaves, were weighed, freeze-dried and reweighed. At the end of the test, the larvae, leaves and feces were freeze-dried to constant weight (for about 5 days). Nutritional indices were calculated based on a dry weight (biomass) basis. Calculated indices of larval performance were growth rate (GR), relative growth rate (RGR), consumption rate (CR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI)[20]. Feeding trials were conducted at 25–29 °C and at a photo:scotophase of 14:10 h.

Table 1. Pupal weight and larval development time of tobacco hornworms on TMV-infected and non-infected plants

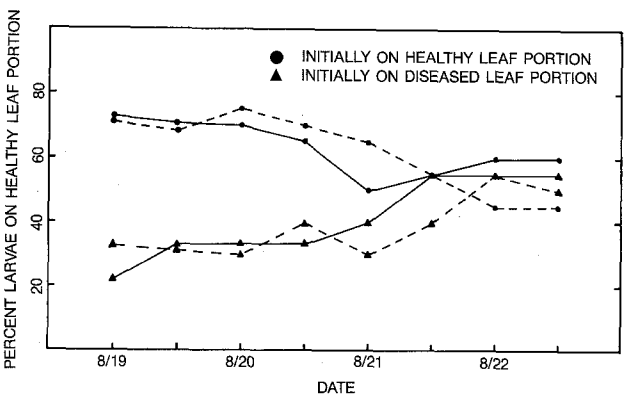
Infection status	Cumulative development time (days) 2nd	3rd	4th	5th	End of wandering stage	Pupal wt. (g)
TMV-infected tobacco	2.9 (0.5)[1] n = 70[2]	5.2 (0.8) n = 68	7.9 (0.7) n = 68	10.6 (0.8) n = 67	15.2 (0.8) n = 65	5.32 (0.54) n = 52
Non-infected tobacco	2.9 (0.4) n = 69	5.2 (0.8) n = 69	7.9 (0.4) n = 64	10.7 (0.8) n = 64	15.1 (0.9) n = 64	5.28 (0.62) n = 49
t-test	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Standard deviations in parentheses; [2] n = sample size.

Results

Development time (i.e. the period between egg hatch and the onset of the larval wandering stage) for hornworms fed TMV-infected and non-infected tobacco plants were 15.2 days and 15.1 days, respectively (table 1) and not significantly different from each other. Similarly, the mean pupal weight of hornworms reared on TMV-infected or non-infected tobacco leaves were not significantly different from each other (table 1).

In spite of large differences in the distribution of larvae on healthy and infected leaf portions at 12 h (about an average of 72% versus an average of 27%, respectively), after 72 h larvae were about evenly distributed (fig.). The infection of leaves with TMV had no significant effect in the amount of leaf disks consumed by 4th instar hornworms (table 2). Previous feeding experience had no influence on the choices of hornworm larvae. The disease status of tobacco had no significant influence on growth rate, food consumption rate, digestion or conversion efficiencies in any of the four treatments (table 3). Thus, systemic infection of the tobacco plant by the tobacco mosaic virus does not significantly affect the growth, development, feeding preference, food consumption and utilization of the tobacco hornworm larvae.



Distribution of tobacco hornworm larvae over time, initially placed on healthy or TMV-diseased leaf semi-circles. Dashed and solid lines represent each of two separate experiments.

Table 2. Feeding preference of the fourth instar tobacco hornworms for TMV-infected and non-infected tobacco leaves and the role of prior feeding experience

Food treatment prior to 4th stadium	Percentage of non-infected leaves eaten 1st exp.	2nd exp.
Infected	46.0 (± 31.1)	52.8 (± 34.4)
Non-infected	45.5 (± 33.9)	52.9 (± 31.7)
t-test	n.s.	n.s.

Discussion

In the present study, newly hatched tobacco hornworm larvae showed no feeding preference for TMV-infected and non-infected tobacco leaves. This lack of preference may not necessarily reflect the larval's inability to distinguish leaf types but may reflect the tobacco hornworm's ability to feed on food of variable quality (or marginal plants)[21]. After feeding on TMV-infected or non-infected tobacco leaves for three stadia prior to preference tests, 4th instars still did not show any feeding preference for either leaf type. This may suggest that the changes caused by TMV systemic infection in the tobacco leaves were not strong enough to affect the larval feeding preference.

Waldbauer[20] reported average (dry weight based) values (for the fourth instar hornworms) for AD, ECI and ECD of 50%, 25%, and 51%, respectively. These values were only based on a very small number of larvae. Waldbauer[22], using tomato and five other species (tobacco not included) as food plants calculated a larval AD ranging from 31.5% to 55.9%, ECI from 12.0% to 35.7%, and ECD from 38.5% to 64.4%. Our values fall within these ranges, although a different host plant (tobacco) was used in our study.

The number of TMV particles per cell in necrotic lesions may reach 10^3 whereas in systemically infected hosts this number can be 3–4 orders of magnitude higher[23]. In the present study, the TMV content in the infected leaves was 650 g viral RNA/g dry leaf. Nevertheless, tobacco hornworm larvae fed on the tobacco leaves with this level of TMV were still unaffected, as reflected in their growth,

Table 3. Growth rate, food consumption rate and food conversion efficiency of the fourth instar tobacco hornworms

Foodplant treatment[1]	Nutritional index[2] Growth rate GR (mg/day)	RGR (mg/day/mg)	Consumption rate CR (mg/day)	RCR (mg/day/mg)	Efficiency AD (%)	ECD (%)	ECI (%)
NON-NON	33.0 a (± 3.2)	0.40 a (± 0.03)	125.8 a (± 9.0)	1.53 a (± 0.16)	49.1 a (± 4.2)	53.5 a (± 2.2)	26.3 a (± 2.9)
TMV-NON	32.7 a (± 3.5)	0.40 a (± 0.04)	125.3 a (± 17.1)	1.51 a (± 0.23)	48.4 (± 4.4)	54.6 a (± 4.1)	26.2 a (± 1.6)
TMV-TMV	28.7 a (± 3.8)	0.38 a (± 0.03)	111.0 a (± 19.5)	1.49 a (± 0.18)	51.2 a (± 4.7)	51.1 a (± 1.9)	26.1 a (± 2.1)
NON-TMV	33.3 a (± 5.3)	0.39 a (± 0.03)	127.2 a (± 14.9)	1.50 a (± 0.12)	54.3 (± 4.2)	48.2 a (± 3.3)	26.1 a (± 1.8)

[1] Values in rows with same letter are not statistically significantly different.
[2] Mean value and standard deviation in parentheses.

development, feeding preference, and food consumption and utilization of tobacco.

In hypersensitive hosts the physiological reactions to TMV infection are more drastic than those with systemic infections, as reflected in the increase and accumulation of toxic quinones at the site of TMV multiplication[24]. Thus, different results might be obtained if hypersensitive host/TMV combinations had been evaluated. The conversion of nicotine to nornicotine[17], if indeed it occurs, may be why the systemic infection of tobacco by TMV does not affect tobacco hornworm larvae. Hare[25] reported that growth rates of fourth instar hornworms were reduced by 27 and 16% when reared on locally and systemically protected tobacco leaves, respectively. These results are not in agreement with those from our study. However, several factors are different between studies. Although not explicitly stated, Hare[25] implies that his experiments were conducted with a hypersensitive tobacco variety, as appears to be in the case in his previous experiments[5]. In addition, differences in levels of TMV infection between locally and systemically protected tobacco leaves are not clear nor is it possible for us to determine if Hare's[25] systemically protected leaves are comparable to our inoculated plants.

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1 Scriber, J. M., and Slansky, F. Jr, A. Rev. Ent. *26* (1981) 183.
2 Lewis, A. C., Ent. exp. appl. *26* (1979) 202.
3 Boiteau, G., and Singh, R. P., Can. Ent. *114* (1982) 473.
4 Kingsley, P., Scriber, J. M., Grau, C. R., and Delwiche, P. A., Prot. Ecol. *5* (1982) 127.
5 McIntyre, J. L., Dodds, J. A., and Hare, J. D., Phytopathology *71* (1981) 297.
6 Whittaker, R. H., and Feeny, P. P., Science *171* (1971) 757.
7 Van Emden, H. F., in: Biochemical Aspects of Plant and Animal Coevolution, p. 309. Ed. J. B. Harborne. Academic Press, London 1978.
8 Scriber, J. M., Oecologia *28* (1977) 269.
9 Reese, J. C., and Beck, S. D., J. Insect Physiol. *24* (1978) 473.
10 Scriber, J. M., and Feeny, P., Ecology *60* (1979) 829.
11 McNeil, S., and Southwood, T. R. E., in: Biochemical Aspects of Plant and Animal Coevolution, p. 77. Ed. J. B. Harborne. Academic Press, London 1978.
12 Owen, P. C., Annls appl. Biol. *46* (1958) 205.
13 Goodman, R. N., Kiraly, Z., and Zailin, N., The Biochemistry and Physiology of Infectious Plant Disease. D. Van Nostrand Inc., Princeton, New Jersey 1967.
14 Wildman, S. G., Cheo, C. C., and Bonner, J., J. biol. Chem. *180* (1949) 985.
15 Sheen, S. J., and Lowe, R. H., Can. J. Plant Sci. *59* (1979) 1099.
16 Commoner, B., and Nehari, V. J., Gen. Physiol. *36* (1953) 791.
17 Schuster, G., Phytopath. Z. *32* (1958) 335.
18 Pirone, T. P., and Davis, D. L., Tob. Sci. *21* (1977) 83.
19 Taniguchi, T., Nature *194* (1962) 708.
20 Waldbauer, G. P., Adv. Insect Physiol. *5* (1968) 229.
21 Yamamoto, R. T., J. Insect Physiol. *20* (1973) 641.
22 Waldbauer, G. P., Ent. exp. appl. *7* (1964) 253.
23 Weintraub, M., and Ragetli, H. W. J., Can. J. Bot. *42* (1964) 533.
24 Farkas, G. L., Kiraly, Z., and Solymosy, F., Virology *12* (1960) 408.
25 Hare, J. D., in: Variable Plants and Herbivores in Natural and Managed Systems, p. 655. Eds R. F. Denno and M. S. McClure. Academic Press, New York 1983.

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Mate choice and male competition in *Orchesella cincta* (Collembola)

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Summary. Both male and female *Orchesella cincta* (Collembola) were able to discriminate between spermatophores of different origin. Females chose spermatophores deposited by closely related males while males preferentially destroyed spermatophores of other males.
Key words. Mate choice; mating; sperm competition; kin recognition; Collembola.

Sexual selection theories usually assume that female mate choice is based on male fitness expressed by secondary sexual characters. It has also been suggested that characters expressing genetic relatedness between mates may determine the choice[1,2]. An optimal level of inbreeding and outbreeding will strike a balance between the cost of reduced individual heterozygosity and the benefits of maintaining associations of alleles of individuals in a locally adapted population[3,4].

We have investigated whether the Collembola *Orchesella cincta* L. are able to discriminate between spermatophores of different origin. Female *O. cincta* are fertilized by spermatophores (a drop of sperm on a 280-μm high stalk)[5] deposited by males on the substrate without prior contact with any female[6]. The female is fertile for approximately two days during each reproductive period, whereas the male produces spermatophores during five days in each reproductive instar[7].

We collected *O. cincta* from two heaps of twigs and litter, 2 m^2 in size and five years old, situated 2 km apart in a pine forest near Lund in southern Sweden. The samples are referred to as groups 1 and 2. We assumed that the individuals were more related within than between the groups, as they were aggregated, and were likely to move 2 km, crossing two gravel roads, during one generation.