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Effect of sinigrin on the efficiency of acquisition of turnip mosaic virus by *Myzus persicae* and *Brevicoryne brassicae*

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Abstract

The efficiency of turnip mosaic virus (TuMV) acqusition from *Sinapis alba* L. without sinigrin and *Brassica nigra* (L.) Koch with a high content of sinigrin by non-fasted *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.) was investigated in order to determine the effect of the phagostimulatory glucoside sinigrin on virus transmission. There was a significantly higher acquisition of TuMV from *S. alba* by *M. persicae* than from *B. nigra*. Conversely, *B. brassicae* acquired the virus more efficiently from *B. nigra* than from *S. alba*. The efficiency of transmission was positively correlated with the response of both aphid species to sinigrin. Saturation of *S. alba* leaves with a 1 % solution of sinigrin did not significantly affect the acquisition of TuMV, in neither *M. persicae* nor *B. brassicae*, whether the aphids were fasted or not.

Introduction

A number of naturally occurring substances have been shown to influence aphid probing and feeding behaviour. Little is known about the effect of these chemicals on the transmission of plant viruses by aphids when they probe and feed on plants (Harris 1977, 1983).

The glucosinolate sinigrin, a secondary plant metabolite occurring in some brassicas, has been extensively investigated in relation to its effects on the feeding behaviour of several aphid species (Herrbach 1985). Matthews (1970) has suggested the use of specific plant chemicals to induce aphids to feed on unnatural hosts for experimentally increasing the range of viruses transmitted by particular aphids. Nault (1975) showed that presence or absence of sinigrin can not be the key factor affecting transmissibility of a virus by an aphid. Nevertheless, the possible effect of the glucosinolate content on the efficiency of virus transmission by aphids is an interesting question with respect to the breeding and cultivation of low glucosinolate cultivars of brassicas.

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In a previous study (Špak 1991), it was shown that the presence of sinigrin in black mustard (*Brassica nigra* (L.) Koch) and its absence in white mustard (*Sinapis alba* L.) had no significant effect on the efficiency of acquisition of turnip mosaic virus (TuMV) by starved *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* L. during short, one-minute test probes.

It was supposed that 2 h of fasting suppressed the priority of food stimuli of aphids and that the test probes on a virus source plant were too short to manifest the phagostimulative effect of sinigrin on TuMV acquisition by aphids. Therefore experiments were conducted to elucidate the effects of phagostimulants on non-persistent virus transmission.

This paper reports experiments on the acquisition of TuMV by non-fasted aphids and on the effects of saturating mustard leaves with a solution of sinigrin on the virus acquisition by fasted and non-fasted aphids.

Material and methods

M. persicae and *B. brassicae* were reared on Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr. cv. Nozaki) and the inoculum of TuMV isolate Ruzyně was prepared using the methods described by Špak (1991). Virus source plants of *Sinapis alba* L. cv. Přerovská and *Brassica nigra* (L.) Koch cv. Ruská were cultivated simultaneously in a *Heraeus-Voetsch* climate chamber at 21 - 23 °C with a photoperiod of 12 h. When the plants had four true leaves, the two lowest leaves were inoculated with the virus.

Acquisition of TuMV by non-fasted aphids: Single adult apterous aphids were moved by means of a moist brush from the Chinese cabbage plants and placed immediately for acquisition of the virus on the fifth true leaf of a *S. alba* or *B. nigra* plant inoculated with TuMV 17 - 19 d previously. For each experiment, only one leaf of a single plant of a species was used. *M. persicae* was allowed 5 or 45 min acquisition feeding, *B. brassicae* 45 min. The aphids were then transferred to young test plants of *Nicotiana tabacum* L. cv. Samsun, on which only the largest and two next leaves below were left. On each of the three leaves one aphid was placed for an 18-h inoculation period. The plants were then sprayed with the aphicide *Pirimor* and placed in an insect-free greenhouse. Twenty plants (60 leaves) and 60 aphids were used for each experiment. Within 5 to 10 d local necrotic lesions appeared on the leaves to which the virus was transmitted by the aphids. The number of lesions per leaf was noted. Each experiment was repeated 3 to 5 times. The significance of differences was evaluated with the *t*-test at $P < 0.01$ level.

Saturation of *S. alba* leaves with sinigrin solution: 17 to 19 d after inoculation, the fifth true leaf of *S. alba* with well developed symptoms of infection was cut off with a blade and the petiole immersed in distilled water immediately thereafter. Fasted and non-fasted aphids of both species were placed on the leaf for virus acquisition. Aphids were 2 h starved in groups of ten in glass vials and then allowed 1 min or 45 min of acquisition feeding. Non-starved aphids of *B. brassicae* were allowed an acquisition period of 45 min, those of *M. persicae* a period of 5 or 45 min. Then single aphids were placed on a leaf of *N. tabacum* and handled in the same way as described above.

At the end of each acquisition feeding the petiole of leaves were dipped in a freshly prepared 1 % sinigrin solution (Na^+-glucosinolate, *Koch Light, Ltd.*). 16-18 h later, fasted and non-fasted aphids of both species were placed on the leaf for virus acquisition as above. The insects were then tested on a *N. tabacum* leaves for infectivity. Sixty aphids were used in the experiments with non-fasted aphids and 30 aphids in those with the fasted ones. Each experiment was repeated 3 times and evaluated as described by Špak (1991).

Estimation of the virus concentration in the leaves of mustard plants: At the end of each experiment the virus concentration in the leaves used for acquisition was estimated. 0.5 g of the leaf of *S. alba* or *B. nigra* was homogenized in a mortar with 2 cm^3 0.1 phosphate buffer, pH 7.0. The infectious sap from *S. alba* was inoculated on one half of a leaf of *N. tabacum* and the sap from *B. nigra* on the other half. Thirty leaves were used for each experiment. 5 to 10 d later the number of local lesions on each half of the leaf were counted and differences evaluated using the dual *t*-test.

Results and discussion

Acquisition of TuMV by non-fasted aphids from *S. alba* and *B. nigra*: The efficiency of acquisition by non-fasted *M. persicae* and *B. brassicae* after 45 min acquisition is presented in Table 1. Statistical evaluation of the results showed that the acquisition of the virus by *M. persicae* was significantly higher ($P < 0.001$) from *S. alba* than from *B. nigra*. Similar results were obtained in experiments with 5 min acquisition of the virus by *M. persicae* from *S. alba* and *B. nigra*. Therefore different periods of acquisition have little influence by this aphid on virus acquisition from the two plants. Non-fasted *B. brassicae* acquired the virus after 45 min acquisition more efficiently ($P < 0.001$) from *B. nigra* than from *S. alba* (Table 1).

Table 1. The efficiency of TuMV acquisition by non-fasted aphids from *Sinapis alba* L. and *Brassica nigra* (L.) Koch.

Aphid	Acquisition period [min]	Virus source plant *Sinapis alba* [%]	*Brassica nigra* [%]
Myzus persicae	5	13.6 ± 2.3	3.1 ± 0.3
Myzus persicae	45	10.7 ± 1.8	3.3 ± 1.3
Brevicoryne brassicae	45	4.8 ± 2.2	16.7 ± 2.4

The results are in accordance with the response of both aphid species to sinigrin. Wensler (1962), Wearing (1968), Tjallingii (1976), Nault and Styer (1972) and Klingauf *et al.* (1972) revealed that there is strong phagostimulation of *B. brassicae* by sinigrin. In polyphagous *M. persicae*, however, the reaction to sinigrin can vary from a slightly positive to a negative reaction (Qin and Ke 1984, Herrbach 1985).

In only 2 out of 13 experiments was a significantly lower ($P < 0.001$) amount of the virus found in the *B. nigra* acquisition leaves than in those of *S. alba*. Also, in these two cases, the transmission of the virus was higher from *S. alba* than from *B. nigra* by *M. persicae*.

The efficiency of acquisition of TuMV by both fasted and non-fasted aphids from sinigrin saturated leaves of *S. alba*: There was no significant difference in virus transmission from an intact *S. alba* leaf and from the same leaf after excision and immersion in 1 % sinigrin solution by both non-fasted aphid species after a 45 min acquisition period (Table 2).

Table 2. The efficiency of acquisition of TuMV by non-fasted and fasted aphids from 1% sinigrin treated and non-treated leaf of *Sinapis alba* L.

Aphid	Non-fasted sinigrin [%]	control [%]	Fasted sinigrin [%]	control [%]
Myzus persicae	8.2 ± 4.3	10.2 ± 3.9	28.6 ± 5.8	41.7 ± 8.2
Brevicoryne brassicae	10.3 ± 1.8	7.6 ± 1.8	31.1 ± 4.2	33.8 ± 3.4

Similar results were obtained by fasted aphids after 1 min acquisition (Table 2). Although differences in TuMV acquisition by both fasted aphid species from *S. alba* and *B. nigra* were not significant, the efficiency of acquisition was correlated with the reaction of aphids to sinigrin. It is evident that saturation of *S. alba* leaves with phagostimulatory glucosinolate does not influence the virus acquisition by *M. persicae* nor by *B. brassicae*.

The most likely explanation of these results is that the location of sinigrin permeating into the leaf through transpiration stream does not correspond with its subcellular compartmentation in intact leaves, as reviewed by Lüthy and Matile (1984). It has frequently been shown that sinigrin acts as a stimulant for stylet penetration and assists the location of the phloem sieve elements by oligophagous aphids with an affinity to *Brassicaceae* (Wearing 1968, Klingauf *et al.* 1972, Nault and Styer 1972). Wensler (1962) found that *B. brassicae* made probes that were of longer duration on leaves of *Vicia faba* L. treated with a 2 % solution of sinigrin than on non-treated leaves. However, all authors mentioned used non-brassicaceous plants in their experiments. The divergence between the final distribution of sinigrin in systemically treated leaves and its natural distribution in *Brassica* leaves could be relevant for feeding site selection by an aphid and consequently for the acquisition of a virus.

It is also possible in *S. alba*, which contains sinalbin as well as enzyme myrosinase and which is a suitable host plant for *M. persicae* and *B. brassicae*, that the presence of sinigrin does not increase its acceptability for *B. brassicae* or lower it for *M. persicae*. Nevertheless, it is difficult to find a plant from another family, which would be a good systemic host for TuMV as well as being a host plant acceptable to both species of aphids.

It appears that plant glucosides or products of their enzymatic degradation which stimulate the intake of food can also enhance the acquisition (transmission) of non-persistent viruses by aphids. Further evidence could be obtained by other techniques, *e.g.* the acquisition of purified virus through a parafilm membrane in the presence/absence of single pure glucosinolates with or without myrosinase. Electrical monitoring of aphid probing behaviour (Tjallingii 1985) in the course of such experiments could further elucidate the interactions.

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