2-Thiouracil-Induced Changes in Alfalfa Mosaic Virus Infectivity and Nucleoprotein Components in Hypersensitive Bean

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ABSTRACT

2-Thiouracil caused the number and size of local lesions produced by alfalfa mosaic virus (AMV) on hypersensitive bean to increase 2.0 and 2.4 times, respectively. When sap extracts were made from infected leaves, thiouracil-treated leaves caused 100-1,000 times more local lesions than control leaves. As bean plants matured from 8 to 13 days after planting, the number of lesions decreased for both thiouracil and water-treated leaves. Reducing light intensity caused lesion size to increase on both treated and untreated leaves, and thiouracil caused approximately a similar increase at all light intensities. Thiouracil increased AMV nucleoprotein in bean by 1.8 times and specific infectivity by 17 times. Density-gradient profiles revealed that the middle and bottom AMV nucleoprotein components could not be observed in virus preparations from nontreated bean but small quantities of each component were present in virus preparations from thiouracil-treated bean. The increased infectivity appeared to be related to the presence of the bottom and middle nucleoprotein components.

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2-Thiouracil can either increase (5, 10) or decrease (4, 7) plant viral biosynthesis and size of local lesions. The mechanism of this ambivalent property is not understood. Enhancement has been established only for cowpea chlorotic mottle virus (CCMV) (5, 10) but this report shows a similar effect on local lesions

caused by alfalfa mosaic virus (AMV). Previously, Matthews (11) reported that thiouracil had little effect on AMV in systemically infected hosts. As will be described in this report, thiouracil caused a clearcut enhancement of AMV lesion number and size, infectivity of sap extracts, viral nucleoprotein, and specific infectivity in the hypersensitive host bean.

MATERIALS AND METHODS.–The test host for AMV (Ac 106 of the ATCC) was hypersensitive bean, *Phaseolus vulgaris* L. 'Bountiful'. The virus was cultured in *Glycine max* (L.) Merr. 'Bragg' and sap inoculum was obtained from the first two trifoliolate leaves 6 to 12 days after inoculation. Half leaf local lesion assays were conducted on Bountiful bean.

Bean plants were grown in pots containing a soil-sand-vermiculite mixture in the greenhouse (24 to 30 C). Immediately after the primary leaves of 9-to 10-day-old plants were inoculated, one-half of each leaf was floated on 0.5×10^{-3} M 2-thiouracil in a petri dish and the other on distilled water. The dishes were incubated at 27 ± 2 C with 8,600-10,764 lx (800-1,000 ft-c) from fluorescent lights (cool-white, extra high intensity) and with a photoperiod of 16 hr.

Lesions were counted 4 to 5 days after inoculation and lesion diameters were measured with a dial caliper. Infectivity of leaves with local lesions was assayed by grinding the leaves in 0.01 M neutral potassium phosphate buffer and inoculating Bountiful bean.

The virus was purified from bean and soybean by extracting diseased tissue in 0.01 M neutral potassium phosphate containing 1% ascorbic acid plus 3.5 ml of 50% K_2HPO_4 per 100 ml of buffer; chloroform; and *n*-butanol (1:1:1:1, w/v/v/v). The aqueous phase was frozen after centrifugation at 5,000 *g*. This was followed by two cycles of centrifugation at 368,000 *g* (1 hr) and 5,000 *g* (10 min). Spectrophotometric measurements, local lesion assays, and density gradient analyses were made with the final pellets suspended in 0.01 M potassium phosphate, pH7. Density-gradient profiles were determined with an ISCO ultraviolet analyzer (Model UA2) after 1 mg of virus was layered on 10, 20, 30, and 40% sucrose and centrifuged 3 hr at 70,000 *g* (SW25.1 Spinco rotor). This procedure isolated the three heaviest nucleoprotein components of AMV and no attempt was made to obtain less dense or smaller components.

TABLE 1. Effect of thiouracil on AMV local lesions on Bountiful bean[a]

Treatment[b]	Number of lesions per half-leaf	Area/lesion (sq mm)	Sap infectivity (lesions/half-leaf)[c]
Water	52[d]	1.18[e]	0-4[f]
Thiouracil	105	2.82	120

[a] Average of 12 tests; 6-12 leaves from different plants/test.

[b] Half-leaves were floated on water or 2-thiouracil (0.5×10^{-3} M) and incubated at 27 C.

[c] Leaves with lesions noted in column 2 were ground in buffer and used as inoculum in local lesion assays. Sap dilution was 1/100.

[d] Two tests were significantly different at the 1% level, six at the 5% level, and four were significantly different.

[e] Nine tests were significantly different at the 1% level and three at the 5% level.

[f] All tests were significantly different at the 1% level.

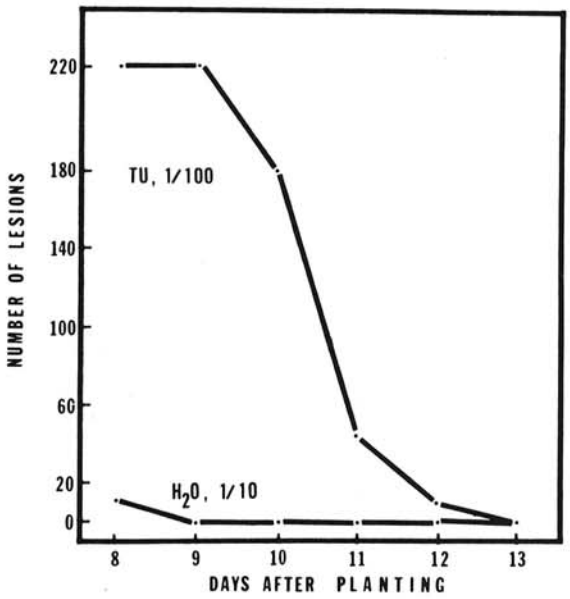

Fig. 1. Effect of host age on alfalfa mosaic virus infectivity in 'Bountiful' bean. After opposite half-leaves were floated on 2-thiouracil (TU) or water, they were ground in buffer and used as inocula for bioassay on Bountiful bean. 1/10 and 1/100 = sap dilution.

RESULTS.–Initially, the effect of thiouracil on AMV local lesions was studied by floating inoculated, opposite half-leaves of bean on water or thiouracil. Thiouracil caused the number of lesions to double and the area/lesion was increased 2.4 times (Table 1). When test leaves were ground in buffer, sap from thiouracil-treated leaves caused several times as many lesions as expected from the increase in lesion size and area (Table 1). The infectivity increase was variable but was usually between 100 and 1,000 times. For example, sap from water-treated leaves, diluted 1/10, caused 5 to 10 lesions/half-leaf and thiouracil-treated leaves, diluted 1/100, caused 75 to 150 lesions/half-leaf.

Host age affected the number and size of AMV local lesions and the amount of infectivity. As bean plants matured from 8 to 13 days after planting, the number of lesions decreased for both thiouracil-treated and control leaves. Treated plants had 94, 132, 126, 72, 64, and 34 lesions/half-leaf and control plants had 56, 58, 49, 27, 20, and 20 at 8, 9, 10, 11, 12, and 13 days, respectively. Lesion size was similar (approximately 0.5 mm^2) on control plants, regardless of host age. For treated plants, the lesion area gradually decreased with age: 2.1, 1.8, 1.4, 1.3, 0.8, and 0.6 mm^2 at 8, 9, 10, 11, 12, and 13 days, respectively. Sap infectivity also declined with increasing host age (Fig. 1). All measurements

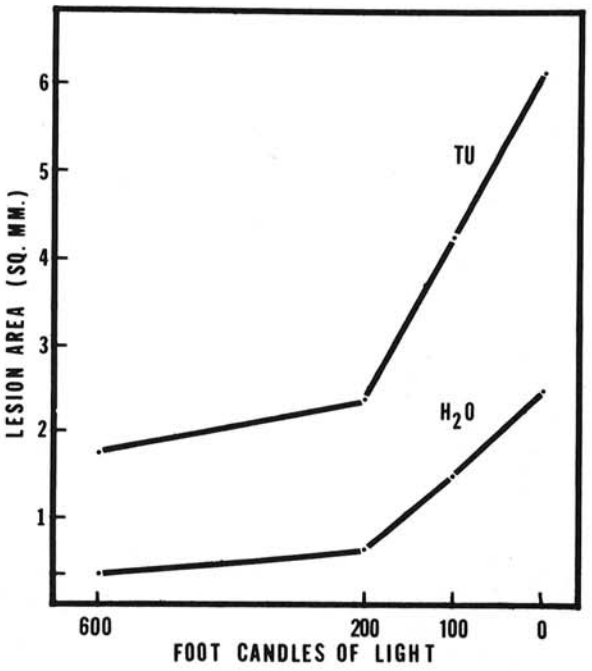

Fig. 2. Effect of light intensity on the size of alfalfa mosaic virus induced local lesions produced on 'Bountiful' bean. Opposite half leaves were floated on 2-thiouracil (TU) or water.

emphasized the need to use bean plants 8 to 10 days after planting to obtain high infectivity levels.

When light intensities were lowered from 6,450 lx (600 ft-c) to darkness, the size of AMV lesions increased on both thiouracil-treated and control leaves (Fig. 2). Lesions on treated leaves were larger than those on control leaves at each of the light intensities tested. When leaves were maintained in petri dishes in darkness, they frequently began to deteriorate after 2 to 3 days and were unsuitable for further testing.

To purify AMV from hypersensitive bean, approximately 100 intact plants in 50 pots were inoculated. One-half of the pots were placed in a tray containing 2-thiouracil (0.5×10^{-3} M) and the other half in water. This method of application increased lesion number, lesion size, and sap infectivity and was more convenient for purification than floating leaves. The amount of AMV nucleoprotein varied from 0.02 to 0.29 mg/g fresh wt for nontreated plants and from 0.09 to 0.40 mg/g fresh wt for thiouracil-treated plants. Under uniform environmental conditions, this nucleoprotein quantity was more variable than was usually found for AMV in systemically infected tissue such as tobacco or soybean. In four tests, thiouracil increased the viral nucleoprotein by 1.8 times, sap infectivity by approximately 29 times, and specific infectivity by approximately 17 times (Table 2). Although the various increases induced by thiouracil in hypersensitive soybean were impressive, the virus was considerably less infective than AMV purified from systemically infected soybean (Table 2).

Density-gradient centrifugation revealed different patterns of viral nucleoprotein components with virus from different sources. Three typical AMV components were observed with virus from systemically infected soybean (Fig. 3-A). Neither of the two heavier components was obvious in AMV preparations from water-treated, hypersensitive bean (Fig. 3-B), and only small quantities of the heavier components were present with virus from thiouracil-treated bean (Fig. 3-C). With the latter two preparations from bean, there was a large amount of the least dense viral component and even lighter, noninfectious material was observed near the meniscus (Fig. 3-B, C).

Using cowpea chlorotic mottle virus ($S_{20,w} = 88$) and southern bean mosaic virus ($S_{20,w} = 115$) as standards, the sedimentation coefficient values for the three AMV components in Fig. 3-A were estimated (3) to be 74 S, 86 S, and 99 S, similar to reported values for the top b, middle, and bottom components of AMV (8).

DISCUSSION.—Thiouracil induced an obvious increase in lesion number and size in AMV-infected, hypersensitive bean. The lesion increase was accompanied by an increase in viral nucleoprotein, sap infectivity, and specific infectivity. Increases in infectivity (100 to 1,000 times) were considerably more than could be expected from the increase in total lesion area (4 to 5 times). A lack of correlation between lesion size and infectivity of leaf extracts was also observed for CCMV in thiouracil-treated

TABLE 2. Comparison of AMV from thiouracil-treated hypersensitive plants, nontreated hypersensitive plants, and nontreated systemic plants[a]

Host and virus reaction	Treatment[b]	Amount of purified virus (mg/g)[c]	Sap infectivity[d]		Specific infectivity[e]	
			Dilution	Lesions/half-leaf	μg/ml	Lesions/half-leaf
Bean, hypersensitive	Water	0.12	1/10	10	75.00	14
Bean, hypersensitive	Thiouracil	0.21	1/100	29	7.50	24
Soybean, systemic	Water	0.82	1/1,000	242	.75	62

[a] Average of four tests.
[b] Pots were placed in pans with water or 2-thiouracil (0.5×10^{-3} M).
[c] 6.0 absorbance @ 260 nm = 1 mg/ml.
[d] The aqueous solution obtained after chloroform-butanol clarification was used as inoculum.
[e] Purified virus was used as inoculum.

hypersensitive soybean (10), but the differences were even greater in this study with AMV.

Although viral nucleoprotein increased in treated plants, its increase (1.8 times) was less than total lesion area (4 to 5 times) and thus, could not account for the large infectivity increase. Density-gradient profiles, however, revealed that substantial quantities of the heavier AMV components, 88 S and 99 S, were missing in virus preparations from nontreated bean plants. It is now established that the AMV infectious unit is multicomponent in nature (2, 8, 9) and that RNA from more than one nucleoprotein component is necessary for infection. Therefore, a deficiency of a component, particularly the bottom one, is probably responsible for the low infectivity of AMV isolated from hypersensitive bean. Small quantities of the middle and bottom components were present in AMV isolated from thiouracil-treated plants and infectivity was enhanced.

The reason for the low quantities of the middle and bottom components is not apparent. Schwenk et al. (12) reported that ratio differences of the three major components varied among strains of AMV. However, substantial quantities of each component were present and the variation was with regard to the amount of each component compared to the others. All strains were purified from systemically infected tobacco. Environmental conditions (12), and the length of the infection period (1, 12), apparently have no effect on the amount of each AMV component produced.

Since infectivity was obtained from AMV in hypersensitive bean, probably small quantities of the middle and bottom components were produced and may account for the low infectivity level. On the other hand, more normal proportions of each component, than was observed, may have been produced and then broken down more quickly than in systemic hosts. A light weight, broad zone occurred in sucrose density gradients with AMV from hypersensitive bean. This zone may be heterogeneous breakdown products. Changes in quantities of enzymes and chemicals occur during a hypersensitive reaction (6, 13), and AMV is known to be relatively unstable and its particle integrity and infectivity are affected by several agents such as divalent cations, monovalent cationic salts, enzymes, and pH (8).

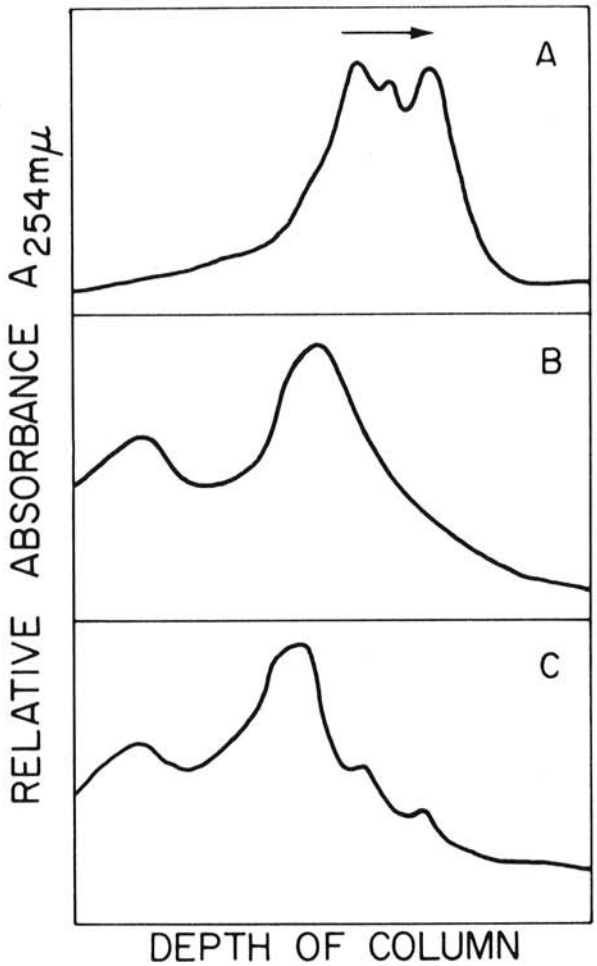

Fig. 3. Density-gradient profiles of purified alfalfa mosaic virus; **A)** Virus from systemically infected 'Bragg' soybean; **B)** Virus from hypersensitive 'Bountiful' bean; **C)** Virus from hypersensitive Bountiful bean treated with 2-thiouracil.

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