Insecticidal Soap Reduces Infection by Two Mechanically Transmitted Plant Viruses

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ABSTRACT

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Safer, an insecticidal soap containing potassium salts of fatty acids, is sprayed on plants to control insects, including aphids. We suspected that soap residue on leaves was reducing plant infection by mechanically inoculated viruses. The soap was tested for its ability to reduce infection by tobacco mosaic virus (TMV) and tobacco ringspot virus and for its ability to induce resistance to virus infection in pinto bean (*Phaseolus vulgaris*) and tobacco (*Nicotiana tabacum* 'Xanthi-nc'). Amending inoculum of TMV with 0.1–10% soap reduced necrotic local lesions by 43–92%. Half-leaves dipped in 1 or 10% solutions of soap and inoculated with one of the two viruses developed 35–82% fewer lesions than opposite half-leaves dipped in water. Leaves treated on the underside with soap and inoculated on the upper side with virus and bean plants treated with soap on primary leaves and inoculated 7 days later on secondary leaves were not significantly more resistant to virus infection than controls.

Many common genera of North American aphids, including *Aphis* and *Myzus*, have become resistant to insecticides. Controlling aphids in commercial and research greenhouses has become increasingly difficult, requiring frequent rotation of insecticides.

After the application of various insecticides failed to end a 6-mo aphid infestation in our greenhouse, we tested the insecticidal soap Safer (Safer, Inc., Wellesley, MA) (hereinafter referred to as "soap"). When diluted 1:50 to the manufacturer's recommended level of 1% active ingredient (potassium salts of fatty acids), the soap was remarkably effective in the immediate eradication and long-term control of aphids. However, we noticed an apparent decrease in our ability to infect tobacco (*Nicotiana tabacum* L. 'Xanthi-nc') by mechanical inoculation with tobacco mosaic virus (TMV).

We used two methods to directly test the effect of the soap on plant virus infection: we treated the virus with soap before using it to inoculate plants, and we treated plants with soap before inoculating them with the virus.

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MATERIALS AND METHODS

Plants were grown in the greenhouse in 10-cm pots containing the commercial soilless potting mix ProMix (Premier Brands, Inc., New Rochelle, NY) and were treated twice weekly with a 0.5% solution of soluble 15-16-17 fertilizer. TMV and tobacco ringspot virus (TRSV) were obtained from R. W. Fulton. TMV was purified from tobacco (4), and TRSV was purified from bean (*Phaseolus vulgaris* L. 'Pinto') (3). Viruses were suspended in 30 mM sodium phosphate buffer, pH 7.7 ("buffer") at concentrations of 0.3 μg/ml for TMV and 1 μg/ml for TRSV.

In inoculum interference experiments, inocula containing both virus and soap were prepared by separately diluting in buffer the desired virus and the soap to 2× the final concentration of each and then mixing equal volumes of both solutions. Inocula were rubbed on corundum-dusted, fully expanded primary leaves of bean or on tobacco leaves with a cheesecloth pad. Whenever possible, soap-amended inocula were applied to half of a leaf, and control inocula not amended with soap were applied to the opposite half-leaf.

To compare plant virus infection of soap-treated leaves to that of untreated leaves, a fully expanded primary bean leaf was sprayed with or dipped in a solution of soap prepared in water, and the opposite leaf was treated with water alone, or one half of a tobacco leaf was dipped in water and the opposite half was dipped in a solution of soap. When leaves were dry (about 20 min), inocula were applied with a fresh cheesecloth pad for each bean primary leaf or each tobacco half-leaf, to prevent contamination of inoculum with soap.

To differentiate between an effect of the soap on the virus and an effect on the plant, we tried a method of treating leaves separately with soap and virus. We sprayed either the underside or the upper side of a bean primary leaf with soap diluted in water and the underside or upper side of the opposite leaf with water. After drying, plants were inoculated on the upper side as described above.

To test for the induction of systemic resistance, we rubbed primary leaves of bean seedlings or the youngest fully expanded leaf of tobacco with a 1% solution of soap in water; similar plants rubbed with water served as controls.

Table 1. Infection of half-leaves dipped in soap, expressed as a ratio of infection of opposite half-leaves dipped in water[a]

Virus and plant	Soap concentration 0.1%	1.0%	10%
Tobacco mosaic virus and pinto bean	1.24 ± 0.126 (n = 23)	0.645 ± 0.056 (n = 18)	0.175 ± 0.038 (n = 22)
Tobacco ringspot virus and Xanthi-nc tobacco			
Trial 1	3.36 ± 1.36 (n = 5)	0.57 ± 0.087 (n = 10)	0.22 ± 0.050 (n = 10)
Trial 2	0.94 ± 0.17 (n = 15)	0.75 ± 0.17 (n = 12)	0.53 ± 0.10 (n = 10)

[a] After dipping, leaves were allowed to dry for about 20 min before inoculation. Data are averages and standard errors of the ratio of necrotic lesions with amended inoculum to lesions with unamended inoculum.

Seven days later, newly developed leaves were inoculated with TMV in buffer.

All experiments were done at least twice, with a total of at least eight replicates per treatment. Where appropriate, lesion counts were analyzed by calculating the average and the standard error of the ratio of lesions with amended inoculum to those with unamended inoculum on opposite half-leaves or opposite primary leaves.

RESULTS AND DISCUSSION

Inoculum containing 0.3 μg of TMV per milliliter and amended with 10, 1, or 0.1% soap reduced necrotic lesion counts by 92 ± 1.8%, 53 ± 4.5%, and 43 ± 6.2%, respectively, on opposite halves of bean primary leaves. The pHs of the amended inocula were 9.5, 8.7, and 7.7, respectively. The unamended inoculum (pH 7.7) produced an average of 69 ± 5 lesions per half-leaf on a total of 60 leaves from two trials (20 leaves per treatment). These data confirmed that soap interfered with plant virus infection when mixed with purified inoculum before application to plants.

In practice, however, soap is sprayed on host plants that are later inoculated with virus. To test whether soap residue on plants reduced virus infection, we inoculated leaves treated with soap solution and untreated leaves with TMV or TRSV. Virus infection was reduced on leaves treated with 1 or 10% soap (Table 1). This result showed that plants recently treated with soap may produce fewer lesions when inoculated with necrotizing viruses than untreated plants.

To test whether the effect of soap endured for longer periods after application, a time course study was done. Pinto bean primary leaves inoculated with TMV either 30 or 60 hr after treatment with 2% soap produced 45 ± 11% and 41 ± 24%, respectively, as many lesions as opposite primary leaves treated with water. Controls averaged 68 ± 11 lesions per leaf. Thus, the effect of the soap persisted for at least 2 days after application. Tests of longer duration were not done because the susceptibility of primary leaves decreases rapidly with aging regardless of treatment.

In the above experiments, the virus and the soap were present at the same place on the leaf. The inhibition of infection may have resulted from an interaction between the soap and the virus or between the soap and the leaf. If the presence of soap on the leaf were sufficient to reduce infection, then leaves treated on the underside with soap and inoculated on the upper side with virus would be expected to develop fewer lesions than controls sprayed with water on the underside. However, leaves so treated with 1% soap developed 93 ± 1.9% as many lesions as controls; leaves both sprayed with soap and inoculated on the upper side developed 43 ± 5.9% as many lesions as controls, which produced on average 138 ± 18 lesions. Apparently, the presence of soap on inoculated leaves was not sufficient to reduce lesion numbers significantly; the soap had to be present at the site of inoculation to decrease infection. These results provided no evidence for the rapid induction of local resistance to infection.

Repeated tests with bean and tobacco produced no evidence for the induction of systemic resistance in soap-treated plants (*data not shown*).

The reduction of plant virus infection by insecticidal soap is primarily of interest to plant pathologists who work with mechanically transmitted viruses. Soap-treated plants may require inoculation with higher concentrations of virus to achieve the same level of local infection as untreated plants. Furthermore, because the degree of local infection often correlates directly with the extent of systemic replication, it is possible that soap indirectly reduces systemic virus infections by reducing local infections following inoculation. Such a systemic effect may be masked and go unnoticed.

The mechanism of action may be a direct effect on the virus particle or the blockage of infection sites of the leaf. Soaps, as well as detergents, are amphipathic molecules: they have both polar and nonpolar regions and therefore can form spherical micelles in aqueous solutions (2). The ionic detergent sodium dodecyl sulfate (SDS) strips coat protein from TMV, exposing the viral RNA to inactivating agents in the environment (1); soaps may behave similarly. However, because soap is composed of the salts of weak carboxylic acids whereas SDS is the salt of a strong sulfated acid, aqueous solutions of soap are slightly alkaline (pH 7.7–9.9), whereas those of SDS are slightly acidic (pH 5.5—approximately the same pH as the distilled water used to make the solutions). Thus, the action of soaps on virus particles may result in part from pH effects. However, the pH of a 0.1% solution of soap prepared in buffer was 7.7, identical to that of the buffer, yet the soap-amended inoculum produced only 57% as many lesions as unamended inoculum also at pH 7.7. This argues against pH effects being the sole cause for the reduction of virus infection.

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