# 19

# Occurrence of Resistance to *Bacillus thuringiensis* in Diamondback Moth, and Results of Trials for Integrated Control in a Watercress Greenhouse

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## Abstract

**High levels of resistance to *Bacillus thuringiensis* Berliner in diamondback moth *Plutella xylostella* (L.), ($LC_{50}$ more than 280 ppm) was observed in a watercress greenhouse in Osaka Prefecture in 1988. Populations of egg, larva, pupa and adult of diamondback moth were monitored weekly throughout 1988. Population of the larva fluctuated from nearly 0 to 1,300,000. Three control methods were attempted: Insect Killer (high voltage electric shocker with luring purple color lamps), Konagakon (a sex pheromone dispenser), and the vacuum cleaner to suck adult moths. The percentage of moths killed by 24 sets of Insect Killer in the greenhouse was estimated at less than 15% of the emerged adults even in summer. The percentage of mating reduction by Konagakon depended on moth density (more than 90% of mating reduction if 1 moth/$m^2$; less than 50% if 3 moths/$m^2$). The vacuum cleaner reduced moth population by 50% in every operation. It is therefore possible to reduce the moth density where Konagakon is effective through five or six operations of vacuum sucker.**

## Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) has developed resistance to most organophosphorus and carbamate insecticides used in Japan since 1971. It has also developed resistance to synthetic pyrethroids only 1 or 2 years after these insecticides were marketed in 1983 (Hama 1987). A *Bacillus thuringiensis* Berliner (*Bt*) formulation was also introduced in 1983 in Japan, and its effectiveness on DBM did not decrease for some time, although Morishita and Azuma (1987) reported a population with slightly higher $LC_{50}$ in Wakayama Prefecture. McGaughey (1985) found resistance to *Bt* formulation in *Plodia interpuctella* (Hübner), and so far it is the only report concerning high levels of *Bt* resistance in any lepidopteran insect.

However, a *Bt* formulation (Toarow CT, 7% *Bt* toxin) became less effective in controlling DBM in watercress grown in greenhouses in Osaka Prefecture in 1987. This decrease in susceptibility was confirmed by leaf-dipping method in 1988.

In the present work, the progress of DBM resistance to *Bt* in the greenhouse is reviewed, and the effectiveness of three other control methods discussed.

## Cultivation

Watercress was grown in two steel-frame plastic houses (20 m × 50 m × 3 m high), located in a small valley in Kishiwada City in Osaka Prefecture. Hydroponic cultivation of watercress was started in 1975, and now three people collect and sell 8,000 to 40,000 shoots/day. Watercress is grown throughout the year, and the houses are heated with kerosene heaters from November to March, and the side vinyl of the houses are kept open from April to October. The houses are surrounded by forest and a pond, and the nearest vegetable garden planted to crucifers is at least 300 m from the houses, so immigration of DBM adult moths is assumed to be minimal. DBM is the only insect pest on the watercress, and diseases are not important economically.

## Materials and Methods

### Resistance to *Bt* formulation

Six tests were done from May 1988 to November 1989. Adult females (30-100 individuals) were collected from the greenhouses on 18 May, 4 and 21 October, 15 November and 13 December 1988 and 16 November 1989, and were given cabbage leaves for oviposition. Hatched larvae were reared on cabbage leaves and the third instar larvae were used for the tests. Susceptibility of Toarow CT was determined by leaf-dipping, i.e. cabbage leaves were dipped in the *Bt* solution for 30 sec, dried in the air and placed into a plastic case (4 cm long, 10 cm diameter). Ten to fifteen larvae were released in each of 2-6 cases, each case being one replicate and dead larvae were counted 3 days later (except in November 1989, 2 days later). Each test consisted of 5-11 concentrations of the *Bt* solution including 70 ppm, and $LC_{50}$ was calculated. All the tests were carried out at 25°C and 12 L:12 D photoperiod except for a test in May 1988 when *Bt* concentration of only 70 ppm was used and the test was carried out at normal room temperature and photoperiod.

### Population dynamics

Weekly population densities of egg, larva, pupa and adult were investigated in the greenhouses from January (egg was from March) to December 1988. Forty-eight shoots of watercress longer than 15 cm were systematically chosen in each of the houses, and the numbers of eggs, larvae and pupae on each shoot were counted. Larvae were counted separately as 1st-2nd, 3rd, and 4th instar. Adult density was expressed as the flying number of moths/$m^2$ by the line transect method, i.e. number of flying moths counted on both sides of 1 m line along a fixed route of 300 m (area 600 $m^2$) in each of the houses.

### Killing moths by electric shocker

Twenty-four sets of high voltage electric shockers with purple color lamps as lures were hung systematically in one of the houses where they were operated from 9 June to 30 September 1988 to kill adult moths. Eight of them were Model YF11990 of Insect Killer (Matsushita Electric Works, Ltd.) and another 16 sets were Model SL-055 (Sun Co. Ltd.). The number of moths killed by each of the electric shockers was counted weekly. Yamada and Kawasaki (1983) showed that the developmental period of larvae and pupae was 9.2 and 3.8 days at 25°C, and the 4th instar larva is assumed to emerge in 6 or 7 days. The number of emerged adults was calculated using the total population of the 4th instar larvae and pupae in the greenhouse, estimated by weekly observations as described earlier, assuming that they (4th instar larva and pupa) emerged in 1 week without any deaths. Killing efficiency ($E_K$) is then calculated as:

$$E_k(\%) = 100 \times N_k/N_e$$

($N_k$: Number of moths killed in a given period)
($N_e$: Number of moths emerged in a given period)

## Communication disruption by sex pheromone

Sex pheromone of DBM was applied in a pheromone dispenser (Konagakon, Shin'Etsu Chemical Co. Ltd.) for communication disruption of moths in both greenhouses throughout 1988, and its effect was estimated. The 250 m long dispensers covered 0.1 ha in each of the houses, and were replaced every 3 months. Tests for estimating communication disruption were carried out 15 times from May to October 1988, in which 8-30 female adults were collected from the houses and each female placed in a plastic case with cabbage leaves for oviposition. This was done for specific air temperatures and adult flying density (number of flying moths/$m^2$, as described in the section on population dynamics). If the female laid eggs and the eggs hatched, the female was regarded as mated. If the laid eggs did not hatch, or if the female survived 3 days without laying any eggs, the female was regarded unmated (all tests were carried out at 25°C). The percentage of mating reduction ($R_m$) was calculated as:

$$R_m(\%) = 100 \times N_u/(N_u + N_m)$$

($N_m$: Number of mated females)
($N_u$: Number of unmated females)

## Elimination of moths by vacuum sucker

Sucking of moths by vacuum cleaner was attempted, and its effect in controlling DBM was estimated. The vacuum pump was an electric single-phase induction motor (Model No. 3 APM of Ebara Co. Ltd., 500 W, capacity: 55 $m^3$/min) and an accordion-like flexible metal conduit (80 cm long and 20 cm diameter) was attached to it. All the moths sucked by the machine were torn to pieces by the fins of the motor fan.

The vacuum cleaner was operated five times in one-fourth of a greenhouse (250 $m^2$) on 18 June 1990. The number of moths sucked by the machine was counted, and flying moth density (number of flying moths/$m^2$) was recorded before every operation and after the last one by line transect method. Weather at the test site was clear and the air temperature was 32°C.

# Results and Discussions

## Resistance to *Bt* formulation

All cabbage leaves in the tests were extensively eaten, and the percentage of dead larvae at 70 ppm concentration was higher than 50% in two tests (87.5% on 18 May and 53.9% on 21 October 1988), and lower than 50% in the other four tests (Fig. 1) (Tanaka and Kimura 1991). In the latter five tests, $LC_{50}$ was 44 ppm on 21 October 1988, and more than 280 ppm in others. On the other hand, the percentage of dead individuals in two other populations on cabbage in April 1988 in Osaka Prefecture was 100% and cabbage leaves had no feeding holes at the 70 ppm concentration. Morishita and Azuma (1987) reported that $LC_{50}$ of Toarow CT to DBM on cabbage in Wakayama Prefecture was 0.2-0.9 ppm in five populations, and slightly higher (3.9-17.6 ppm) in one population. Therefore, $LC_{50}$ of DBM on watercress in Kishiwada was 20-1000 times higher than that observed by Morishita and Azuma (1987).

Toarow CT was sprayed 15-20 times a year since June 1986 in the houses, and the spraying did not cease even after losing most of its effectiveness, because DBM has developed resistance to all organophosphorus, carbamate, pyrethroid and cartap insecticides. This frequent spraying of Toarow CT, associated with slight immigration of DBM adults, is assumed to cause the resistance in the greenhouses earlier than any other areas.

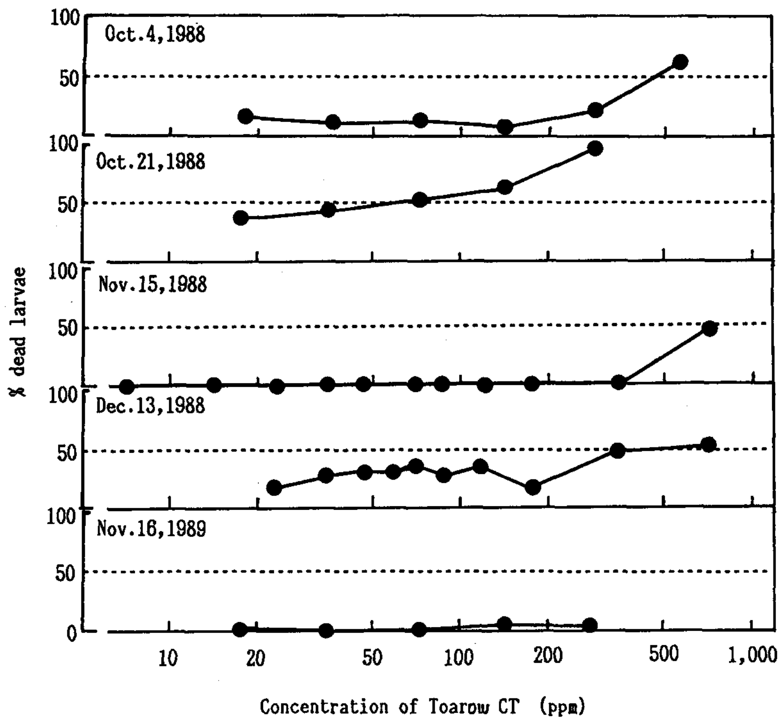


Fig. 1. Susceptibility of 3rd instar larvae of DBM on watercress to *Bt* formulation (Toarow CT) by leaf-dipping method using cabbage leaf. Collection dates of adult moths are shown. (Tanaka and Kimura 1991)

## Population dynamics

Population dynamics was quite similar in both houses throughout the year, and the results of a house with a generally higher population are shown in Fig. 2 (Tanaka and Kimura, in press). Population peaks of the flying moth were observed in late March, early May and mid June (10.8 moths/$m^2$) in the year. The interval between peaks is thought to be the period of one life cycle. Six vague peaks were observed: early July, late July, mid August, early September, late September and early November. Peaks of the eggs, larvae and pupae succeeded those of adults, though they were not so clear from July onwards.

Susceptibility of DBM to Toarow CT decreased markedly in 1987 and 1988 and this formulation is not thought to have been a major factor in suppressing the population in the greenhouses in 1988. Predator and parasitoid wasps of DBM were rarely observed throughout the investigation, possibly because of the occasional spraying of cartap and other pesticides. Disease or other factors may suppress DBM density, though we have not studied this in detail.

Spatial distribution patterns of the eggs and larvae of DBM in the greenhouses were analyzed with Iwao's $\overset{*}{m}$ regression method, and they were both contiguous, i.e., $\alpha = 1.62$ and $\theta = 1.59$ in egg, and $\alpha = 0.07$ and $\beta = 1.60$ in larva (Tanaka and Takahara 1989). The total number of watercress shoots longer than 15 cm was estimated as 680,000 in each of the greenhouses. Short shoots were not suitable for estimating the egg and larval densities, because these stages concentrate on canopy of watercress (Tanaka, unpublished data). Thus the total population of eggs in the greenhouse was estimated to fluctuate from 0 to 6,600,000 $\pm$ 1,800,000 (mean $\pm$ SD), and that of the larva from 0 to 1,300,000 $\pm$ 400,000. Zero in the estimation means undetectable, when the total population was estimated to be under 10,000 in a greenhouse.

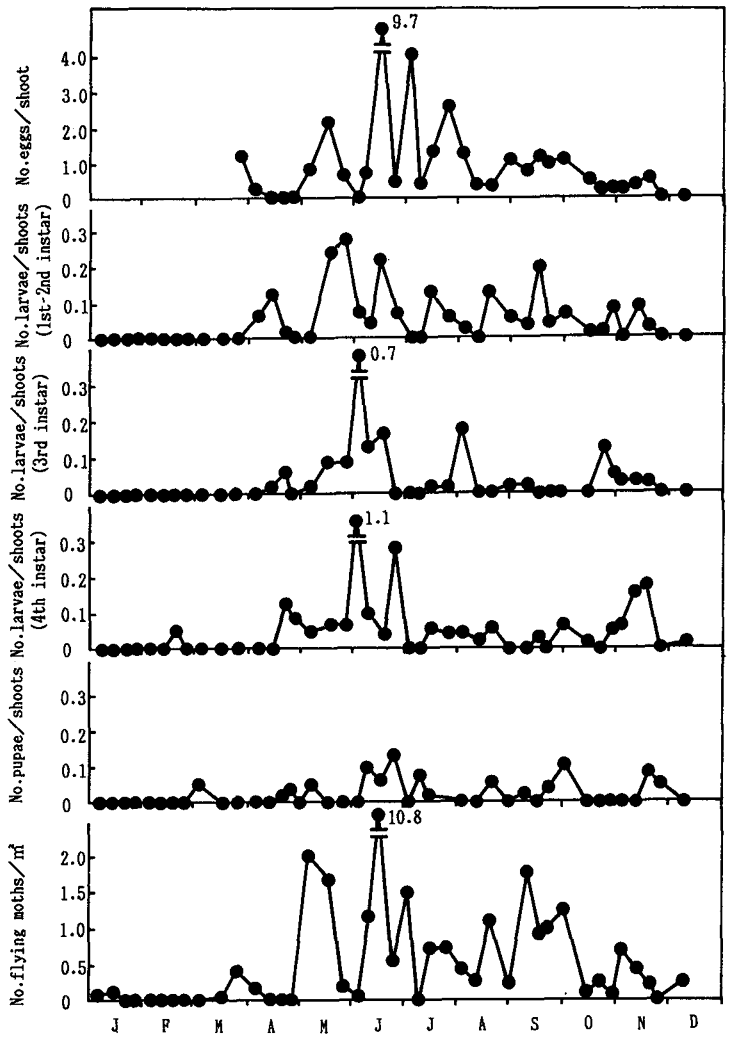


Fig. 2. Population fluctuation of DBM on watercress in 1988; investigation of the egg started in March (Tanaka and Kimura, in press).

## Electric shocker

The number of moths emerged, killed, and the killing efficiency are shown in Fig. 3 (Tanaka et al. 1989). The number of emerged adults was 390,000 in July (24 June-24 July), 210,000 in August (25 July-29 Aug.) and 220,000 in September (29 Aug.-30 Sept.), and the killing efficiency was 8.6, 13.6 and 3.7% in the respective months. The total number of emerged adults in 3 months (24 June-30 Sept.) was 820,000 and the average killing efficiency during the period was 8.6%. If the percentage of deaths were 30 or 40%, the estimated values of killing efficiency would increase 43% or 67%. The developmental period of larva and pupa at 20 and 30°C increases by 45% and decreases by 15% respectively, compared with that of 25°C (Yamada and Kawasaki 1983), and killing efficiency decreases by 31% at 20°C and increases by 18% at 30°C compared with 25°C. So the killing efficiency is not high enough to suppress the moth density.

During this test, neither the larval density nor the damage to watercress was suppressed by 24 sets of electric shockers. Moreover, high killing efficiency cannot be expected from October to May, because of low flying activity of moths at night. These results suggest that control of moths with the electric shocker is not practical in the greenhouses.

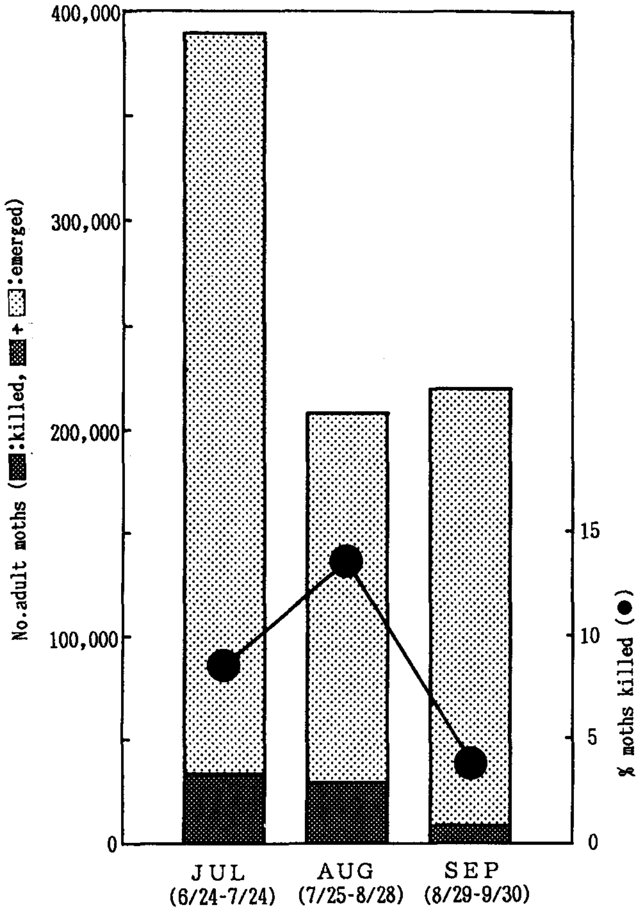


Fig. 3.
Percentage of DBM adults killed by high voltage electric shockers in greenhouse of watercress. (Tanaka et al. 1989).

## Communication disruption by sex pheromone

The relation between the flying moth density and the percentage of mating reduction varied depending upon the air temperature (Fig. 4) (Tanaka and Kimura 1990). At temperatures above 20°C, the mating reduction was 100% when the flying moth density (number of flying moths/$m^2$) was less than 0.1, 50% when the flying moth density was 0.3-0.4 and as low as 10% when the flying moth density was more than 1.0. At temperatures below 20°C, the percentage of mating reduction was less than 30% when the flying moth density was about 0.1, and 0% when the flying moth density was more than 0.2. Estimated mating reduction at each temperature could be drawn as shown by lines in the figure.

Mating reduction achieved in the houses in 1988 can be estimated from the lines in Fig. 4, in association with the fluctuation of the flying moth density as shown in Fig. 2 and the temperature data. The estimated percentage of mating reduction was nearly 100% from January to early March except for mid January (50%), less than 20% from late March to early April, more than 90% in mid and late April, and nearly 0% at the peak of the moth density in May and June.

DBM is not uniformly distributed in the houses, and high adult density is often found at the fringe of the houses, where rather high rates of mating is assumed to occur, even if average adult density in each of the houses is very low.

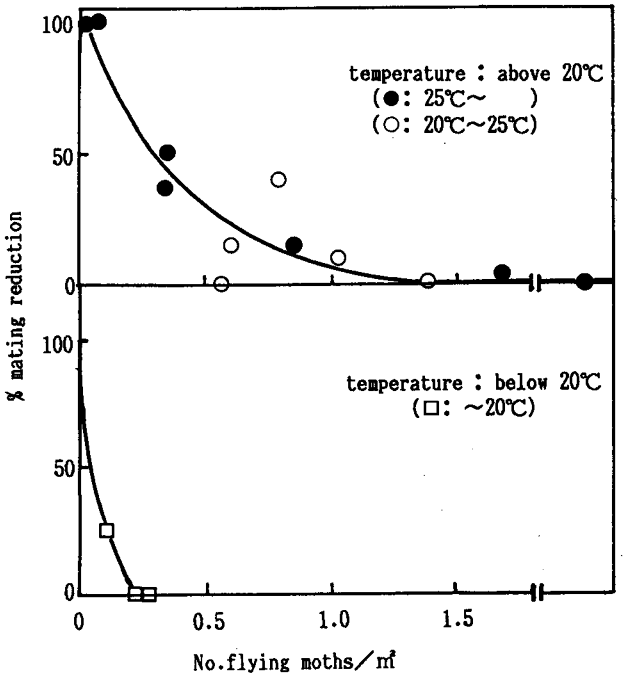


Fig. 4.
Relationship between density of flying moth and percentage of mating reduction by pheromone dispenser in DBM on watercress (Tanaka and Kimura 1990).

## Vacuum suction

The flying moth density before the start of the test was $1.78/m^2$ and it decreased to $0.91/m^2$ after the first operation which sucked 11.07 individuals/$m^2$ (Fig. 5). The density reduced by about one-half in each operation. The peak of the flying moth density in the houses in summer was $1.0\text{-}10.0/m^2$ (Fig. 2) and 4-7 operations can reduce the density to less than $0.1/m^2$, the level at which the pheromone dispenser effectively reduces the rate of mating.

Since it took 15 min for one operation in one-quarter of a greenhouse in this test, it would take 4-7 hours in a house to reduce the moth density to a level at which the pheromone dispenser is effective. The vacuum cleaner itself was too heavy to be carried on the back for long periods. If the vacuum cleaner is mounted on a rail, for example, and if it takes 20 min for every operation in a house (one-third of the time now required for it), control of DBM using the vacuum cleaner might be practical.

The relation between the accumulated number of eliminated moths ($N_e$) and the flying moth density ($N_f$: number of flying moths/$m^2$) is expressed as a regression line.

$$N_f = -0.083N_e + 1.792 \ (r = -0.9995).$$

The X-intercept of the regression line means the point at which all of the moths are eliminated by the vacuum cleaner, its value (= 21.59) also means the actual number of moths/$m^2$ at the start of the test. The ratio of the X-intercept to the Y-intercept is 12.05. This is the ratio of the actual moths density to the flying moth density at the start of the test. So the relation between the flying moth density and the percentage of mating reduction as shown in Fig. 4 can be substituted by another relation, using the actual moth density. A high percentage (more than 90%) of mating reduction then occurs in the actual moth density of less than $1.0/m^2$.

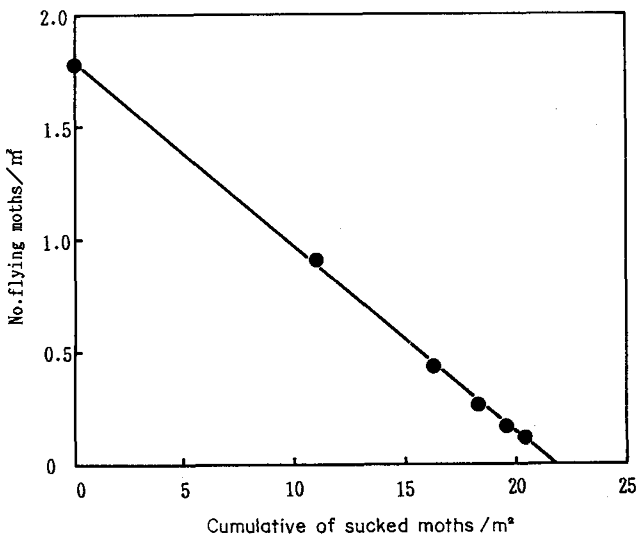


Fig. 5.
Relationship between cumulative number of DBM moths sucked by vacuum cleaner and density of flying moths on watercress.

## Conclusions

Growing cruciferous vegetables throughout the year in greenhouses with only chemical control is assumed to create resistance to all insecticides in DBM. So we must seek to develop some physical, biological and cultural control methods, as well as chemical ones, and to estimate the efficiency of each. I investigated the population dynamics and the spatial distribution of DBM in greenhouses of watercress, and estimated the efficiency of three kinds of control methods. The control efficiency of the methods was not satisfactory, though the pheromone dispenser proved to be most effective in situations of low moth density and the vacuum cleaner in situations of high moth density. It is therefore most promising to combine the pheromone dispenser and vacuum cleaner. There is certainly room for improvement in the efficiency of the vacuum cleaner, and the combination may overcome the DBM problem in the greenhouses in the near future. It is important to consider the cost of the control methods. The dispenser-sucker combination is not too costly in the cultivation of watercress, so it could possibly be adopted. Nakahara et al. (1986) reported that DBM in watercress in Hawaii was controlled by use of an intermittent overhead sprinkler system, which disturbed the mating and egg-laying activities of the moths. However the system was not adopted for watercress in Osaka because the sprinkler spraying would dilute the nutrient concentration in hyrdoponics.

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