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Urea Application on Tobacco Stumps for the Control of Tobacco Mosaic Virus Infection

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담배 잔근의 요소처리에 의한 담배 모자이크 바이러스 방제

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ABSTRACT : Tobacco stalks were cut and removed from the field after harvest, and urea was treated by placing it on the cutting portions of the remaining tobacco stumps. Relative virus infectivity of the root residue(compared to the fresh root residue infected with TMV) was reduced to 14.6% in December, 1993(before overwintering) and to 8.5% in March, 1994 just before transplanting, indicating that the TMV infectivity decreased remarkably, but was preserved still in the root residue in the field soil. There was no significant difference in infectivity of remaining root tissue between the treated and untreated root residue. However, as roots with urea treatment had been extensively decayed, only about one-fifth of the initial root volume remained after overwintering. TMV occurred less(by one-third) in the urea treatment than in the control, suggesting that urea treatment effectively prevented tobacco from TMV infection by reducing the inoculum potential.

Tobacco mosaic virus(TMV) is the most important disease of tobacco in Korea. It causes great economic losses, especially in flue-cured tobacco, by reduction in both yield and quality. In recent years(from 1988 to 1991), nation-wide disease incidences have ranged from 2.1% to 11.5% in average(8), but in some tobacco fields, particularly with the previous TMV infection, are damaged to near-devastation by the virus.

It is well known that TMV is very easily transmitted through contact. The virus can be transmitted via wounds made by contact with tools, and workers' hands or clothes that carry the sap of previously infected plants(5). Also TMV in plant debris may remain infectious in soil, and be mechanically introduced into plants through wounds(1).

Probably it is one of the best ways in controlling TMV to eliminate or inactivate virus inocula before

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starting cultural practices, keeping the crop TMV - free. Bed soils are fumigated by a fumigant to kill weed, and thus viruses in the weed are inactivated (7). But fumigation in most cases is inefficient and impractical in field conditions. In fields, roguing out infected plants or plant residue prior to the first cultural practice is an effective way in preventing initial TMV infection. Nevertheless, in most fields with catch crops, as in Korea, tobacco stumps are not plowed out until the next spring so that enough initial inoculum sources still remain available. Therefore, this study was designed to develop a control measure to reduce the TMV inoculum source. Tobacco stumps were treated with urea for the rapid decomposition of the tobacco root debris, by which TMV loses its infectivity.

MATERIALS AND METHODS

Experimental field and urea treatment. We selected a 0.2 - ha field located in Doan - Myun, Goesan - Kun, Chungbuk where TMV had occurred about 93% at the harvest time of 1993, showing mosaic symptoms. After the completion of harvest in August, the remaining plant stalks were cut and 3 - 4 g of urea was placed on top(became concave after the cut surface dried)of the stumps in the half of the field. The other half of the field was left without the urea treatment for the untreated control.

Infectivity of plant root residue. Plant roots with TMV symptoms on the leaves were sampled at the time of the urea treatment, and freeze - dried. They were stored at - 70℃, and used later as the standard inoculum for local lesion assays. Root samples both treated or untreated were also collected and freeze - dried in mid December of 1993 and early March of 1994. Three root samples were used for each assay. The root samples were ground in 0.05 M phosphate buffer(pH 7.2) to prepare inocula for the local - lesion assays with tobacco (*Nicotiana tabacum* 'Xanthi - nc') plants. The tobacco leaves were inoculated with the preparations by dusting 500 - mesh carborundum, using cotton swabs. For each assay, one half of the leaf was inoculated by a sample preparation and the other half by the standard preparation as the control check. Five to six leaves were used for each assay. Inoculated plants were placed in a greenhouse at 23 - 27℃. Five days after inoculation, local lesions produced on a half leaf were counted, and its relative infectivity was measured by comparing the number of local lesions on the half leaf with that of the standard inoculum.

Field experiment. Tobacco(*N. tabacum* cv. NC 82) seeds were planted in steam sterilized soil composed of a 1 : 1 mixture of sand and organic compost on February 17, 1994. On April 8, tobacco seedlings were transplanted into the field. They were dipped in milk suspension to prevent accidental infection while handling. Each row was 120 cm apart, and mulched with polyethylene film after transplanting. A total of 29 rows, 13 for the urea - treatment plot, 13 for the untreated and 3 border rows in between, were planted with tobacco seedlings spaced 30cm in the row. TMV occurrence in the field was examined by the visual mosaic symptoms on April 27, May 7, May 19, May 30, and June 14, 1994.

RESULTS

Infectivity of plant root residue. Four to five days after the inoculation with the infected root tissue preparations, necrotic local lesions appeared on the inoculated 'Xanthi - nc' leaves. Regardless of the urea treatment, the number of local lesions formed by the inoculation of the root residue left in the field for months was much less than that by the standard inoculum. The relative infectivities of the urea treated and untreated root samples were 12.6% and 14.6 % in December, 1993(before overwintering), and 6.9% and 8.5% in March, 1994(after overwintering and just before transplanting) respectively (Fig. 1).

There was no significant difference in infectivity between the samples treated and untreated with urea. However, the urea treated root residue was decayed so extensively that only a small portion remained, while in the untreated root remained with most of the main root system intact(Fig. 2). The remaining portion of the treated root was approxima-

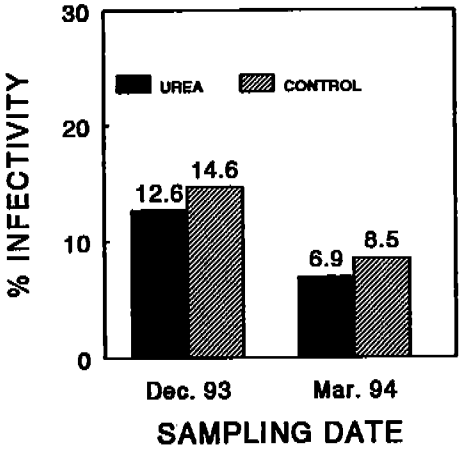

Fig. 1. Infectivity of tobacco root residue with or without urea treatment sampled before (Dec, 1993) and after(Mar. 1994) overwintering as evaluated by the percentage of local lesions produced on tobacco leaves relative to that of infected fresh root residue sampled in Aug., 1993.

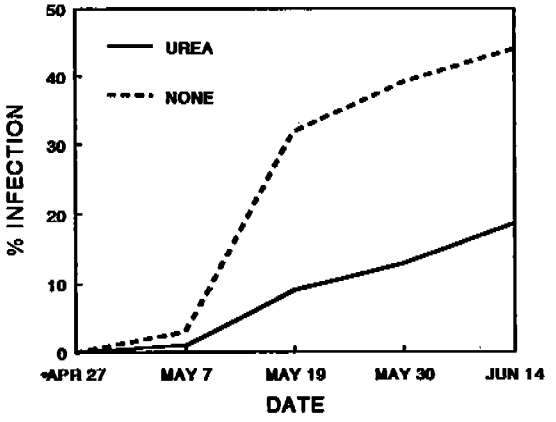

Fig. 3. Incidence of TMV during the growing season of 1994 in tobacco cv. NC 82 in plots in which the previous plant root residue was treated with(urea) or without(control) urea in Aug., 1993. TMV infection was determined by mosaic symptoms on tobacco leaves. Percentages of infection were the relative numbers of infected plants out of 2,865(urea) and 2,787(control)plants.

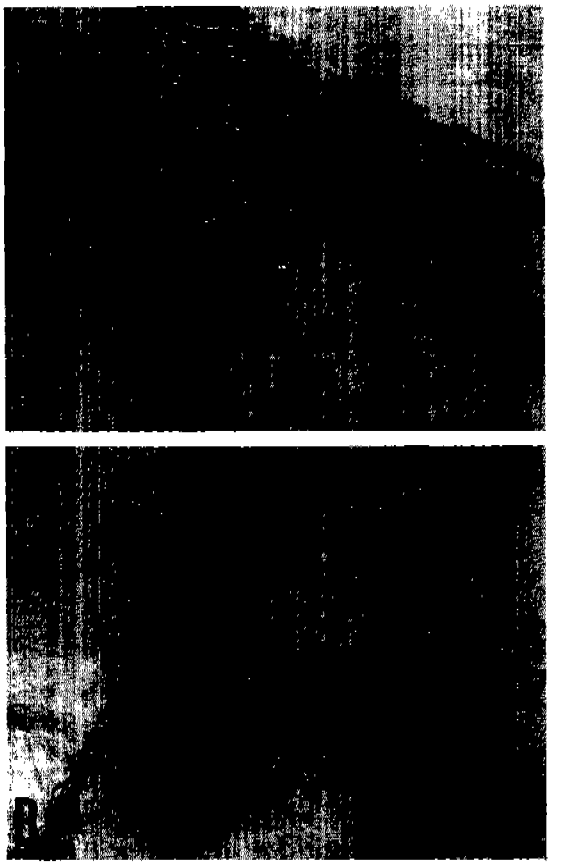

Fig. 2. Plant remnants of the previous tobacco plants showing near-intact root residue(A) from the control plot and severely decayed root residue(B) sampled in the urea-treated plot.

tely one-fifth of the untreated one. Therefore, combining the relative viral infectivity and reduction of the root mass by urea treatment, the viral infectivity of the overwintered roots can be speculated to be decreased 50 times more than that of the fresh roots of the tobacco plants infected with TMV.

Field experiment. No visual symptoms were noted on the tobacco seedlings at the time of transplanting. A month after transplanting(May 9), TMV occurrence was 1.1% in the urea treated plot and 3.1% in the untreated(control) plot. In both plots, the disease occurrence increased thereafter(Fig. 3). Ten days later(May 19), the disease incidence was inc-

Table 1. Disease severity of TMV in tobacco cv. NC 82 in plots with or without urea treatment on the previous plant root residue

Treatment[1]	No. of plants observed	% no. of plants for disease severity index[2]				Average index
		0	1	2	3	
Urea	2865	80.9	4.9	5.2	9.0	0.41
Control	2878	55.9	4.0	7.5	32.6	1.16

[1] Urea was treated 3 - 4 g per plant on root residue in August, 1993.

[2] Disease severity index ; 0, no mosaic symptom ; 1, mosaic symptoms on 1 or 2 upper leaves ; 2, mosaic symptoms on 3 - 10 leaves ; and 3, mosaic symptoms on 11 or more leaves. Disease severity was examined on June 14, 1994(2 months after transplanting).

reased remarkably, making the difference between the two plots become much greater(9.2% in the urea treated plot and 32.0% in the control plot). The disease progression in the two plots was kept at the same rate through the end of May when the disease incidence was about 3 times higher in the untreated control plot than the treated plot. In June 14, the disease incidence was 18.7 in the treated plot, and 44.1 in the control plot.

The disease severity estimated 2 months after transplanting was also significantly different between the treated plot and the control plot(Table 1). In the treated plot, 80.9% of the total 2,865 tobacco plants had no visual mosaic symptoms on leaves, and 9.0% were graded as severely diseased. In the control plot, however, 55.9% of 2,787 plants had no visual virus symptoms, and 32.6% were severely diseased.

DISCUSSION

TMV infection occurs mostly by the contact with farmers' tools and hands during various cultural practices such as raising and transplanting seedlings and weeding. Milk treatment has been recommended to prevent such mechanical spread of TMV(4). Dipping seedlings and workers' hands in milk during transplanting efficiently protects plants from TMV infection. Also some surfactants such as α - olefin, linear alkyl benzene, dioctyl sulfosuccinate and dodecyl benzene sulfonic acid were proved to be excellent protectants against TMV(6). However, milk and such surfactants are little effective in controlling soil - borne TMV transmission.

Initial soil - borne TMV infection occurs during transplanting when plants are inserted into soil and exposed to infected plant debris. When healthy tobacco plants susceptible to TMV are transplanted into a field in which the previous crops were mostly infected, approximately 0.1 - 5% of the plants become infected and show symptoms 3 - 4 weeks after transplanting(2). In our study, 3.1% of the plants were initially infected with TMV, probaly by the soil - borne manner. Under field conditions, TMV can survive in roots of infected plants until the succeeding crop is planted(2). TMV may survive as long as 2 years in the soil or in large fragments of stalks and/or roots unless they are not completely rotted. On the other hand, TMV may be inactivated when the plant remmants are fully exposed to weathering and decay for 5 or 6 months(5). Based on our experiment, TMV in root residue rapidly lost its infectivity or the viral concentration decreased to a great extent during the period between the harvest time and the next cropping time under the field conditions. Nevertheless, TMV in the root residue was not completely inactivated, but still infectious, preserving approximately 10 % inoculum potential as that of fresh roots of the infected plants. This may be sufficient inoculum source for the succeeding crop. Destruction or decay of the root debris may not be compete under the field conditions. Therefore, elimination of the primary inoculum source is crucial for the control of TMV in fields.

It has been suggested that crop residue should be removed by cutting the stalks and plowing out

the root system soon after harvest to eliminate inoculum sources. There were significant differences in TMV occurrence between the fields whose plant remnants were removed and the fields otherwise (Park and Kim, unpublished). In Korea, however, this cultural practice is not made in many fields, especially where a catch crop grows. In 1994, in about 75% of the tobacco farms in a province, tobacco root residue was not removed from fields(9).

Gooding and Lucas(3) indicated that herbicides could accelerate root destruction, and reduce the amount of overwintering TMV. Herbicides cannot be applied in fields with catch crops due to their phytotoxicity. In our experiment, urea treatment was effective in accelerating root rotting which subsequently reduced the inoculum potential. Plant tissues come in contact with urea were promptly killed, and thereby rotting of the stumps seemed to have been proceeded with ease. Urea treatment is relatively simple and economic, and gives no significant phytotoxic harms to the plants, It might be somewhat laborious to treat urea plant by plant in a large farm, but it is a recommendable way of TMV control for small - scale farms and in tobacco fields with catch crops. The effect of the urea application in terms of soil fertilization will be studied.

요 약

담배 수확후 담배 모자이크 바이러스에 감염된 식물체의 줄기를 절단하고 절단한 그루터기 표면에 요소를 주당 3 - 4g 처리하였다. 담배 잔근의 바이러스 감염율은 처리시기인 1993년 8월에 비해 월동전 12월에는 14.6%, 이듬해 담배를 심기 전 3월에는 8.5%로 나타나 전염력이 현저히 감소하였으나, 여전히 전염력이 유지되었다. 바이러스의 불활성화에 있어서 요소처리와 무처리간에는 유의성 있는 차이가 없었으나, 요소처리에 의해 담배 뿌리가 심하게 부패하여 무처리 담배 뿌리의 1/5 정도만 남아 있었다. 요소를 처리한 포장에서의 담배 모자이크 바이러스 발생율은 무처리에 비해 약 1/3정도로 낮아, 요소처리가 바이러스의 전염을 차단하는데 효과가 있는 것으로 나타났다.

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