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Plant Virus Transmission-Tests Using Electron Beam Irradiated Green Peach Aphids, *Myzus persicae* (Sulzer) (Homoptera : Aphididae)

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Abstract: The potentiality of insect-borne plant virus infection by the sterile green peach aphid, *Myzus persicae* irradiated at 400 Gy was studied to determine the efficacy of electron beam irradiation as a quarantine treatment for cut flowers infested with aphids. The two model tests of plant virus transmission with persistent and non-persistent manners by irradiated aphids were performed on *Potato leafroll virus* (PLRV) and *Cucumber mosaic virus* (CMV). The aphids irradiated at 400 Gy one day after irradiation transmitted PLRV, but did not transmit CMV.
Key words: radiation, *Myzus persicae*, sterility, plant virus vector, PLRV, CMV

Introduction

Import cut flowers including cut foliage to Japan have been increasing year by year. The amount of them was about 360 million stalks in 1989 soared up to about 1,540 million stalks in 1999 and the increase ratio is more than 4 times as many in past one decade. According to the inspection statistics, 10-25% of the total stalks were disinfested because of infestation of quarantine pests in the past several years and were subjected to methyl bromide or hydrogen cyanide fumigation (Yokohama Plant Protection Station, MAFF, 1990, 1996-2000).

As methyl bromide was regarded as an ozone depleting substance in the 4th Meeting of Montreal Protocol in November 1992, it is urgently required to develop other alternatives to methyl bromide for disinfestation of quarantine pests. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has sponsored an international project, "The FAO/IAEA Coordinated Research Programme on Irradiation as a Quarantine Treatment of Mites, Nematodes and Insects Other Than Fruit Flies" between 1992 and 1997 in order to accumulate data on the effects of irradiation on pests and host commodities because there is little data to demonstrate the efficacy of irradiation against a range of other arthropod pests of quarantine importance but tephritid fruit flies and codling moth (IAEA, 1999).

In view of current situation on irradiation as a quarantine treatment as mentioned above, we have studied the efficacy of electron beam irradiation for cut flowers. However, irradiation dose without deterioration of the cut flowers cannot provide 100% mortality and can sterilize the pests (DOHINO *et al.*, 1998a; TANABE and DOHINO, 1993, 1995). In our

previous study conducted with the green peach aphid, *Myzus persicae* irradiated at 200-600 Gy, sterilized aphids could still feed their host plants and even the maximum dose, 600 Gy could not prohibit their feeding immediately after irradiation although the feeding behavior was depressed by the irradiation (DOHINO *et al.*, 1998b). In addition, PHATAK *et al.* (1994) reported plant viruses were much tolerant to irradiation. These stimulated us if irradiated and sterile aphids transmit plant viruses to their host plants.

There are two transmission manners of insect-borne plant virus; persistent and non-persistent transmission manners. We studied whether irradiated aphids will be able to transmit plant viruses through the two model tests of virus transmission with *Potato leafroll virus* (PLRV) and *Cucumber mosaic virus* (CMV) for persistent and non-persistent transmission manners, respectively, which are also important plant viruses in many countries.

Materials and Methods

1. Test insects and irradiation

Test insects were used from a laboratory colony of *M. persicae* and this colony founded with aphids obtained from Yokohama Research Center, Mitsubishi Chemical Corporation. Aphids were irradiated with an electron beam accelerator (Nissin High Voltage Co. Ltd., 2.5 MeV, 1.5×10^6 Gy/hr) at National Food Research Institute (Tsukuba, Ibaraki, Japan) according to our previous study (DOHINO *et al.*, 1998b). Absorbed dose 400 Gy was selected in the following insect-borne virus infection tests by irradiated aphids because our previous studies found that a dose of 400 Gy sterilized these pests in a lump; spider mite, *Tetranychus urticae*, mealybug, *Pseudococcus comstocki*, leaf miner, *Liriomyza triforii*, thrips, *Thrips palmi* and *T. tabaci*, and tobacco cutworm, *Spodoptera litura*, green peach aphid, *M. persicae* (DOHINO *et al.*, 1998a). Furthermore, the dose did not cause severe injury for many kinds of flowers (TANABE and DOHINO, 1993, 1995; TANABE *et al.*, 1994). Even if 400 Gy-irradiation induces injury to cut flowers, *e.g.* floral preservative solution and aqueous solution of sucrose, glucose, fructose or maltose are effective in preventing deterioration of irradiated chrysanthemum which is extremely sensitive to radiation (KIKUCHI *et al.*, 1995; HAYASHI and DOHINO, 1995; HAYASHI *et al.*, 1998).

2. Plant virus transmission tests using irradiated aphids

The flow charts of plant virus transmission tests using aphids with persistent and non-persistent viruses are shown in Fig. 1 and Fig. 2, respectively. Twelve plants were tested for a replication of treatment and control in both tests and the tests were replicated 3 times. All experiments were done in 1996-1997.

a) Test of persistent transmission in combination with *M. persicae* and PLRV

PLRV infected potatoes (cv. 'Waseshiro' and 'Nohrin Ichigou') were obtained from Tsumagoi Station, National Center for Seeds and Seedlings (Tsumagoi, Gunma, Japan). Aphids were released on the potted PLRV-infected potato plants in a cage ($29\times44\times60$ cm) covered with Bemberg® nets under 25 ± 2℃, 60-80% RH and 16L:8D, thus being al-

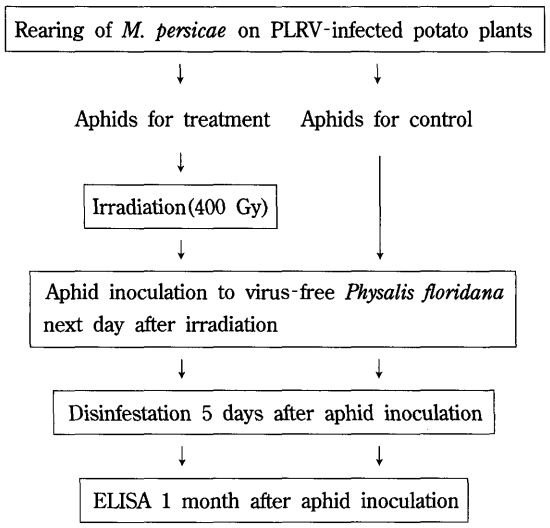

Fig. 1. Flow chart of plant virus transmission-test with persistent virus (PLRV)

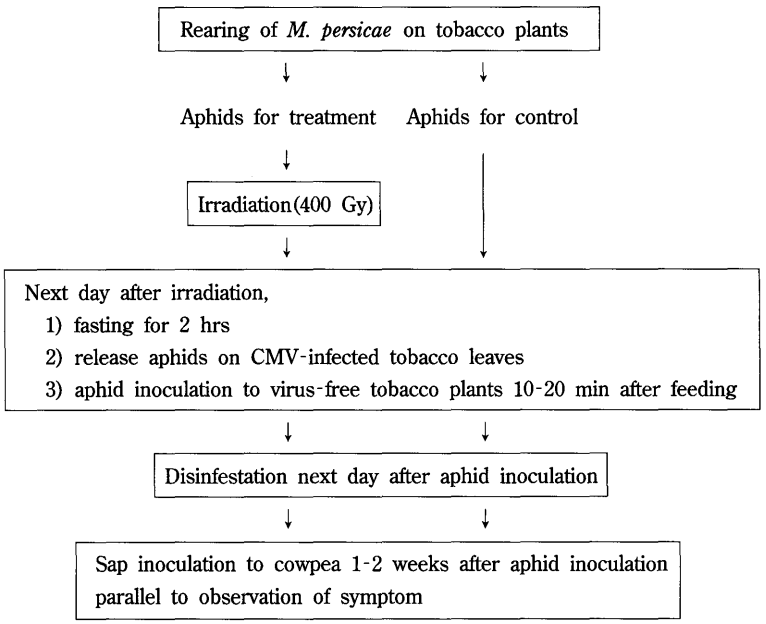

Fig. 2. Flow chart of plant virus transmission-test with non-persistent virus (CMV)

lowed to acquire PLRV in 10-11 days on host plants.

The potato stem's buds or leaves, which were infested with aphids, were cut. The base was then covered with moistened cotton and aluminum foil, and later placed in a polyethylene cup (4 cm in height, 10 cm in diameter). The infested potato buds or leaves in the cup were irradiated at 400 Gy. After irradiation, the cups with their aphids were held in the environmental chamber set at 25℃ and 16L: 8D until aphid inoculation test.

On the next day, 5 irradiated aphids were transferred to a virus free indicator plant, *Physalis floridana* by fine brush, and the 6 inoculated plants were placed in a cage with Bemberg® nets. Similarly, 5 unirradiated aphids per *P. floridana* plant were used for control and the 6 plants were placed in another cage. Plants were held in an environmentally-controlled room set at 25℃, 70% RH and 16L: 8D. The aphids were killed in spraying of insecticide (acephate: orthene) 5 days after inoculation. One month after inoculation, confirmation of PLRV infection was carried out by enzyme-linked immunosorbent assay (ELISA).

b) Test of non-persistent transmission in combination with *M. persicae* and CMV

Under the same condition as well as the persistent transmission test, aphids were reared on potted tobacco plants (cv. 'White Burley') in a cage (29×44×30 cm).

The tobacco leaf infested with the aphids was cut. The base was then covered with moistened cotton and aluminum foil and later placed in a polyethylene cup. The aphids on tobacco leaf in the cup were irradiated at 400 Gy. After irradiation, the aphids were kept in the same manner as the persistent transmission test until aphid inoculation test.

On the next day, the aphids were moved from the leaves into a 100 m*l* beaker in order to fast for 2 hours and the beaker was covered with Parafilm 'M'® to prevent aphids from escaping. After abstinence from food, these aphids were released on CMV-O strain infected tobacco leaves and allowed to feed for 10-20 min. Five irradiated aphids were transferred to a virus free tobacco plant and 6 thus inoculated plants were placed in a cage. Similarly, 5 unirradiated aphids per a tobacco plant were used for control and the 6 plants were placed in another cage. Plants were held in an environmentally-controlled room as mentioned above. The aphids were killed in spraying of insecticide the next day after inoculation.

The confirmation of CMV infection was carried out by sap inoculation to *Vigna sinensis* 1-2 weeks after the aphid inoculation in addition to the observation of symptoms on tobacco leaves.

Results and Discussion

a) Persistent transmission test

ELISA result indicated that both unirradiated and irradiated aphids transmitted PLRV to all tested *P. floridana* plants (Table 1), although the plants did not develop typical symptoms of PLRV, *e.g.* stunt, interveinal chlorosis, leafroll (HARRISON, 1984). The aphids kept the transmission ability of PLRV one day after irradiation at 400 Gy. However, it is sug-

Table 1. Effect of electron beam irradiation on PLRV transmission of *M. persicae*[1]

Absorbed dose	No. of aphids inoculated/a plant	Transmission frequency[2]				Transmission
		Rep 1	Rep 2	Rep 3	Total	
0 Gy	5	12/12	12/12	12/12	36/36	100%
400 Gy	5	12/12	12/12	12/12	36/36	100%

[1] ELISA was conducted one month after aphid inoculation to confirm PLRV transmission.
[2] Number of test plants infested with PLRV / number of test plants.

Table 2. Effect of electron beam irradiation on CMV transmission of *M. persicae*[1]

Absorbed dose	No. of aphids inoculated/a plant	Transmission frequency[2]				Transmission
		Rep 1	Rep 2	Rep 3	Total	
0 Gy	5	1/12	3/12	2/12	6/36	16.7%
400 Gy	5	0/12	0/12	0/12	0/36	0%

[1] The confirmation of CMV transmission was conducted by sap inoculation to cowpea 1-2 weeks after aphid inoculation parallel to observation of symptoms on tobacco leaves.
[2] Number of test plants infected with CMV / number of test plants.

gested that the possibility of plant virus transmission by irradiated aphids decreased with the lapse of time after irradiation since the number of honeydew droplets excreted by irradiated aphids decreased with time (DOHINO *et al.*, 1998b).

b) Non-persistent transmission test

Unirradiated aphids transmitted CMV to 6 of 36 tobacco plants while the irradiated aphids did not (Table 2). NAKAZAWA (1972) reported that the percentage of CMV transmission depended on the number of aphids used and that the percentage of actual transmission in his experiments coincided reasonably well with the percentage of expected transmission calculated from the following formula (SYLVESTER, 1955) ; $P=1-Q^X$ (P ; probability of obtaining an infection, Q ; probability of not obtaining, X ; number of insects used). Relying on the formula, 45.2% was expected for transmission when the experiment was conducted with 5 aphids. However, in our experiment, 16.7% was obtained for actual transmission. So it cannot be concluded that the irradiated aphids would not transmit CMV one day after irradiation, because the value of actual transmission in our control was quite small comparing to the expected value. It cannot be denied that the irradiated aphids might transmit CMV one day after irradiation since there was no significant difference in the feeding one day after irradiation between control group and 400 Gy-irradiated group (DOHINO *et al.*, 1998b).

The result of these tests indicated the potentialities that sterile aphids by irradiation will work as plant virus vectors. Further studies as comparison of the locomotor activity between control aphids and irradiated aphids, will be needed to determine the risk of irra-

diated aphids as plant virus vectors.

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和 文 摘 要

電子線照射されたモモアカアブラムシ *Myzus persicae* (Sulzer) (Homoptera: Aphididae)は植物ウイルスのベクターになるか?

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植物検疫くん蒸剤として主に用いられている臭化メチルがオゾン層破壊物質に指定され，その全廃に向けて世界的な規制がかかっている。そのため，ガスくん蒸に代わる輸入切花の消毒方法を開発することを目的として，加速器から発生させた電子線をハダニ，アザミウマ，カイガラムシ，アブラムシ等の切花付着害虫に照射して，殺虫・不妊化データを蓄積してきた。

モモアカアブラムシの場合，他の害虫と同様，吸収線量400Gy の照射で不妊化されたが,その摂食行動は照射により抑制されたものの，完全に吸汁を阻止することはできなかった(土肥野ら,1998b)。このことは,400Gy で照射されたモモアカアブラムシは植物ウイルスのベクターになり得ることを示唆した。

そのため，植物ウイルスのアブラムシによる伝搬様式として知られる「永続型伝搬」と「非永続型伝搬」の2ケースについて,代表的な植物ウイルスを,前者ではジャガイモ葉巻ウイルス(PLRV)を,後者ではキュウリモザイクウイルス(CMV)を選定し,これらのウイルスが400Gy で照射されたモモアカアブラムシによって伝搬されるか否かの試験を実施した。

その結果，対照区とほぼ同様の吸汁活動を示した400Gy 照射後1日目のモモアカアブラムシは，永続伝搬する PLRV を100%伝搬したが，CMV については伝搬しなかった。前回報告（土肥野ら，1998b）において，照射後日数を経るにつれ，モモアカアブラムシの吸汁活動は急激に抑制されたことを報告した。照射されたモモアカアブラムシが植物ウイルスのベクターになるか否かについては,照射された害虫のその後の移動性も含めて検討する必要があると思われた。