Integrated Management of Sudden Wilt in Melons, Caused by *Monosporascus cannonballus*, Using Grafting and Reduced Rates of Methyl Bromide

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ABSTRACT

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Sudden wilt of melons induced by *Monosporascus cannonballus* is a worldwide problem, mainly in arid and semi-arid regions. Soil disinfection by fumigation with methyl bromide is a common and very effective treatment for disease control. However, methyl bromide is expected to be banned from use within the next 5 to 10 years; therefore, alternative measures for disease management are urgently needed. In this study, the efficacy of methyl bromide at a reduced rate (15 g/m² instead of 50 g/m²) and grafted plants, alone or in combination, was examined. Experiments were conducted in the 'Arava region in southern Israel in naturally infested fields. First, various rootstock accessions were tested in three experiments. Of the eight rootstocks evaluated, cv. Brava (*Cucurbita maxima*) was selected to serve as a rootstock in the subsequent experiments due to its effects on wilt reduction (63 to 100% reduction in two experiments and 35% reduction in one experiment) and its horticultural performance. Next, integration of grafting and a reduced rate of methyl bromide was tested in two experiments. The reduced methyl bromide rate suppressed disease, but results were variable (32 and 91% reduction in the two experiments). Grafting, when used alone, significantly reduced wilt incidence by 84 to 87% compared to untreated plots. Integration of the two control measures provided adequate and reliable results and wilt incidence was always significantly lower (75 to 100% reduction) than incidence in untreated plots. Because control by each measure alone varies, integrated application is preferable.

Additional keywords: fumigation, grafting, integrated pest management

Sudden wilt of melons (*Cucumis melo*), also known as melon collapse or vine decline, is a worldwide problem, mainly in arid and semi-arid regions (12). This disease is a major problem in the arid 'Arava region of southern Israel, which is the main out-of-season melon production area for the local market and for export. The sudden wilt syndrome is characterized by rapid wilt of plants bearing mature fruit and may result in total loss of the crop. Field trials and inoculation experiments conducted by Reuveni et al. in the early 1980s (15) showed that a *Monosporascus* sp., identified probably by mistake as *M. eutypoides*, was a primary agent of melon collapse in the Jordan Valley. Pathogenic-

ity tests performed in 1995 and 1996 (14) suggested that the most aggressive fungus involved in the sudden wilt syndrome of melons in the 'Arava Valley of southern Israel is *M. cannonballus* (1). *M. cannonballus* was reported to induce melon sudden wilt also in the southern United States, Central America, Japan, Tunisia, Saudi Arabia, and Spain (2,6,11,12,17). Soil disinfection by fumigation with methyl bromide is the common treatment used in the 'Arava to control this disease (18). However, methyl bromide is expected to be banned from use worldwide within the next 5 to 10 years (16), yet growing melons without methyl bromide is impossible. Therefore, alternative measures for disease management are urgently needed.

Melons and watermelons grafted on *Cucurbita* spp. rootstocks to combat diseases are common in various regions in the Mediterranean Basin and in southeastern Asia (9,10). Grafted cucurbits are used mainly to overcome root diseases caused by *Fusarium* spp. (10). We found no data on the response of *Cucurbita* spp. used commercially as rootstocks to prevent *M. cannonballus* damage. Grafting is currently not used in Israel commercially, probably due to the current availability and high

efficiency of methyl bromide. It is possible that grafted plants alone or integration of grafting with reduced rates of methyl bromide may suppress sudden wilt as effectively as a full rate of methyl bromide. Reduced rates of methyl bromide will be allowed in certain places during the phase-out period (16). In this study, various *Cucurbita* spp. were screened as a potential source of rootstock for grafting, and the efficacy of grafting and pre-plant fumigation of the soil with methyl bromide at reduced rates, solely and in combination, were studied.

MATERIALS AND METHODS

Grafting melon plants on *Cucurbita* sp. rootstocks. Seeds of melon (scion) and *Cucurbita* sp. (rootstocks; Table 1) were sown in seedlings trays (Polyvid, Mishmar HaNegev, Israel, 37 mm/cell, 128 cells/tray) filled with a 1:1 (vol/vol) mixture of peat and vermiculite. The plants were grown in an environment-controlled greenhouse at 25 and 20°C day and night, respectively. Plants were grafted at the two-leaf stage. True leaves of the rootstocks were removed with a razor blade, creating a "V"-shaped cut between the rootstock's cotyledons, and an inverse "V"-shaped cut was made on the stem of the scion, approximately 2 cm below the cotyledons, to fit the cut in the rootstock. Scion and rootstock were held with a grafting clip (Sakata Seed Corp. Yokohama, Japan). Grafted plants were transferred to a mist chamber (>95% relative humidity) for 8 days, after which the relative humidity was reduced gradually for acclimatization.

Evaluation of rootstock response to sudden wilt. The response of eight rootstocks belonging to different species of *Cucurbita* (Table 1) to sudden wilt were evaluated in three field experiments, conducted in fields naturally infested with *M. cannonballus* (14). Of the eight rootstocks evaluated, only three ('TZ-148', 'Brava,' and 'Tetsukabuto') were tested in all three field experiments; the others were tested only once due to a limited number of seeds. The first two experiments were carried out in the autumn 1995, one at the Zohar Experiment Station at 'En Tamar (northern 'Arava) and the other in an organic field at 'En Yahav (Central 'Arava). Susceptible melon plants (cv. 'Arava,

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grafted and non-grafted) were transplanted on 12 September. Intra-row spacing was 50 cm and beds were 190 cm apart. Each experimental plot was 3.5 m long and consisted of seven transplants. There were four replicates per treatment (rootstocks) and the experiments were laid out in a randomized complete block design. The third experiment was conducted at 'En Yahav in the spring cropping season of 1996, on the same plot used for the autumn experiment. Melon plants were transplanted on 27 January. Plant spacing and the number of transplants per experimental plot were the same as for the autumn experiment, but there were only two replicate plots for each of the tested rootstocks.

Integrated management of sudden wilt. The efficacy of grafting and pre-plant fumigation with methyl bromide in suppression of sudden wilt was tested in two field experiments conducted at the Zohar Experiment Station. The first experiment was carried out in the 1997 spring cropping season (transplanted 23 January and harvested 26 April to 11 May). The second experiment was conducted in the 1997 autumn cropping season (transplanted 15 September and harvested 4 to 21 November). Cv. 'Arava, which is highly susceptible to *M. cannonballus*, was used in each experiment. The spring experiment consisted of a factorial design with two main factors, (i) grafting (cv. 'Arava scions were either not grafted or were grafted on cv. Brava rootstock) and (ii) pre-planting treatment with methyl bromide. The second factor consisted of four levels: (i) untreated, (ii) methyl bromide which had been applied at a rate of 50 g/m² before the previous season (before the autumn cropping of 1996), (iii) methyl bromide applied at a reduced rate (15 g/m²), and (iv) methyl bromide applied at the full rate (50 g/m²). The autumn experiment consisted of a factorial design with two main factors: (i) grafting cv. 'Arava scions were either not grafted or were grafted on cv. Brava rootstock and (ii) pre-planting treatment with methyl bromide. The second factor consisted of three levels: (i) untreated, (ii) methyl bromide applied at a reduced rate

(15 g/m²), and (iii) methyl bromide applied at the full rate (50 g/m²).

Methyl bromide was applied via drip irrigation tubes, which were placed on beds prior to covering the beds with plastic sheets (Polyon, Barqay, Israel). Methyl bromide (Dead Sea Bromine Group, Be'er Sheva', Israel) was applied through the irrigation tubes at the desired rate (15 or 50 g/m²) employing the hot gas method (8).

Disease assessments and yield quality measurements. All experiments were conducted in fields with a history of sudden wilt caused by *M. cannonballus* (14). Wilt was evaluated visually in each experiment at harvest. A plant was considered dead when the whole plant appeared irreversibly wilted. Involvement of *M. cannonballus* in wilting was confirmed by visual evaluation of perithecial development on wilted plant root samples after 3 weeks of incubation in paper bags at room temperature. The number of wilted plants and the total number of plants per plot were used to calculate the incidence of wilting (percent). Fruit were harvested twice a week upon ripening; there were four to six harvests in the different experiments. The fruit in each plot were counted and weighed. Total soluble solids (TSS, an indicator of fruit sugar content) was evaluated from a sample of five fruit per plot using an Atago digital refractometer. Samples (approximately 20 g) were randomly taken from each fruit, manually squeezed, and the drops of juice were placed on the refractometer for analysis. Results were analyzed statistically by appropriate (one- or two-way) analysis of variance (ANOVA) tests. Data for disease incidence were transformed using arcsin function prior to analyses. When the *F* values were significant at *p* > 0.05, differences among the treatments were determined by Fisher's protected least significant difference (LSD) test.

RESULTS

Evaluation of rootstock response to sudden wilt. Wilt intensity in all three experiments was high and 89 to 100% of the non-grafted cv. 'Arava plants wilted by

the end of the season (Table 1). The three rootstocks tested exhibited similar responses to the disease within each experiment with a significant reduction (68 to 100%) in wilt incidence in the experiments conducted at 'En Tamar, but an insignificant reduction (4 to 35%) in the 'En Yahav experiment (Table 1). Because the horticultural performance of the melons grafted on cv. Brava seemed to be better than that of the other transplant combinations, Brava was selected to serve as a rootstock in the subsequent experiments.

Integrated management of sudden wilt. Severe wilt developed in each experiment in untreated plants (94 to 100% wilt; Tables 2 and 3). The full rate of methyl bromide (50 g/m²) was highly effective and sudden wilt was rare in this treatment. The reduced methyl bromide rate (15 g/m²) was effective but results were variable (32% control in the 1997 spring experiment and 91% control in the 1997 to 1998 autumn experiment). A previous-season methyl bromide treatment was highly effective as well (96% control). Grafting, when employed as the sole control measure, significantly reduced wilt incidence by 84 and 87% in the two experiments. Integration of grafting and reduced rate of methyl bromide improved wilt suppression further in the fall but not in the spring experiment, when compared with each of the measures applied alone (Tables 2 and 3).

Effects on yield, yield components, and fruit quality. Yield was significantly affected by the sudden wilt disease, as reflected by the significant yield increase in treated plots. In the 'En Tamar spring 1997 experiment, for example, the yield in methyl bromide-treated plots with grafted plants were 5.7× higher than that in the untreated plots (28.6 versus 0.51 kg/m², respectively; Table 2). Each of the control measures, when examined alone, resulted in a significant increase in yield (*P* ≤ 0.05) in comparison with untreated plots. When methyl bromide at the reduced rate and grafting were combined, yield was significantly higher as compared to each treatment applied alone (Table 2).

Table 1. Sudden wilt incidence (%) caused by *Monosporascus cannonballus* in non-grafted and grafted melons (cv. 'Arava) on various rootstocks in three experiments³

Rootstock cultivar	Rootstock species	Source of rootstock	Ein Tamar autumn 1995 ²	Ein Yahav autumn 1995 ²	Ein Yahav spring 1996 ²
Non grafted	<i>Cucumis melo</i>	Hazera, Israel	93 a	100 a	89 a
TZ -148	<i>Cucurbita maxima</i>	Tezier, France	10 b	96 a	28 b
Brava	<i>Cucurbita maxima</i>	Petoseed, United States	0 b	65 a	33 b
Tetsukabuto	<i>C. maxima</i> × <i>C. moschata</i>	Takii Seed, Japan	13 b	65 a	50 b
Marukanpyou	<i>Lagenaria siceraria</i>	Takii Seed, Japan	76 a	nt	nt
Feggous	<i>Cucumis melo</i>	Inter Semences, Morocco	nt	100 a	nt
Slaoui	<i>Lagenaria siceraria</i>	(Unknown)	nt	100 a	nt
Just	<i>Cucurbita</i> sp.	Takii Seed, Japan	14 b	nt	nt
Butternut	<i>Cucurbita moschata</i>	Petoseed, United States	67 a	nt	nt

³ Experiments were conducted in soil naturally infested at 'En Tamar (northern 'Arava) and at 'En Yahav (central 'Arava) in southern Israel. Disease incidence was evaluated 60 days after transplanting.

² Within columns, values followed by the same letters, do not differ significantly (*P* = 0.05), as determined by Fisher's protected least significant difference test; nt = not tested.

The number of fruit per unit area was the yield component affected most by the disease and increased by the treatments. In general, the effects of the different treatments on the number of fruit resembled their effects on yield. A large increase (up to 4.4×) was observed in the 1997 spring experiment with additive effects of methyl bromide and grafting; in the 1997 autumn experiment, only soil fumigation increased the number of fruit significantly. Fruit weight also was increased by the treatments significantly, but this increase was smaller (up to 1.3×) than the increase in the number of fruit. Fruit quality (as measured by TSS) in the 1997 autumn experiment was not affected by the treatments (Table 3).

DISCUSSION

Growing grafted vegetables, including cucurbits, is a common method employed in Japan, Korea, the Mediterranean Basin, and some countries in Europe (10). The purpose of growing grafted melons is mainly to control Fusarium wilt caused by

various races of *F. oxysporum* f. sp. *melonis*; to the best of our knowledge, no data are currently available on the possibility of using grafting against *M. cannonballus*.

Genetic sources for rootstocks resistant or tolerant to *M. cannonballus* may exist in various plants of the Cucurbitaceae. *Cucurbita*, as a genus, was defined by Mertely et al. (13) as a host for *M. cannonballus*. Indeed, perithecia of *M. cannonballus* could be detected on various *Cucurbita* sp. roots recovered from the soil after harvest, although no wilt symptoms were evident throughout the growing season (S. Pivonia, unpublished). However, not much is known about the rate of infection of *Cucurbita* spp. compared with melons or other cucurbits and the response of different *Cucurbita* accessions to the pathogen.

In general, results obtained in this study suggest that grafting is an effective measure against melon sudden wilt. When cv. 'Arava scions were grafted on cv. Brava rootstocks, wilt incidence was significantly reduced (by 63 to 100%) in four of the five

experiments. However, in one experiment ('En Yahav, autumn 1995) where disease pressure was very high, wilt incidence in the grafted plants did not differ significantly (35% reduction) from that in non-grafted plants. The variable performance of grafting is a known phenomenon (10). Moreover, a significant reduction in wilt incidence in a certain year does not necessarily reflect the whole picture. The pathogen population can still increase gradually in the soil and, once it reaches a certain level, grafted plants might be endangered and provide limited control. In addition, differences in environmental conditions, especially high temperatures, can enhance symptom development (3,7) and cause disease in grafted plants. The differences in the response to the disease of grafted plants in the autumn of 1995 at 'En Yahav versus 'En Tamar is an example of one such possible variation. Thus, occasional incidence of wilt should be expected and additional measures for disease control should be implemented.

Table 2. Effects of methyl bromide and grafting on sudden wilt incidence, yield and yield components, in an experiment conducted at Ein Tamar in spring 1997^w

Soil, analysis	Grafting, variables ^x	Wilt incidence (%)	Marketable fruit (no./m ²)	Fruit wt. (kg)	Yield (kg/m ²)
Untreated	—	100	0.82	0.61	0.51
	+	16	2.57	0.71	1.82
Methyl bromide					
Previous season	—	4	2.62	0.65	1.70
	+	2	3.46	0.77	2.66
15 g/m ²	—	68	2.01	0.66	1.33
	+	25	3.57	0.79	2.82
50 g/m ²	—	2	2.89	0.69	1.99
	+	0	3.62	0.79	2.86
ANOVA ^y					
Main effects	S	0.0001	0.0225	0.0004	0.0001
	G	0.0001	0.0042	0.0001	0.0001
Interaction	S × G	0.0001	0.7738	0.3970	0.8476
LSD ^z	...	17.4	0.7747	0.08	4.93

^w Melon (cv. 'Arava) transplants were grown in soil naturally infested with *Monosporascus cannonballus*. Methyl bromide at the commercial rate (50 g/m²) was applied before the autumn cropping season of 1996.

^x No graft (—) or graft (+); S = soil and G = grafting.

^y ANOVA = analysis of variance (*P* values).

^z LSD = least significant difference.

Table 3. Effects of methyl bromide and grafting on *Monosporascus* wilt, yield, yield components, and fruit quality in an experiment conducted at Ein Tamar in autumn 1997^w

Soil, analysis	Grafting, variables ^w	Wilt incidence (%)	Marketable fruits (no./m ²)	Fruit wt. (kg)	Yield (kg/m ²)	TSS (%) ^x
Untreated	—	94	1.40	1.00	1.40	10.2
	+	12	1.79	1.23	2.20	11.6
Methyl bromide						
15 g/m ²	—	8	2.47	1.06	2.61	11.7
	+	0	2.29	1.19	2.73	10.3
50 g/m ²	—	7	2.39	1.07	2.56	11.3
	+	0	2.44	1.08	2.63	11.5
ANOVA ^y						
Main effects	S	0.0001	0.0001	0.7142	0.0059	0.3317
	G	0.0001	0.6443	0.0380	0.1360	0.5729
Interaction	S × G	0.0001	0.3801	0.2981	0.2469	0.1083
LSD ^z	...	10.3	0.18	0.18	0.76	2.1

^w Melon (cv. 'Arava) transplants were grown in soil naturally infested with *Monosporascus cannonballus*.

^w No graft (—) or graft (+); S = soil and G = grafting.

^x TSS = total soluble solids.

^y ANOVA = analysis of variance.

^z LSD = least significant difference.

Effects of the treatments on yield resemble those obtained on wilt suppression. In general, treatments that resulted in reduced wilt also significantly increased yield. The main yield component that was affected by the disease (and thus was increased by the control treatments) was the number of fruit per unit area. Fruit quality (tested on marketable fruit only), which is determined mostly by its TSS, was not affected. These results reflect the expression of the epidemic of the disease in the field, where infected plants collapse shortly before harvest and do not produce any marketable fruit.

Part of the yield increase observed following soil fumigation with methyl bromide and the use of grafted plants is attributed to disease control. However, increased growth response, which is not related to the control of a major pathogen, is a well-known phenomenon in both soil-fumigation and grafted plants (10). The increased growth response effect following soil fumigation results largely from the decrease in, or elimination of, minor pathogens (5). In grafted plants, the rootstock's vigorous root system is often capable of absorbing water and nutrients more efficiently compared to the non-grafted plant, and may serve as a good supplier of endogenous plant hormones (5). However, the rootstock effect varies greatly with scion cultivar and growing season (10). An increased growth response effect may have been more pronounced in the 1997 spring experiment, when wilt incidence in the grafted and non-grafted plants treated with two of the methyl bromide treatments (previous season, and full rate methyl bromide) was similar (0 to 4% wilt). However, yield in the grafted plants was significantly higher (by 0.87 to 0.98 kg/m²) than in the non-grafted plants. This effect was not apparent, however, in the 1997 autumn experiment.

Grafted plants are expected to be expensive. Seeds of both rootstock and scion

may be expensive hybrids. The preparation of the transplant includes manual labor and expensive post-grafting handling (9,10). Extensive efforts to overcome the labor problem are being made in Japan, where private companies, institutions, and universities are attempting to develop feasible grafting robots. In the near future, it may be common practice for growers to buy seedlings grafted by robot (9).

In this study integration of two control measures was tested: grafting and pre-planting treatments with methyl bromide at reduced rate. Integration of the two control measures provided an adequate and reliable solution to sudden wilt of melon. Wilt incidence in plots treated with both measures was always significantly lower (by 75 to 100%) than that in untreated plots. Yield in these plots was always similar to (or even higher than) yield in the plots treated with full rate methyl bromide. The overall conclusion is that reduced rates of methyl bromide and grafting can be used as alternative measures to full-rate application of methyl bromide. Furthermore, post-planting application of the fungicide fluazinam, which was found effective against *Monosporascus* sudden wilt (4), may be combined as well.

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