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Efficiency of insect exclusion screens for preventing whitefly transmission of tomato yellow leaf curl virus of tomatoes in Israel

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Abstract

Tomato yellow leaf curl virus (TYLCV) is the most frequently occurring virus in tomatoes in the Middle East, and the most harmful one. It is transmitted solely by the whitefly *Bemisia tabaci* (Gennadius). Within 4–6 h of inoculative feeding, a whitefly can transmit TYLCV to a healthy plant with 80% probability. The symptoms are apparent after two to three weeks whereupon fruit-set is effectively terminated. The only means of controlling TYLCV is by controlling the whitefly. Until 1990 this was exclusively by insecticides. Starting in 1990, growers of greenhouse tomatoes in Israel began adopting insect exclusion screens to prevent inoculation of TYLCV. This article reports on the methods used in the search for efficient screening materials and presents data on their relative efficiencies in excluding *B. tabaci* and several other greenhouse pests. Ten materials were tested, of which five were found to be effective in excluding *B. tabaci* under laboratory conditions. This number was reduced to three following field trials and trials in commercial tomato greenhouses. These materials are now in widespread use in Israel: by 2000 practically all table tomatoes in Israel were grown under exclusion screens. The use of exclusion screens has been shown to be an economically viable pest management method.

Introduction

In Israel, tomatoes are an important crop both for export and for domestic consumption and the market demand for table tomatoes is very inelastic and quite stable year round. Consequently, declines in the supply of fresh tomatoes to the local market can lead to substantial price increases. This happened throughout the 1980s and early 1990s when tomato yellow leaf curl virus (TYLCV) drastically reduced Israel's tomato yields (Cohen & Berlinger, 1986). Tomato yellow leaf curl virus is the most frequently occurring virus on tomatoes in the Middle East, and the most harmful one (Avidov, 1956). It is vectored exclusively by the sweet potato (= cotton or tobacco) whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). A viruliferous whitefly can inoculate a healthy plant with 80% probability within 4–6 h of inoculative feeding, increasing to 100% infection after 12 h feeding (Cohen & Nitzani, 1966). Early infection by TYLCV can effectively destroy a crop because fruit set terminates when virus symptoms appear in the plant two to three weeks after inoculation. The *B. tabaci* population grows substantially through the summer months on cotton and

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field tomatoes, peaking in September at which time migrant viruliferous *B. tabaci* invade newly planted greenhouse tomatoes effectively destroying the crop (Berlinger *et al.*, 1984).

In the 1970s and 1980s when the *B. tabaci* and TYLCV problem began, it was unique to Israel. Since then *B. tabaci* has emerged as a near global pest on a range of greenhouse crops (Martin *et al.*, 2000). Although the tomato is not its preferred host, *B. tabaci* transmits several viruses in addition to TYLCV, including tomato pale chlorosis disease (TPCD) in Israel and tomato dwarf necrotic virus (TDNV) in California (Oliveira *et al.*, 2001). Altogether, *B. tabaci* is able to transmit over 70 different plant viruses to various crops, and thus has become a limiting factor in vegetable production in many areas around the world. It is widely distributed in tropical and sub-tropical countries. In the last decade it has expanded its range into western Mediterranean countries and into temperate regions of northern America and western Europe (Martin *et al.*, 2000). In warm countries it survives year-round outdoors and in greenhouses. In temperate regions it is mainly confined to greenhouses.

Because TYLCV cannot be controlled directly, the only practical strategy has been by chemical control of the *B. tabaci*. Long term effective chemical control of *B. tabaci* has proved impossible to achieve because virus acquisition requires so little time and insecticide resistance develops very rapidly in this multivoltine and highly mobile species. *Bemisia tabaci* quickly developed resistance to nearly all chemical pesticides used against it in Israel so that, by 1986, all labelled insecticides (all of the few effective pyrethroids; Berlinger *et al.*, 1987) had become ineffective and new products were not being released fast enough to replace the old insecticides. As a result, growers began to spray more frequently.

A programme to combat TYLCV was initiated at the Gilat Regional Experiment Station in 1977. Initially, this programme emphasized chemical control of the vector (Berlinger *et al.*, 1996a), but was supplemented by basic research on *B. tabaci* (Berlinger *et al.*, 1996c). As a better understand of the biology and ecology of *B. tabaci* was acquired, it became apparent that only mechanical exclusion would solve the TYLCV problem economically.

In 1990, a whitefly exclusion technique using screens was proposed as an economically viable solution (Berlinger *et al.*, 1990). Since then, all winter tomatoes have been protected by whitefly exclusion screen, resulting in an expansion in the total area of greenhouse tomato cultivation (Taylor *et al.*, 2001). Greenhouse screening has now become a standard pest management strategy for tomatoes, vegetables and herbs in Israel (Berlinger *et al.*, 1996b) and the technology is increasingly being adopted elsewhere (Bethke *et al.*, 1994; Arsenio *et al.*, 1999). The efficiency of various greenhouse screening materials were compared by Bell & Baker (2000) and a detailed economic analysis by Taylor *et al.* (2001) has shown the technology to be economically beneficial for both producers and consumers. In this article the methods used for selecting effective greenhouse screen materials and the determination of their relative efficiency are reported.

Materials and methods

Experiments to validate the efficacy of the screening concept began in 1980 by testing the only product then available, Agryl® (see table 1 for the manufacturer of

Table 1. Summary of properties of screen materials and efficacy of laboratory, field, and greenhouse trials.

	Product	Manufacturer (importer)[1]	Screen mesh[2] and gauge		Laboratory			Field[3]			Greenhouse[4]	
			Holes per inch (cm)	mm	Reps	Φ%	SE	N per trap	Φ%	SE	N per trap	SE
1	Shade screen	Klayman-Meteor Ltd	14×16 (6×6)	0.22	30	99.3	0.015	30.4	24.2	13.5		
2	KML 22×50	Klayman-Meteor Ltd	22×50 (9×20)	0.22	62	23.2	0.077					
3	Anti-Virus	Klayman-Meteor Ltd	25×50 (10×20)	0.22	12	6.2	0.044	0.59	0.58	0.29	0.62	0.23
4	KML 25×50	Klayman-Meteor Ltd	25×50 (10×20)	0.24	28	6.8	0.046					
5	BTD '50 mesh'	Ben Tsur-Drouianoff Ltd	25×54 (10×22)	0.22	46	8.8	0.052	0.36	0.41	0.27	0.79	0.35
6	GGL 27×58	Gil-Gad 1982 Ltd	27×58 (11×23)	0.20	6	1.2	0.020					
7	GGL '50 mesh'	Gil-Gad 1982 Ltd	28×58 (11×23)	0.20	36	0.5	0.013	0.74	0.84	0.31	0.44	0.27
8	GGL 29×58	Gil-Gad 1982 Ltd	29×58 (12×23)	0.20	6	0.1	0.006					
9	TL 26×23	Tama Ltd	26×23 (10×21)	0.28	12	2.1	0.026					
10	Agryl	Sodoca (Steinberg)	–	–	12	5.8	0.043	0.4	0.5	0.10		
	No screen							88.5	100	0	40.2	6.3

[1] All materials are made in Israel, except no. 10, Agryl, which is made by Sodoca, France and imported to Israel by A. Steinberg, Ltd.
[2] Screen mesh is measured in holes per inch in the weft × weave directions; the approximate number of holes per cm is given in brackets.
[3] Field experiment with low tunnels was a randomized block experiment with 5 treatments and 6 replicates; the shade cloth treatment was used to calculate a penetration index (Φ, equation 3).
[4] Greenhouse experiment was a fully randomized design with 4 replicates except for 2 replicates of material no. 7.

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materials used), a non-woven polypropylene fabric. Delicate and prone to tearing, Agryl proved to be unsuited to outdoor conditions, and so could only be used inside greenhouses. However, it was immediately adopted for commercial use in greenhouses of organic vegetables, during the most critical growth period in Israel – September to December (Berlinger *et al*., 1988). Prior to planting, Agryl was installed above the training wires to form a 2 m high tent. It was kept in place while the greenhouse vents were kept open for cooling until November–December when the whitefly migration was over. However, it reduced ventilation, resulting in increased daytime temperature and night time humidity. These climatic changes caused other problems, and stimulated a search for more suitable screening materials. As a result of intensive collaborative research among screen manufacturers, agricultural advisors and growers, more than 20 screening materials were submitted for consideration of which nine were tested at Gilat and Besor between 1982 and 1990. Potential screen materials were of four basic types (Berlinger *et al*., 1999): knitted, woven, knitted-woven, and non-woven. Examples of woven screen materials tested are Anti-Whitefly® and Anti-Virus®; a knitted-woven material is SuperNet®; non-woven screens are Agryl®, Reemay®, FastStart® and a micro-perforated polypropylene material, Agronet® (table 1).

Laboratory trials

The test apparatus consisted of two 750 ml clear plastic cups (A and B). The bottom of both cups was removed such that the approximately 7 cm diameter of one (A) was slightly smaller than the aperture of cup B. A 10 × 10 cm screen sample was stretched across and glued across the bottom of cup A which was then inserted into the bottom of cup B. Semitransparent yellow plastic sheet was taped to the outside surface of a 9 cm plastic Petri dish, while insect glue was smeared on the inner surface. The Petri dish was placed over the top of cup B, glue side down. About 120–150 viable adult *B. tabaci*, reared on cotton seedlings, were introduced into cup A by holding it with its opening upward beneath whitefly-infested leaves and gently shaking the adults into the open cup. A cover was then snapped on and the cup inverted. With cup A inserted into cup B, the space between the fabric and the Petri dish created a cell with a yellow light source above and a cell containing whiteflies below. The yellow light source attracted whiteflies from the lower cell (A) into the upper cell (B) and the insect glue in the Petri dish ensured that whiteflies that passed through the test screen could not return to the lower cup A.

Samples of screen materials of various mesh were received for testing (table 1). Each candidate material was compared against two controls. A 17% shade screen (mesh density of 14 × 16 (approximately 6 holes per cm by 6 holes per cm); mesh size is conventionally measured in holes per inch in the weft × weave directions) with a passage rate of > 99% offering little impediment to the passage of whiteflies was used as a control to test for viability (control I). The second control was a dense screen (28 × 58) with a passage rate of < 1% and was used to control for variations in whitefly size and possible selection for smaller whiteflies (control II). Each type of screen was tested in this way 12 times in environmentally-controlled conditions of 28 ± 1°C and 60 ± 5% relative humidity. After 24 h all whiteflies had died, and the number in both chambers was counted and the proportion of *B. tabaci* penetrating the material (Φ) was estimated from

$$\Phi = \frac{1}{n}\sum \frac{p_i}{r_i + p_i} = \frac{1}{n}\sum \frac{p_i}{T_i} \qquad (1)$$

with standard error

$$\mathrm{se}(\Phi) = \sqrt{\frac{1}{n} \cdot \Phi(1 - \Phi)} \qquad (2)$$

(Finney, 1980) where n = 12 is the number of replicates, p_i is the number of whiteflies in the ith container and $r_i = T_i - p_i$ ($T_i \approx 120$) is the number remaining in the ith cup at the end of the experiment.

Field trials

Following the laboratory tests, four promising materials, all of woven type, were tested for use in greenhouses and low tunnel-like (45 cm high × 45 cm wide × 3 m long) field cages ('screenhouses') (Berlinger *et al*., 1990). The field cage tests were conducted during the peak whitefly flight period in September 1988. Field cages were constructed using materials nos. 1 (the shade screen standard), 3, 5, 7 and 10 (table 1). The screens were placed, before planting, over a row of field tomato plants in a randomized block design with six replicates per material.

Sampling inside and outside the screens was by yellow sticky traps. Plastic petri dishes (9 cm) were smeared inside with insect glue. They were put singly, with their opening upward, horizontally on yellow backgrounds usually at a height of ~ 0.4 m above ground and 0.1 m inside the screenhouses. Four Petri dish traps were placed inside and four outside each cage and were changed once or twice weekly. The collected traps were examined in the laboratory and the pest species counted. The average number of *B. tabaci* caught per trap per day was calculated and the ability of whiteflies to penetrate each material was calculated from

$$\Phi = 100 \cdot \left\{ 1 - \frac{X_s \cdot Y_t}{X_t \cdot Y_s} \right\} \qquad (3)$$

where X is the number outside a screened field cage, Y is the number inside the same cage, and the subscripts s and t denote the standard (shade cloth screen) and test materials, respectively. This index is equivalent to Abbotts' (1925) method for standardizing pesticide efficacy data (Taylor, 1987). The index Φ was transformed using the arcsine square root transformation commonly used for proportions and percentages:

$$Z = \sin^{-1}(\sqrt{\Phi / 100}) \qquad (4)$$

Arcsine square root transformed Φ was analysed as the dependent variable in a randomized complete block design to test for differences in the ability of *B. tabaci* to penetrate the four test materials.

Commercial greenhouse trials

In the greenhouse experiment, three materials were tested at ten commercial greenhouses of at least 1000 m^2. Two houses were fitted with screen 5 and screens 3 and 7 were fitted to four houses each (table 1). Ten yellow traps were placed inside each greenhouse and ten placed outside were used as controls. The shade screen standard was not

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included in this experiment because its inclusion would have added no new information. The yellow traps were changed weekly from 26 September 1988 to 21 November 1988. The average number of *B. tabaci* caught per trap per day inside and outside the cages was calculated. The log of average inside trap catch was used as the dependent variable with log of average outside trap catch as a covariate to remove the effect of differences in the background density between the sites. The data were analysed as a fully randomized design comparing the ability of the three materials to exclude whiteflies. This experiment is partially replicated because screen 5 was employed in only two houses whereas the other materials were tested on four houses. Consequently, non-linear estimation was used for this analysis (NAg, 1978).

Other insects

The greenhouse trial was repeated at a greenhouse in Ranen starting in September 1993 to determine if screening excluded other insects. Ten yellow sticky cards (or blue for thrips) were placed inside and ten outside the greenhouse equipped with Anti-Virus screens (no. 3). Traps were changed weekly and sampling continued for about six months. Insect pest species were identified on cards both inside and outside the greenhouse and the numbers of the most commonly caught pests were recorded as numbers per card and compared graphically.

Results

Laboratory trials

The experiments to test the efficacy of screening materials showed large differences in the ability of *B. tabaci* to penetrate the various materials (table 1). The differences ranged from 99.3% with the shade cloth standard (screen 1) to 0.1% with the densest mesh screen (no. 8). Under laboratory conditions, penetration through the screens in commercial use (nos. 3, 4, 5, 7 and 10) ranged from 8.8% to 0.5%. Penetration through all screening materials was not significantly greater than 0 for all materials except screen 2 (t = 3.0; df = 29; $P < 0.01$) and the shade cloth control (t = 65.2; df = 29; $P < 0.001$), which was not significantly different from 100% penetration (t = 0.78; df = 29; $P > 0.45$).

Field trials

Four materials were tested (screens 3, 5, 7 and 10) and compared to the shade cloth standard (screen 1) and an uncovered control (table 1). The average number of *B. tabaci* caught inside the test field cages was extremely low, not exceeding 0.7 whiteflies per trap per day compared to catches under the shade cloth screen tunnel (no. 1) (30.4 whiteflies per trap per day) and the unscreened control (88.5 whiteflies per trap per day). Arcsine square root transformed percent penetration (Φ, equation 3), was calculated for each cage relative to the shade-cloth standard and was analysed as the dependent variable in a randomized complete block design. Analysis of variance showed no significant difference (F = 2.91; df = 3,15; $P > 0.1$) between the four screen materials. A contrast to test the joint difference of the screens from the shade cloth was highly significant (F = 4309; df = 1,15; P = 0) indicating that all four screen materials were highly effective barriers to whiteflies. However, the penetration of the screen materials by whiteflies was significantly greater than zero (F = 24.3; df = 1,15; $P < 0.01$) reminding us that biological significance of this is limited because it has been found that as few as five viruliferous whiteflies are needed to ensure 100% virus infection of healthy plants within 24 h of exposure: TYLCV incidence is highly correlated ($r^2 > 0.95$) with whitefly population density (Berlinger *et al.*, 1990).

Commercial greenhouse trials

The average number of *B. tabaci* caught inside screened commercial houses was also very low; ranging between 0.4 and 0.9 whiteflies per trap per day compared to an average of 40 whiteflies per trap per day trapped outside (fig. 1a). Analysis of variance with the log of whitefly catch per trap per day inside the greenhouses as the dependent variable and the log of catch per trap per day outside as the covariate showed a significant difference (F = 23.1; df = 2,9; $P < 0.001$) between the three screens. However, the difference between the log of catch per trap per day taken within the screened houses and the outside control traps was highly significant (F = 102.6; df = 1,9; $P < 0.001$) indicating that all three screen materials were highly effective in excluding whiteflies.

Other insects

As with the previous experiments, the whiteflies caught outside the greenhouse at Besor were primarily *B. tabaci* but some greenhouse whitefly *Trialeurodes vaporariorum* (Westwood)) (Hemiptera: Aleyrodidae) were also present. Other insects caught on the sticky cards were western flower thrips *Frankliniella occidentalis* (Pergande), onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), melon or cotton aphid *Aphis gossypii* (Glover), peach–potato aphid *Myzus persicae* (Sulzer)) (Hemiptera: Aphididae), and leafminers of the *Liriomyza brassicae* (Riley) complex (Diptera: Agromyzidae). In addition, leafhoppers of the genus *Empoasca* (Hemiptera: Cicadellidae) and some unidentified psyllids (Hemiptera: Psylloidea) were caught outside the greenhouse but not inside. Very few insects were caught on the cards inside the greenhouse. Figure 1 shows trajectories of the numbers of the most common species caught inside and outside the greenhouse at Ranen. The figures show a marked difference between the catches inside and outside the greenhouse indicating probable benefits beyond the exclusion of *B. tabaci* and TYLCV.

Discussion

One of the main objectives of greenhouses is to protect crops from unfavourable climatic conditions. Unfortunately, the optimal plant conditions maintained in greenhouses also tend to accelerate the development of insects and insect-borne diseases. Invasion of greenhouses by pests is especially acute where pest populations develop on outdoor crops during the summer and emigrate in autumn in search of over-wintering sites or hosts.

Greenhouse ventilation systems strongly affect the influx of insects. Active (negative pressure) ventilation based on sucking air out of the greenhouse causes under-pressure which can significantly increase the influx of insects compared with passive ventilation. Screens to exclude

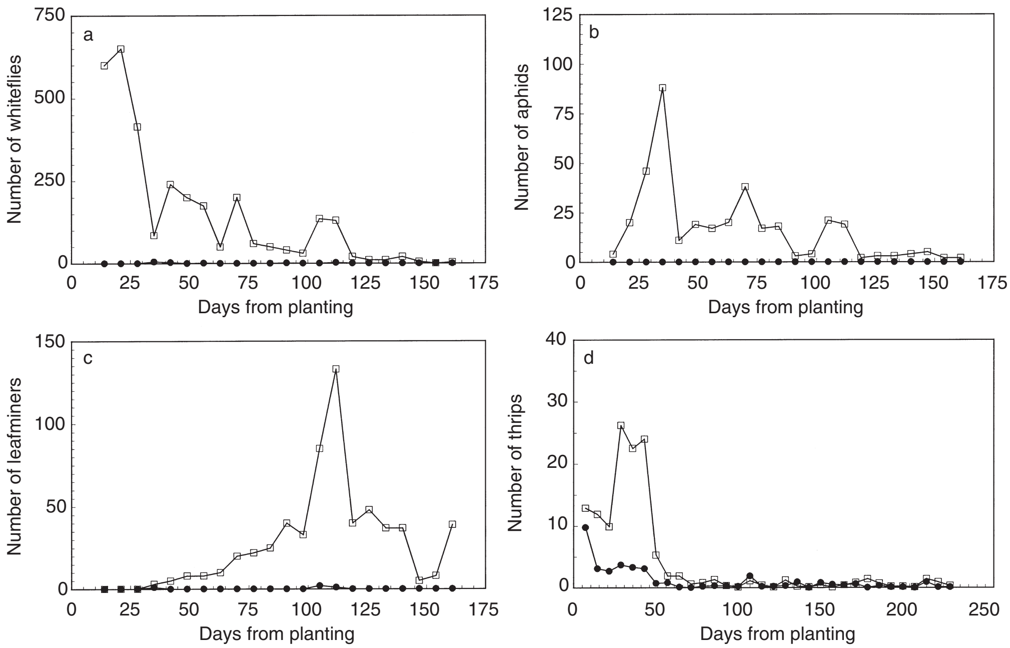

Fig. 1. The aerial densities of (a) whiteflies (*Bemisia tabaci* and *Trialeurodes vaporariorum*), (b) aphids (*Aphis gossypii* and *Myzus persicae*), (c) leafminers of the *Liriomyza brassicae* complex, and (d) thrips (*Frankliniella occidentalis* and *Thrips tabaci*) inside (●) screened greenhouses are essentially independent of the density outside (□). Samples are expressed as numbers per trap per week; there were ten sticky cards inside and ten outside the greenhouse.

insects from greenhouses with negative air pressure must have smaller holes to compensate for the increased draught drawing insects through the screens which further reduces ventilation efficiency and increases the power requirements for the fans which in turn reduces the efficiency of the screens (Price & Evans, 1992). Attempts to use less dense screens and to compensate by repellent colours have not proven to be effective in excluding whiteflies (Antignus *et al.*, 1998; Costa & Robb, 1999). Conversely, positive air pressure developed by actively pushing air through an insect-proof filter into the greenhouse can reduce insect influx to 33% of the level of a passively ventilated screened greenhouse. Although this method consumes more energy than passive or negative pressure ventilation, it reduces insect immigration while ventilating and cooling the greenhouse.

To be effective, screens must necessarily be installed prior to the pests' appearance and all openings must be totally covered by screens, including entrances which need to be in the form of air locks. Furthermore, plants must be quarantined before they go into production areas to be sure they are pest-free before planting. Even with these precautions, which are not without cost, some penetration by pests will usually occur. Thus, complementary pest control measures may be required.

The economic threshold for TYLCV infection is 10% virus-infected plants at the end of the season. The accepted economic threshold for whitefly incidence in greenhouses for primary TYLCV infection is 1.4 whitefly per trap per day or 10 whiteflies per trap per week. As long as whitefly catches are below this threshold, growers do not need to apply chemical controls. Towards the end of the season a whitefly population building up and causing a secondary virus transmission can be prevented by using biorational insecticides or biocontrol agents (Berlinger *et al.*, 1988). Furthermore, the absence of hard chemicals permits the introduction of bumblebees (*Bombus* spp.) for pollination. Bee pollination of tomatoes is much less expensive than hand pollination; it greatly increases yields by increasing the number of fruit per plant, and improves fruit quality (Pressman *et al.*, 1999). It is possible that this indirect benefit of screening alone could justify the installation costs.

The rate of penetration by insects is directly proportional to a screen's mesh ($r^2 = 0.85$, $P < 0.001$; Berlinger & Lebuish-Mordechi, 1995). However, an insect's ability to pass through any barrier can not be predicted solely from its thoracic width and hole size (Bethke & Paine, 1991). Some laboratory tests revealed an unexpectedly high proportion of whitefly penetration, accompanied by a great variability among the samples of the same screen. This variability may have been due to sliding of unevenly woven yarn. Screens that do prevent passage of whiteflies are likely to inhibit the influx of bigger insects such as moths, leafhoppers, psyllids, leaf miners, spider mites, and aphids as well as whiteflies (fig. 1). Screening can also reduce the influx of smaller insects like western flower thrips (fig. 1d). Another important virus vector, western flower thrips transmits impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV), both serious horticultural and floricultural problems (Allen & Broadbent, 1986). A reduction in immigration rate of this cosmopolitan pest would contribute to the control of this pest and make complementary control measures, like biocontrol, more efficient.

There are, however, disadvantages to screening; without active ventilation, the screens can substantially increase temperatures and humidity (Baker & Shearin, 1994). Increased humidity may necessitate more frequent fungicide sprayings than are required in a comparable unscreened greenhouse. Usually, two to three sprayings per season are required in Israel, but this can increase to five or six with screening (Y. Sachs, Israel Ministry of Agriculture, Plant Protection, personal communication). The cost of screening does not end with the installation: they require maintenance. The screens must be inspected periodically for accidental punctures and dusty screens must be cleaned regularly to remove dust that reduces ventilation and light. Also, an insect monitoring system is crucial for warning of successful immigration. However, the cost of screen maintenance, if incorporated into the routine maintenance of the greenhouse, should be no more expensive than the maintenance of spray application machinery.

Generally, screens are a very reliable and environmentally safe means, fit well into integrated pest management programmes, and greatly reduce the need for chemical control. Thus, the application of whitefly-proof screens, despite the extra management and maintenance costs, has become the most effective method to protect greenhouse tomatoes and other whitefly susceptible crops in Israel. Since 1998 it has been applied by all tomato growers and by many other vegetable and flower growers who expect to double the area of screened greenhouse within the next three years. The importance of screening and its acceptance by growers is illustrated by the rate of increase in the number of greenhouses protected by insect-proof screen.

This article has described and brought together for the first time all the results of the search for a suitable screening material for excluding *B. tabaci* and TYLCV from greenhouses. The search took over five years of effort and co-operation by research, extension, growers and fabric manufacturers. Of the ten materials tested in the laboratory, field and commercial operations, four were found to be effective exclusion materials and three were both effective and durable under production conditions. These materials are now in common use in greenhouses and field screenhouses throughout Israel and have resulted in substantial reductions in pesticide use and cost of production (Taylor *et al*., 2001).

Regardless of the target insect pest, exclusion screens offer several other benefits. In addition to the immediate plant protection benefit, exclusion screens address additional problems: they eliminate the development of insecticide-resistance; they satisfy public demand for pesticide-free produce; they permit the more efficient use of biocontrol agents and bees for pollination. Finally, the main advantage to insect screening is the enormous reduction in pesticide expenditure and exposure. Adding screens to existing greenhouses, building new screened greenhouses and intensively-managed, totally enclosed greenhouses with positive pressure can virtually eliminate the damage caused by the TYLCV.

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