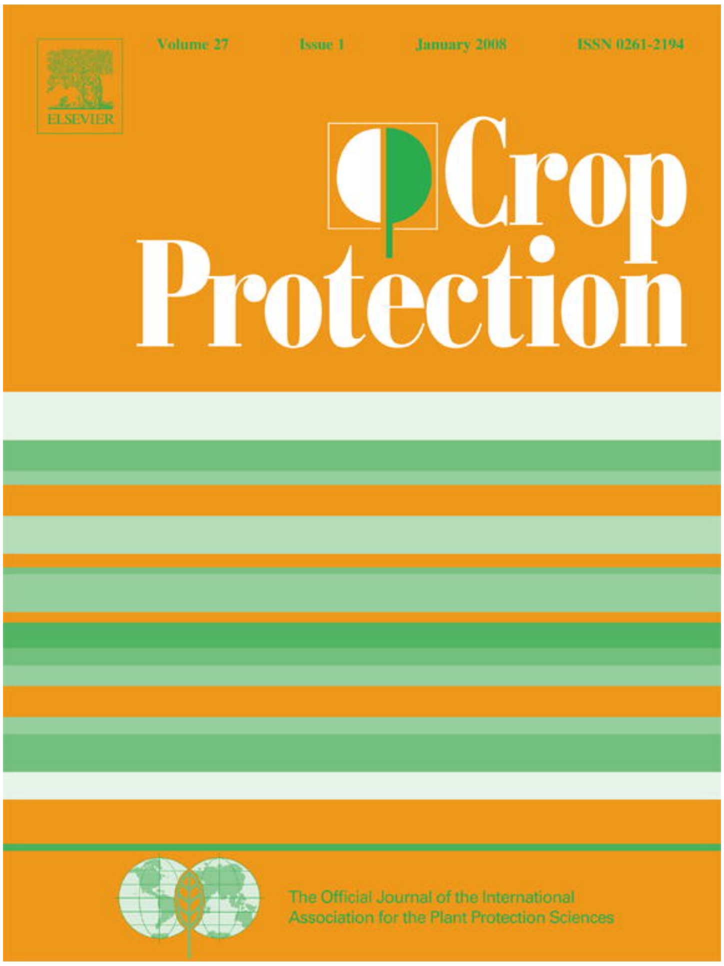
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Living ground covers for management of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and tomato yellow mottle virus (ToYMoV) in Costa Rica

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Abstract

The impact of whitefly-transmitted begomoviruses (*Geminiviridae*) on tomato yields depends on plant age at time of infection, and is greatest during the first 5 weeks after germination (critical period). A preventative strategy designed to minimize contact between the vector and the tomato plant by masking the crop with living ground covers during the critical period was promoted among small-scale Costa Rican farmers. A large-scale field experiment replicated over time and location was conducted to evaluate this strategy by assessing the effect of different living ground covers in comparison to the conventional control methods (insecticide or reflective mulch). Living covers including perennial peanuts (*Arachis pintoi*, Fabaceae), "cinquillo" (*Drymaria cordata*, Caryophyllaceae) and coriander (*Coriandrum sativum*, Umbelliferae) reduced the number of incoming whitefly adults, delayed the onset of tomato yellow mottle virus (ToYMoV), and decreased disease severity, resulting in higher yields and profits, compared to the bare soil control. Coriander provided additional economic returns when sold and was easier to establish and remove than the other living covers. Whitefly and begomovirus management with ground covers or reflective polyethylene mulch compared favorably with the conventional insecticide treatment using imidacloprid Therefore, living ground covers appeared to offer a viable and economic management alternative for resource-poor farmers.

Keywords: *Bemisia tabaci*; Begomoviruses; *Geminiviridae*; ToYMoV; Living ground covers; Tomatoes; Prevention; Integrated pest management (IPM)

1. Introduction

Bemisia tabaci (Gennadius) is a cosmopolitan insect and a key pest in many tropical and subtropical cropping systems (Brown and Bird, 1992; Brown, 1994), as well as greenhouses in temperate climates. Estimated annual economic losses amount to several hundred million or even billion dollars worldwide (Oliveira et al., 2001). This highly polyphagous pest has been reported to develop or reproduce on over 500 different plant species, belonging to 74 families (Greathead, 1986), including some 30 cash and staple crops worldwide, such as tomato, pepper, melon, watermelon, soybean, cotton, beans and cassava.

Crop damage may occur directly through excessive sap removal, or indirectly by either promoting the growth of sooty mold, inducing physiological disorders through feeding, or vectoring plant viruses (Schuster et al., 1996). Among many begomoviruses (*Geminiviridae*, formerly known as geminiviruses) reported for tomato in the Americas, tomato yellow mottle virus (ToYMoV) is present in Costa Rica (Polston and Anderson, 1997).

The impact of these begomoviruses on tomato yields depends on plant age at time of infection: the earlier the infection, the greater the severity of the disease and its effect on yield (Schuster et al., 1996). Field and greenhouse studies indicate that the critical period of susceptibility for

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several tomato begomoviruses encompasses the first 50–60 days after emergence, and particularly, the first 5 weeks (Franke et al., 1983; Schuster et al., 1996). Therefore, management should focus on this critical early period to minimize contact between the vector (*B. tabaci*) and the host plant.

In Costa Rica, a two-phase strategy was promoted for integrated management of whitefly-borne begomoviruses in tomato (Hilje, 2001), emphasizing production of virus-free seedlings for 30 days under tunnels covered by fine nets, and post-transplant protection by combinations of several cultural practices. Additional evaluation was required for the use of living ground covers or mulches as a means of delaying virus dissemination into tomato fields.

The use of reflective mulches to delay the onset of infestations of whitefly and associated virus is well established (Berlinger and Lebiush-Mordechi, 1996; Smith et al., 2000; Summers and Stapleton, 2002; Summers et al., 2004). Less has been documented on the effects of living mulches or ground covers (Amador and Hilje, 1993; Blanco and Hilje, 1995; Cubillo et al., 1999; Hooks et al., 1998; Frank and Liburd, 2005). A theoretical framework to explain these results with living mulches was provided by Finch and Collier (2000), who stated that insect herbivores locate host plants initially through indiscriminant visual attraction. Only after landing is there discrimination between "appropriate" (acceptable) and "inappropriate" (unacceptable) hosts. If the host is not acceptable, the insect flies a short distance and lands again. After a number of such inappropriate landings, the insect is likely to leave the general area entirely. The presence of numerous non-host plants in the form of living mulch greatly increases the likelihood of successive inappropriate landings that eventually lead to abandonment of the search and exit from the area.

Preliminary observations in Costa Rica showed that a number of different living ground covers could reduce whitefly adult numbers, slow down virus spread, reduce disease severity and provide higher yields. Covers included a mixture of spontaneous weeds, the legumes *Arachis pintoi* (perennial peanuts) and *Styzolobium deeringianum* (mucuna) (Fabaceae), "cinquillo" (*Drymaria cordata*, Caryophyllaceae), and coriander (*Coriandrum sativum*, Umbelliferae) (Amador and Hilje, 1993; Blanco and Hilje, 1995; Cubillo et al., 1999). Although promising from an applied standpoint, confirmation over a wider range of agro-climatic settings and testing on a larger scale was lacking. Moreover, the potential role of living covers as sources of begomoviruses and other plant pathogens had not been investigated.

The objective of this research was to evaluate the effects of several living ground covers compared to a synthetic (polyethylene) ground cover, and a conventional insecticide treatment on whitefly incidence, spread of whitefly-borne ToYMoV and yield of tomatoes in plots typical of small-scale vegetable growers in Central America.

2. Materials and methods

2.1. Locations

Plots were established in commercial farms representing two contrasting agro-climatic settings in Costa Rica, in Guayabo (Turrialba) and Santa Gertrudis (Grecia). Guayabo is located in the Caribbean watershed, within the premontane wet forest life zone (Tosi, 1969), at 09°58′N, 83°38′O and 840 m a.s.l., with annual averages of 21 °C, 2762 mm rainfall and 87% RH. Santa Gertrudis is in the Pacific watershed, within the premontane moist forest life zone, at 10°05′N, 84°17′O and 1074 m a.s.l., with annual averages of 23 °C, 2196 mm rainfall and 75% RH.

2.2. Treatments and experimental design

A randomized complete block design was used, with six treatments and four replicates. Replicates referred to as Guayabo and Grecia I, II and III consisted of rectangular blocks of 2000, 1400, 1500 and 2000 m^2 at Guayabo (March–June, 1998) and Grecia (December 1997–March 1998, June–September, 1998 and April–July, 1999), respectively. This design allowed us to optimize resources while still maintaining realistic plot sizes under typical conditions of commercial production.

Replicates were divided into six plots of 230–400 m^2 separated by 1 m^2 empty space. For each occasion, plots were consigned at random to the following treatments: (1) a mulch of the low-growing weed "cinquillo" (*D. cordata*, Caryophyllaceae) (DC); (2) a mulch of coriander (*C. sativum*, Umbelliferae) (CO); (3) a mulch of perennial peanuts (*A. pintoi*, Fabaceae) (PP); (4) an absolute control (C = bare ground); (5) a silver on black co-extruded polyethylene mulch 56 in × 1.25 Mls (Olefinas S.A., Guatemala) (SP, the commercial standard); and (6) imidacloprid (IM, Confidor 70 WG; Bayer) applied to the foliage at the recommended rate (9 g/40 m^2 of seedbed surface) a week before transplanting, and in two drench applications (250 g/ha) 2 and 4 weeks later as a chemical standard on bare soil. Living covers were established well before tomato was transplanted, whereas silver plastic was put in place over the 30-cm wide bed 2 weeks before transplanting. All covers remained in the field throughout the season, except for coriander, which was removed when ready for market at 35 days after transplanting (dat), once the critical period was over.

2.3. Agronomic practices

Tomato seedlings (var. 'Hayslip', Asgrow Seed Co., Michigan) were grown in newspaper cups (Cubillo et al., 1994), placed under field tunnels covered with Tildenet IN50 (Tildenet Ltd., Bristol, UK) insect netting to exclude whiteflies. Seedlings (22-day-old) were transplanted at 1.2 m between rows and 0.4 m between plants, and a weed-free area was continuously maintained around each tomato plant.

Soil was prepared before transplanting, according to local practices. Lime (0.135 t/ha) and chicken manure (0.92 t/ha), was added a month before transplanting. Inorganic fertilizer (10-30-10, N-P-K) was applied at transplanting, as well as 15 and 30 days later and an additional nitrogen-rich fertilizer was applied at 45 dat. Fungicides and bactericides were used as needed. At Guayabo, the following insecticides were applied: permethrin (17 dat) against flea beetles (*Epitrix* sp.) in the *Drymaria* and control treatments, and Javelin (*Bacillus thuringiensis* var. *kurstaki*) (24 and 27 dat) against the tomato pinworm (*Keiferia lycopersicella* (Walsingham)) and other lepidopteran larvae (*Heliothis* spp. and *Spodoptera* spp.) in all treatments. No insecticides were applied at Grecia.

2.4. Sampling

Adult whitefly abundance was monitored weekly in both tomato and living covers by selecting 30 plants per plot at random. Sampling took place between 8:30 and 10:30 h. For tomato, adults were counted on the underside of the topmost, fully expanded leaf of each plant. Because of varying growth habit, adults were monitored on living covers using a bucket of 34 cm diameter provided with a vial on top and a lateral sleeve. The bucket was placed over plants at 30 randomly selected locations per plot. Plants were shaken to dislodge the adults, which flew to the vial where they were caught and counted. Monitoring continued through harvest except in the Guayabo replicate, where a severe attack of bacterial wilt (*Pseudomonas solanacearum*) occurred at 63 dat in the control and at 70 dat in the imidacloprid treatment.

Eggs and nymphs were counted on a leaflet of the 6th leaf (from top to bottom) (Stansly et al., 1998) from each of the same 30 tomato plants. Leaflets were collected into paper bags, held inside plastic bags and refrigerated at 8–12 °C until examined under a stereoscopic microscope. For living covers, four leaves from the upper stratum of the plants were taken at random from those points sampled with the bucket, and held as described for the tomato foliage samples.

Disease incidence (viral symptom appearance) was assessed by marking 100 tomato plants in each plot and inspecting these weekly for symptoms of ToYMoV. Late in the season, leaves of diseased tomato plants, as well as from the living covers (selected at random), were analyzed with a specific probe for ToYMoV at the Cellular and Molecular Biology Research Center, University of Costa Rica (Jovel et al., 1999).

The same 100 tomato plants were evaluated weekly for disease severity, according to the following visual scale (Ioannou, 1985): symptomless (0) (no physiological or morphological effects on plants); light (1) (mild symptoms on top leaves); mild (2) (marginal and interveinal chlorosis on top leaves, with no apparent effect on plant growth); severe (3) (typical yellow leaf curl symptoms, plus evident reduction in plant growth); death (4) (complete necrosis).

Yields were evaluated from the same 100 tomato plants harvested at crop maturity. The number and weight of fruits were recorded according to local quality standards or categories, as follows: I (>180 g, undamaged and with good shape and color), II (120–160 g but otherwise of similar quality), and III (<120 g and often misshapen or unevenly colored) (Kopper et al., 1991). Production costs (including costs of establishing living mulches) by treatment and gross income, both based upon current market prices, were used to evaluate profits.

2.5. Analysis

Disease incidence and severity were assessed by determining the area under the disease progress curve (AUDPC) (Fry, 1978). Whitefly days, representing a cumulative count, were calculated for the crops by multiplying the average number of whiteflies over each sample interval (week) by the number of days in the interval (Stansly et al., 2005). Disease incidence and severity, whitefly days and yield were analyzed by ANOVA (SAS Institute Inc., 1985), and mean values were compared using the LSD test (least significant difference) (SAS Institute Inc., 1985). Economic analysis by treatment was performed by means of partial budgeting and marginal net benefit analyses (Perrin et al., 1976).

3. Results

3.1. Adult abundance

Significantly more whitefly adult days were observed on plants in the bare soil (control) treatment compared to any of the other treatments, with no differences among the latter (Table 1). The number of adult days accumulated on plants in both coriander and silver plastic plots increased

Table 1
Average (±SD) whitefly days per plant, and tomato yellow mottle (ToYMoV) incidence and severity on tomato plants grown on different types of ground cover, at the end of the season

Treatment	Whitefly days	Incidence (AUDPC)	Severity (AUDPC)
Control	3911±2296 a	4505±590 a	2919±467 a
Silver plastic	401±338 b	1703±123 b	812±580 c
Imidacloprid	756±493 b	2867±600 b	1643±379 b
Coriander	791±810 b	1741±897 b	838±546 bc
Perennial peanuts	602±389 b	2004±125 b	951±555 bc
Drymaria	522±306 b	1632±119 b	799±583 c

Means followed by the same lower case letter in a column are not significantly different at $p = 0.05$ level (Fisher t-test LSD).
AUDPC: area under the disease progress curve.

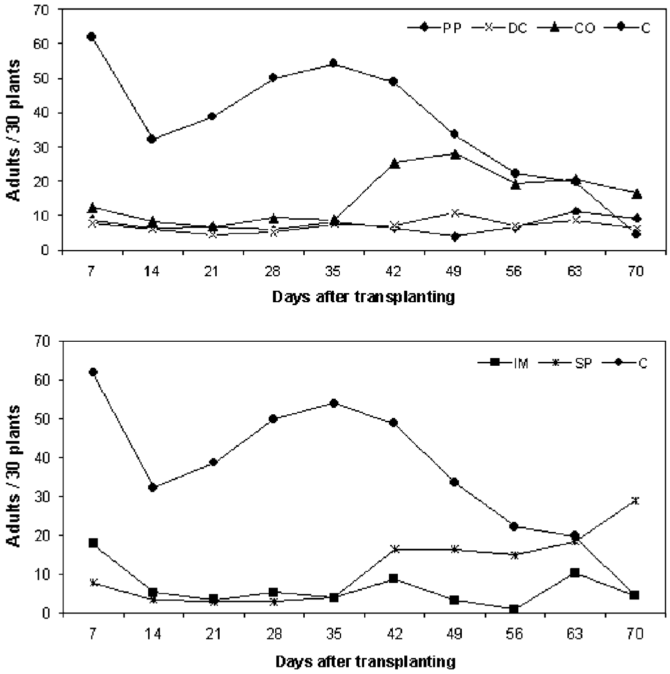

Fig. 1. Average numbers of *B. tabaci* adults on tomato plants grown with either living or inert ground covers, in comparison to bare ground, in Costa Rica. Treatments were perennial peanuts (*Arachis pintoi*) (PP), "cinquillo" (*Drymaria cordata*) (DC), coriander (*Coriandrum sativum*) (CO), imidacloprid (IM), silver plastic (SP) and bare ground (C). The figure is split into two panels in order to avoid excessive data overlap.

markedly after 35 dat, surpassing numbers seen on plants grown in bare soil at 63 dat (Fig. 1). Very few adults were seen on cover crop plants, the highest value corresponding to 0.33 adults/bucket. The number of adult days during the critical period for begomovirus infection (up to 35 dat) was greater for the control treatment than for all the remaining treatments on all sampling dates (Fig. 1).

Egg and nymph numbers on tomato plants were negligible on all sampling dates, but were greatest on plants in bare soil, with maximum averages of 0.56 eggs and 0.6 nymphs/leaflet, compared to all other treatments. No eggs or nymphs were found on living cover plants.

3.2. Disease incidence and severity

ToYMoV spread rapidly in spite of relatively low-whitefly numbers, especially in control plots (Fig. 2). Accumulated disease incidence followed the same pattern as adult whitefly incidence, being greatest in the bare ground control, with no significant differences among the remaining treatments (Table 1). Disease incidence was quite high in control plots, reaching nearly 100% at the end of the season (Fig. 2). ToYMoV was not detected in any of the living ground cover plants tested.

Significantly greater disease severity was also observed in control plots relative to all other treatments, with plants on the silver plastic or associated with *Drymaria* significantly less affected by ToYMoV than those treated with imidacloprid (Table 1, Fig. 3).

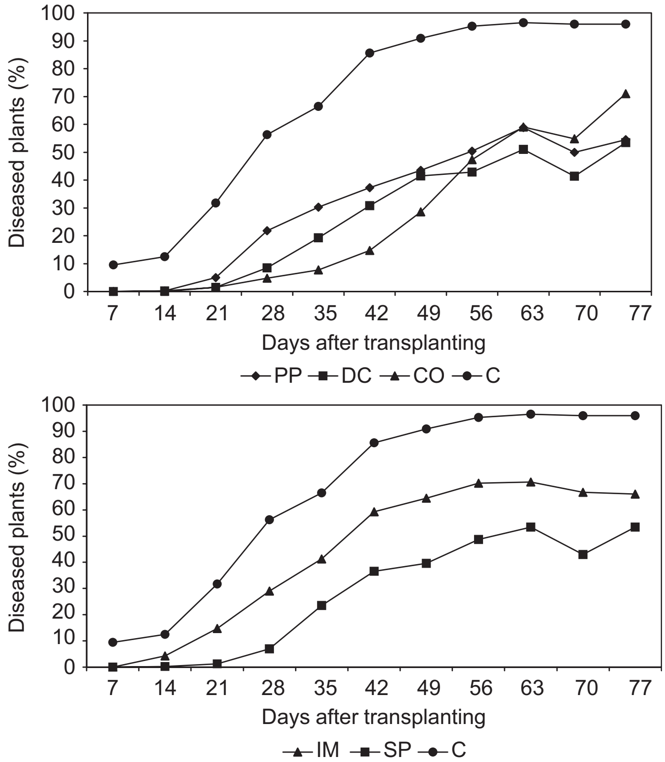

Fig. 2. Average percentages of plants affected by the Tomato Yellow Mottle (ToYMoV) disease in tomato plants grown with either living or inert ground covers, in comparison to bare ground, in Costa Rica. Treatments were perennial peanuts (*Arachis pintoi*) (PP), "cinquillo" (*Drymaria cordata*) (DC), coriander (*Coriandrum sativum*) (CO), imidacloprid (IM), silver plastic (SP) and bare ground (C). The figure is split into two panels in order to avoid excessive data overlap.

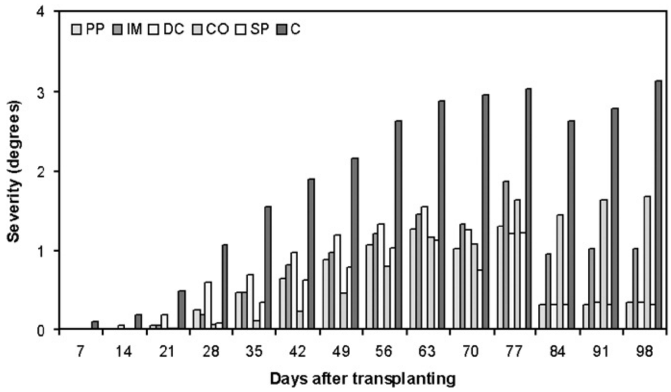

Fig. 3. Mean severity rating of Tomato Yellow Mottle (ToYMoV) disease in tomato plants grown with either living or inert ground covers, in comparison to bare ground, in Costa Rica. Treatments were perennial peanuts (*Arachis pintoi*) (PP), "cinquillo" (*Drymaria cordata*) (DC), coriander (*Coriandrum sativum*) (CO), imidacloprid (IM), silver plastic (SP) and bare ground (C).

3.3. Yields

Yields generally followed trends in disease severity, although with more differentiation among treatments. The number of fruit per plant, the weight of fruit per plant and yield (kg/ha) were least from untreated plants on bare

Table 2
Average yield (±SD) and net profit of tomatoes grown on several types of ground covers

Treatment	No. of fruits per plant	Weight of fruit per plant (g)	Yield (kg/ha)	Net profit ($/ha)
Control	2.14±1.14 c	252.16±177.58 c	5148±3780 c	−2564.9
Silver plastic	10.34±5.36 a	1755.46±1004.27 a	36,378±21,227 a	30,347.4
Imidacloprid	5.31±1.67 bc	771.95±290.44 bc	15,701±6155 bc	5752.4
Coriander	5.96±2.82 bc	901.28±479.78 bc	18,698±10,130 bc	9831.1*
Perennial peanuts	7.02±3.10 ab	1068.24±643.67 ab	21,996±13,763 ab	16,078.5
Drymaria	5.43±3.22 bc	810.76±610.02 bc	16,714±12,812 bc	6017.04

Means followed by the same letter in a column are not significantly different at $p = 0.05$ level (Fisher t-test LSD).
*Does not include $5000 from sale of coriander.

Table 3
Average weight (g) of tomatoes per plant (±SD), according to commercial category, yielded by tomato plants grown on several types of ground covers

Treatment	Commercial categories		
	I	II	III
Control	111.69±117.47 c	79.19±56.84 c	61.28±10.42 b
Silver plastic	1228.11±780.77 a	310.74±193.21 a	208.95±124.42 a
Imidacloprid	472.84±240.52 bc	162.60±75.10 bc	136.50±63.49 ab
Coriander	535.33±341.51 bc	212.07±127.42 abc	153.87±80.01 ab
Perennial peanuts	671.50±535.31 b	236.95±155.34 ab	159.77±47.55 ab
Drymaria	478.30±473.47 bc	204.29±169.38 abc	130.78±83.67 ab

Means followed by the same lower case letter in a column are not significantly different at $p = 0.05$ level (Fisher t-test LSD).

ground and greatest on silver plastic (Table 2), the latter closely followed by and not different from plants associated with perennial peanuts ($p<0.05$). The other treatments (imidacloprid, coriander and *Drymaria*) performed similarly but were not significantly different from either the perennial peanut and control treatments.

When fruit were classified by commercial categories, the greatest weight in categories I and II (marketable tomatoes) were harvested from plants on silver plastic, and least from the control, which was not, however, significantly different ($p>0.05$) from imidacloprid, coriander or *Drymaria* (Table 3). Fruit weight was greater ($p<0.05$) in the silver plastic treatment for category III (unmarketable tomatoes) compared to the control, but no differences ($p>0.05$) were seen from any of the other treatments. Generally, there were no differences among living covers in any of the three categories ($p>0.05$), nor with the imidacloprid treatment.

3.4. Economic analysis

Production costs were greatest for the silver plastic and the imidacloprid treatments due to external inputs (plastic or insecticide), whereas most costs for the living covers came from labor. Costs associated with seedbeds were common for all treatments, amounting to $1011 ($827 in labor and $184 in inputs), and field preparation, etc., which amounted to $3941 ($2552 and $1388). Costs varied among living cover treatments as follows (figures between parentheses refer to labor and inputs, respectively): $8574 for coriander ($5941 and $2632), $7695 for *Drymaria* ($6568 and $1126), $6439 for perennial peanuts ($5092 and $1346), $10,160 for silver plastic ($3321 and $6838), $8897 for imidacloprid ($2989 and $5907) and $4038 for the control treatment ($2857 and $1181).

Net profit was highest in the silver plastic treatment (US$30,347/ha), followed by perennial peanuts (US$16,078/ha) (Table 2). In contrast, losses amounting to US$2565 were estimated for the control treatment. In the case of the coriander treatment, returns were very favorable, due to a net benefit of US$9831/ha for tomatoes plus an additional US$5000/ha from the sale of the coriander used as cover plants.

4. Discussion

This study demonstrated that association with living ground covers reduced numbers of whitefly adults on tomato, reduced the incidence of the ToYMoV, and consequently increased yields and economic benefits. These results are in concert with those obtained with *B. tabaci* and *Aphis gossypii* on zucchini in Hawaii and Florida (Hooks et al., 1998; Frank and Liburd, 2005), although only aphid-borne plant viruses were observed in those studies.

Numbers of adult *B. tabaci*, as well as ToYMoV incidence and severity were higher on tomato plants grown in bare soil compared to plants associated with either living or inert mulches (silver plastic). While results with reflective and living mulches were similar in this and other cited studies, the actual mechanisms may be different. Contrast between plant rows and bare soil could serve to guide the whitefly to the crop, as has been shown with aphids and other homopterous insects (Kennedy et al., 1961; A'Brook, 1968; Kring, 1972; Smith, 1976). Reflective mulches presumably function through repellence by interfering with orientation and host location through reflection of UV light (Csizinszky et al., 1995, 1997, 1999). The living mulch could serve to efface this contrast that guides the whitefly its target.

Without bare ground as a guide, the likelihood of a visually searching whitefly descending on any particular type of plant would be simply a function of the area occupied by that plant type. Filling the space early with "inappropriate" hosts as defined by Finch and Collier (2000) would naturally decrease the probability of an encounter between whitefly and crop. Although encounters with the crop increased as the crop matured and occupied more space relative to the living cover, the potential damage from virus disease decreased proportionately.

Removing the coriander after 35 days, while leaving the crop exposed, still provided protection as well as an extra source of income and reduction of crop competition. Furthermore, an increase in whiteflies on the crop at about 5 weeks was also observed in the other treatments, and is consistent with previously documented observations that adults commonly abandon senescent and/or diseased low-quality hosts in preference for high-quality plants (van Lenteren and Noldus, 1990). On silver mulch, abundant growth of the tomato plant canopy spared from virus hid the silver mulch by 35 dat, so that it too no longer diverted whiteflies.

The living mulches we used were clearly not preferred as hosts of *B. tabaci*, as evidenced by the lack of eggs and nymphs as well as negligible adult numbers. Likewise, ToYMoV was not detected in any of them, indicating that they did not act as ToYMoV reservoirs. Moreover, we also observed few eggs or nymphs on tomato itself. Tomato is a poor host for indigenous *B. tabaci* in Central America, as evidenced by poor development on this crop (CATIE, 1990) prior to the invasion of biotype "B" in some parts of Costa Rica. However, a survey conducted just prior to this study showed that the original "A" biotype still predominated throughout most of the country (Hilje, unpublished).

Average yields of >36 t/ha (and up to 60 t/ha) obtained with plastic mulch are remarkable in Costa Rica compared to normal yields for var. Hayslip, that range from 21 to 35 t/ha (Gustavo Calvo, personal communication). Additional advantages of reflective plastic mulch included reduced competition from weeds, favorable temperatures for root and plant development, and retention of moisture and nutrients below the plastic film. In addition, we saw reduced incidence of late blight on plastic mulch. We were able to demonstrate the cost-effectiveness of reflective mulch for tomato production, producing average net profits of $30,000/ha, i.e. five times higher than those obtained with imidacloprid alone, considered a premier product for whitefly control (Stansly et al., 1998). However, plastic mulch is costly for resource-poor farmers and could be an environmental liability if not disposed of properly or recycled.

Low-growing ground covers provided an economical alternative to plastic mulches, without the environmental liability. Tomato production associated with living covers, competition from which was mitigated by leaving a voided area around each tomato plant, was low cost and profitable, critical factors for farmers with little access to credit. Although labor costs were rather high, this could also be considered a social benefit by providing employment opportunities to agricultural workers. Yields obtained with living ground covers did not differ statistically from those obtained with imidacloprid, although net benefits were sometimes greater, especially for the perennial peanut treatment ($16,000/ha).

Naturally, there was considerable variation among replicates, given different locations and/or growing seasons. For instance, in the Grecia III replicate tomato plants associated with *Drymaria* were injured by late blight, which was further compounded in coriander plots at Grecia II by root knot nematode *Meloidogyne* sp. Although these results were not repeated in all replicates, they may represent real tendencies associated with the particular cover crop, and should be further investigated. However, the consistency of our overall results demonstrates that living covers provided needed protection during the critical period of susceptibility to begomovirus infection (Schuster et al., 1996). Coriander proved to be an especially attractive choice due to its quick establishment and removal after the critical period, and additional market value of $5000/ha.

For these reasons, living covers were further selected for validation by small farmers in Guayabo and Grecia, as a part of a new project at CATIE leaded by an interdisciplinary team. Selected members of farmer organizations were exposed to our findings through meetings facilitated by a rural sociologist, using a participatory research approach. Coriander was the preferred ground cover to be tested in their commercial plots, where virus-free seedlings produced under tunnels covered by fine nets were transplanted. After 3 years of on-farm experimentation the technique was readily adopted (Hilje, unpublished) and widely disseminated by local extension agents through field days and appropriate brochures.

Nonetheless, the failure to eliminate significant crop losses due to whitefly-borne ToYMoV demonstrated the need to integrate the use of mulches, whether living or synthetic, with other preventative and curative approaches aimed at reducing inoculum pressure, including use of clean transplants, area wide coordination of planting dates and crop-free periods, and insecticidal control.

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